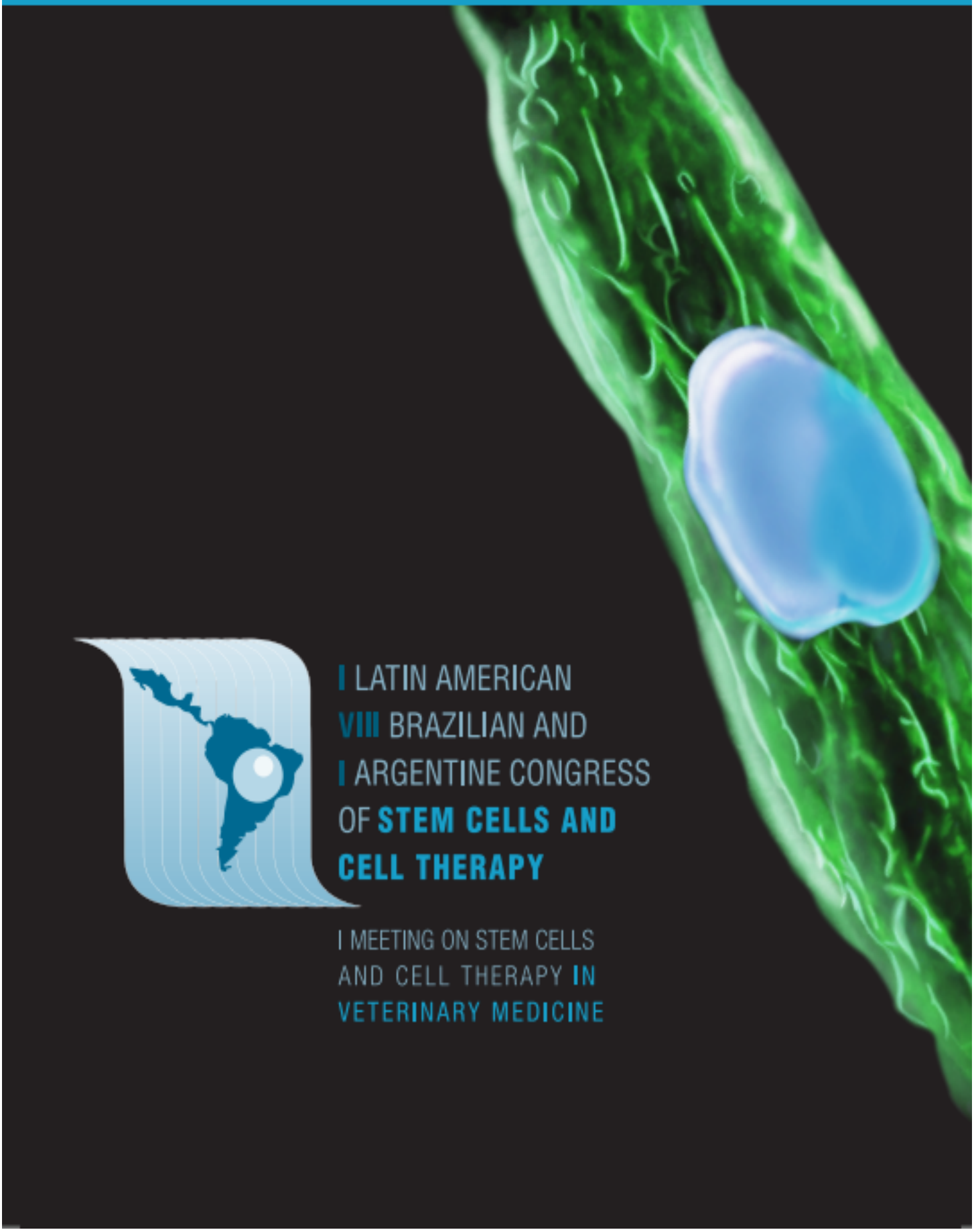
ABSTRACTS



I LATIN AMERICAN
VIII BRAZILIAN AND
I ARGENTINE CONGRESS
OF **STEM CELLS AND**
CELL THERAPY

I MEETING ON STEM CELLS
AND CELL THERAPY IN
VETERINARY MEDICINE

SUMMARY

Veterinary Medicine

1 - INHIBITION OF OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS OF THE OFFSPRING OF RATS TREATED WITH CAFFEINE DURING PREGNANCY AND LACTATION

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Caffeine is an alkaloid widely consumed by being present in medications, coffees, teas and chocolates. This compound through the placenta and milk pass to offspring and can cause teratogenic alterations and reduces the formation, growth and bone mass. How mesenchymal stem cells (MSCs) are responsible for originating the entire skeleton, we hypothesize that these cells are the target of caffeine.
The objective was to evaluate the osteogenic differentiation of MSCs from offspring of rats treated with caffeine during pregnancy and lactation. 24 adults Wistar rats were divided randomly and equally into four groups: one group without caffeine (control) and three groups with caffeine in following doses 25, 50 and 100mg/kg.
Caffeine was diluted in distilled water and administered to mothers by oro-gastric tube throughout pregnancy and lactation. The control group received distilled water as placebo. At weaning, three puppies of 21 days old of each dam and group were euthanized for extraction of bone marrow cells. On the third passage and before osteogenic differentiation, the phenotypic characterization of cells by flow cytometry using the following antibodies: anti-CD11, anti-CD90, anti-CD34, anti-CD73, anti-RT1A and antiCD54 was performed. Then, the MSC from all groups were cultured in osteogenic differentiation. At 7, 14, and 21 days of osteogenic differentiation, MTT tests and the activity of alkaline phosphatase by BCIP/NBT were performed. At 21 days the quantification of number of mineralization nodules stained by Von Kossa and quantitative assessment of the expression of gene transcripts for osteocalcin, osteopontin, sialoprotein, type I collagen, alkaline phosphatase, and Runx-2 by RT-real time PCR were performed. Data were subjected to analysis of variance with comparison of means by t test after logarithmic transformation of the data. Differences were considered to be significant if $p \leq 0.05$.
The extracted bone marrow cells of all groups showed phenotypic features consistent with MSC. The doses of 50 and 100mg/kg of caffeine significantly reduced the activity of alkaline phosphatase in all periods and the expression of collagen I at 21 days. But the expression of gene transcripts for alkaline phosphatase, RUNX-2 and bone sialoprotein and synthesis mineralization nodules decreased significantly in all groups treated with caffeine. The expression of osteocalcin decreased significantly only in the group treated with 50mg/kg of caffeine.
It is concluded that caffeine passing from mother to offspring during pregnancy and lactation inhibits osteogenic differentiation of mesenchymal stem cells. It is postulated that this reduction in the osteogenic potential of mesenchymal stem cells may be involved in the genesis of bone changes observed in the offspring of mothers who received caffeine.
Financial support: FAPEMIG and CNPq.

Ethics Committee: Comitê de Ética em Experimentação Animal (CETEA) – 177/2010

2 - FAILURE IN THE INDUCTION OF PLURIPOTENCY IN RABBIT ADIPOSE STEM CELLS: A HIGH PROLIFERATION PROBLEM?

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By the pluripotency induction, adult somatic cells acquire very similar behavior to embryonic stem cells, eliminating ethical issues related to the use of such research. However, the mechanisms involved in induced pluripotent stem (iPS) cells are not yet fully elucidated. The aim of this study was to induce pluripotency in rabbit adipose stem (ADS) cells. Rabbit ADS cells were collected and characterized by their morphology, dynamic growth, differentiation potential, viability under cryopreservation and phenotypic profile. The ADS cells were transduced with a lentiviral vector containing four human or murine pluripotency factors (OCT4, KLF4, SOX2 and c-MYC). Cells were maintained in IMDM medium, supplemented with 10% bovine fetal serum for six days, when they were trypsinized and 5x103 cells were platted on mitomycin-treated murine embryonic fibroblasts (MEFs) feeder layers with iPS medium. Several protocols were tested to acquire the pluripotent state, including the addition of MEK and GSK inhibitors or human LIF, but just partial reprogramming was detected. In order to verify the integration efficiency of pluripotent factors into the rabbit ADS cells, ADS cells were transduced with only one lentiviral vector containing an individual factor (OCT4 or SOX2), or with a combination of the two factors, those were conjugated with fluorescent reporters, vexGFP (OCT4) and mCitrine (SOX2). The integration efficiency was analyzed and sorted by flow cytometer, and analyzed by confocal microscopy.

Rabbit ADS cells were viable after cryopreservation and demonstrated a mesenchymal stem cell phenotype as fibroblastic morphology, differentiation potential in adipocytes, osteocytes and chondrocytes and a positive expression of CD73 and CD90 and negative expression of CD34 and CD45. An exceptional high proliferation potential was observed. The pluripotency induction resulted in only partial reprogramming morphology in all protocols tested. By the integration efficiency assay, OCT4 factor was detected into the cells, but not the SOX2.

Rabbit ADS are easy and fast to collect, isolate and proliferate in vitro and share the same characteristics of other mesenchymal stem cells, with a exceptional high proliferation rate. The pluripotent state could not be reached using several strategies, and just partial reprogramming cells were detected. By the integration assay, we verified that the OCT4 factor was properly transduced into the ADS cell, but the SOX2 factor could not be detected. The failure in the pluripotency induction and in the integration of SOX2 into the cells remains unclear, and further studies are necessary to better explain the mechanisms involved in the reprogramming. A possible link between proliferation and integration of SOX2 should be further investigated. We expect that managing the proliferation rate of rabbit ADS cells, the iPS cells can be generated. Sponsored by FAPESP.

Ethics Committee: Protocolo nº2560/2012 da Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

3 - OCT-4 AND P63 IMMUNOHISTOCHEMICAL IMPLICATION IN CANINE PROSTATIC CARCINOGENIC PROCESS

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In human medicine, OCT-4 is an important prognostic marker for prostate cancer (PCa) and the loss of this protein is associated with poor prognosis. Despite being a marker for steam cell, normal prostatic epithelial cells are positive for OCT-4 in humans.
In canine PCa no studies with this marker were performed. Due to importance of canine model for prostatic human disease, we performed immunohistochemistry stain for OCT-4 and P63 in canine prostatic lesions, to verify the role of steam cells in canine PCa.
We selected 10 normal prostatic tissue, 15 proliferative inflammatory atrophy (PIA) and 14 PCa for immunohistochemical staining using peroxidase method and 3,3' diaminobenzidine tetrachloride (DAB) in DakoCytomation autostainer Classic platform. For antigen retrieval the slides were incubated in TRIS-EDTA buffer (pH 9.0) in a pressure cooker. The antibody against OCT-4 and P63 were monoclonal mouse antibodies, used at a 1:50 and 1:100, respectively. The immunolabelling was performed by a polymer method. A negative control was performed for both antibodies by omitting the primary antibody and substituting with Tris-buffered saline. For each lesion a score was given: 1- 0-25% of positive cells; 2- 26 to 50% of positive cells; 3- 51 to 75% of positive cells and 4- more than 76% of positive cells. The slides were read by two pathologists at the same time. Chi-square or Fisher exact test was used to determine the association between the categorical variables.
Concerning the p63 protein expression, we found a higher number of p63-positive basal cells (>75%) in the PCa and PIA tissues when compared with the normal prostate (P=0.0002). All secretory epithelial cells of normal samples (10/10) were positive for OCT-4 and the basal cells were P63 positive. Basal cells of normal prostate tissue were negative for OCT-4. In PCa there was a loss of OCT-4 expression with P63 positive tumors cells. In PIA we found cells with P63+ and OCT-4+ phenotype.
In dogs there is an involvement of P63 positive cells in PCa and like in humans, prostatic carcinogenesis process of canine prostate is associated with loss of OCT-4.
Financial support: FAPESP/CNPq

Ethics Committee: FMVZ/Unesp/Botucatu: Protocolo 92/2011-CEUA

4 - ALLOGENIC DELIVERY OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS FOR PAIN CONTROL IN DOGS WITH BILATERAL HIP DYSPLASIA: CASE STUDY

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Studies have reported similar results of the use of mesenchymal stem cells from allogenous adipose tissue (MSCs-AT), compared to an autologous treatment, for the treatment of joint diseases in dogs, with no risks of immunogenicity. As there are many dogs with osteoarthritis (OA) and there is evidence suggesting that in many cases non-steroidal anti-inflammatory drugs cannot eliminate pain completely, the treatment with MSCs-ATs may be a therapeutic alternative. Bilateral hip dysplasia (BHD) is a disease of great incidence in dogs and its development is related to OA, the reason why it was chosen as a parameter in this study.
To assess the efficacy of intra-articular injection of MSCs-ATs in the control of pain in dogs with BHD.
An eleven-year-old Labrador Retriever dog weighing 39 kg with primary complaint of pelvic limb claudication was referred to our practice. The owner reported that the dog was feeling pain, which was evidenced by the animal struggle to climb stairs, jump, run and rise from rest. After performing physical and complementary tests (clinical examination, joint ultrasound and X-rays, blood count and biochemical tests), BHD was diagnosed based on joint ultrasound and X-rays (irregular femoral head, bone proliferation, and shallow acetabulum on both sides). Twenty million allogeneic MSCs-ATs were used for the implantation (cell bank properly characterized from a single donor). Two applications were performed in the impaired joints with a 30-day interval (days 0, 30). The dog in the study was filmed during walking, running, jumping and climbing stairs on days 0, 30 and 60 for subsequent assessment. Clinical pain assessment were performed pre-treatment on day 0 and post-treatment on days 7, 30 and 60, according to Helsinki Chronic Pain Index (HCPI) questionnaire and the 0-10 Visual Analogue Scale of pain (VAS) (0 = no pain, 10 = maximum pain level), as well as a baropodometric examination using a pressure platform (days 0, 30, 60).
Gradual improvement in locomotion was reported within 7 days from the first application (VAS Day 0 = 6.8, VAS Day 7 = 4.1, VAS Day 30 = 4.1, VAS Day 60 = 3.1), and it was easier for the dog to jump and climb stairs. Two veterinary doctors (1 and 2) examined the dog by recording non-dated videos and using the HCPI questionnaire. According to that there was improvement in pain response to palpation, claudication and ability to climb stairs. The VAS score was also established by the assessing veterinary doctors (VAS-1 Day 0 = 7.1 / VAS-2 Day 0 = 4.1; VAS-1 Day 30 = 4.6 / VAS-2 Day 30 = 5.2; VAS-1 Day 60 = 1.5 / VAS-2 Day 60 = 3.1). There were no significant changes in baropodometric examination to the moment, once weight compensation mechanism between hind limbs and forelimbs was very similar on days 0, 30 and 60.
The MSC-AT therapy has apparently been beneficial to reduce pain and to improve the quality of life in a dog with BHD.

Ethics Committee: CEUA FMVZ, UNESP- Campus Botucatu 35/2013

5 - NOT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN THE TREATMENT OF BONE MARROW DISORDER IN DOGS.

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Using other sources of mesenchymal stem cells (MSC) rather than bone marrow-derived to treat bone marrow hypoplasia in dogs in order to offer an alternative therapy to animals undergoing weekly blood transfusions.
Randomly 18 canine patients with bone marrow disorder (BMD), including 4 males and 11 females were selected of a veterinarian hemotherapy center. Most of the animals were diagnosed by myelogram with erythrocytic hypoplasia and only three with pancytopenia hypoplasia. The causes of the BMD varied between immune-mediated and sequel of canine monocytic ehrlichiosis. Only MSC from dental pulp (DPSC) and adipose tissue (ATSC) were used, provided by a heterologous canine bank. The animals were divided into four groups according to the route of administration: intraosseous (IO) or intravenous (IV); and source of MSC: DPSC or ATSC. The protocol constituted of 4 MSC applications with a range of 30 days. Biweekly blood counts were performed and hematocrit value was compared between ratings.
Surprisingly and contrary to previously reported, the animals that received IV MSC transplantation had their hematocrit increased earlier than animals from IO group. Eigth animals (44,4%) had their hematocrit normalized after the first or second transplant. Other five (27,7%) had decreased the interval between blood transfusions during the experiment and owners report improved overall health status. There was no significant difference between the sources of MSC used.
With the data obtained so far we can conclude that the use of not bone marrow-derived MSC can contribute to the regeneration of bone marrow hypoplasia. Furthermore, IV MSC transplant has many advantages over IO, such as ease of administration and not requirement of anesthesia to be performed. Once again the heterologous MSC transplantation has proven effective and without any contraindications for use. We hope our findings will contribute in the treatment of human bone marrow degenerative disorders.

6 - MORPHOLOGY AND MORPHOMETRY OF FELINE ADIPOSE-DERIVED MESENCHYMAL STEM CELLS IN CULTURE.

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The mesenchymal stem cells (MSC) are a very promising subpopulation of adult stem cells for cell based regenerative therapies in veterinary medicine. The MSC have been isolated from adipose tissue (AT), however, very few data exist about the morphology of these cells and no data were found about the morphology and morphometry of feline AT-derived mesenchymal stem cells (AT-MSC) has not been described.
The study objective was isolate, cultivate and differentiate AT-MSC into osteocytes, chondrocytes, and adipocytes and conduct clonogenic and morphological assays.
The cats ranged in age from five months to eight years. Adipose tissue was collected from subcutaneous fat depots of cats. For differentiation of AT-MSC into adipocytes, osteoblasts and chondrocytes, we used cells at third passage or fourth passage. It was assessed the morphology of 50 cells from the same animal at first passage (P1) and third (P3) and measured the length and width of the cells and of their nuclei. Cells were measured at 24, 48, 72 and 120 h of culture. The measurement results were submitted to ANOVA and the means were compared by Tukey's test.
The number of isolated cells ranged from 12,857 to 510,204 cells/g of fat, with an average of 283,793 (±219,946). In the clonogenic assays, obtained colonies varied in size and morphology. The mean CFU-F observed after the culture of 470 and 752 cells/cm2 was 20.62 ± 16.38 colonies at P1 and 21.87 ± 17.64 colonies at P3. In qualitative assays, we showed the in vitro differentiation of AT-MSC of cats into osteoblasts, chondrocytes and adipocytes. We observed the predominance of spindle-shaped and widespread cells with abundant cytoplasm. Spindle-shaped cells are longer, have less cytoplasm and a round central nucleus, with varying shapes of cytoplasmic extensions. We also found slender cells with long cytoplasmic extensions at two ends, with Y-shaped cytoplasmic extensions and smaller cells with larger abundance of cytoplasm only at one end, in addition to rare multinucleated cells. Widespread cells also varied in shape, some of which were rectangular while others were round. In terms of morphometry, we observed a significant increase in the mean length of cells during culture, both at first and third passages. The cell lengths were 109.61±56.86 µm and 155.47±74.68 µm, respectively, at first and third passages (24 h). The cell widths were 24.72±7.05 µm and 34.86±20.45 µm, respectively, at first and third passages (24 h). The nucleus length of the feline AT-MSCs increased from 17.92 µm (24h) to 23.06 µm (120h) and from 22.13 µm (24h) to 32.17 µm (120h), respectively, at P1 and P3.
This study has demonstrated the isolation of feline AT-MSC and proliferation potential. Cultures of feline MSCs cultures undergo changes as they are expanded, as observed in cultures of human MSCs. This information will be important for future qualitative investigations of feline AT-MSC.

7 - MONONUCLEAR CELLS COUNT ON DIFFERENT MOMENTS OF EQUINE BONE MARROW ASPIRATION

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The cell therapy is used for different horse diseases, and bone marrow-derived mononuclear cells (BMDMCs) are a common cell type applied for this therapy obtained by bone marrow aspiration.
To demonstrate the number of BMDMCs on initial and final moments of bone marrow aspiration in the equine sternum. Methods: Two horses were used, the first one (A1) was an 18 years old, and the second one (A2) of 21 years old, both with normal physical examination. They were sedated with acepromazine (0,035 mg/kg, IV), followed by 10% xylazine (0,35 mg/kg, IV) and pethidine hydrochloride 50mg/mL The sites of aspiration at the fourth and fifth sternebrae were cleaned, shaved and anesthetized with 2% lidocaine, followed by antisepsis undertaken using povidone-iodine. Bone-marrow was aspirated using Bone Marrow Harvest Needles, 11ga x 4in, Angiotech in 15 syringes containing IMDM and heparin, for each site, on two different sites (fourth and fifth sternebrae) for each animal. The syringes with samples were maintained in ice. Aliquots of 500µL of the first and final syringes, obtained from each site, were separated for total cell number count (TCNC) performed mechanically using a Cell-Dyn 1300 counter, Abbott Laboratories .Statistical analysis was performed using the paired t test, considering $p<0.05$.
Volumes of 458.5 mL and 408 mL of bone marrow was retrieved from aspirations of A1 and A2, respectively. After the counts were obtained per ml of bone marrow collected an average for first aspirations 51,48 x 10^{6} cells, and in last aspirations 7,22 x 10^{6}, where we found a significant difference between the first and the last aspiration in each collection point bone marrow of the sternum, $p <0.05$.
There was a significant difference between the mean aspirations.

Ethics Committee: CEUA. Protocolo 788 em 06 de junho de 2013

8 - THE USE OF STEM CELL THERAPY IN THE TREATMENT OF BONE MARROW APLASIA IMPROVES BLOOD BIOCHEMICAL PARAMETERS IN A DOG

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Animals suffering from bone marrow aplasia (BMA) exhibit clinical signs such as lower tissue oxygenation, decreased production or destruction of red blood cells and blood cells loss due to hemorrhage. As a result, it is observed pallor of mucosal membranes, lethargy, reduced exercise tolerance, dyspnea, increased heart rate and puffs induced by increased turbulence of blood. Nowadays is not yet described an effective treatment to cure BMA. We studied a female dog, Pekingese breed, with 4-year-old showed symptoms of apathy and lack of estrous cycle. The clinical examination showed severe pale mucosal membranes. The dog also presented reticulocytosis insufficient for the recovery of erythroid values, normocytic/normochromic red cells characteristic of anemic non-regenerative processes. Blood count indicated severe anemia and hematocrit value of 4%. The myelogram showed the presence of myeloid anemia. The dog was treated with transfusions, as well as, standard protocols for the control of hemolytic anemia. No improvement was observed with this treatment.
To investigate the blood biochemical parameters of a dog with BMA treated with stem cell therapy. Methods and Results: Stem cells from dental pulp were isolated and evaluate their proliferative potential. It was also assessed their ability to differentiate into osteogenic, chondrogenic or adipogenic. The data showed that after stem cells from dental pulp melt, they remained "fibroblast-like" morphology. The osteogenic differentiation was evidenced by the mineralization of extracellular matrix at day 11, which became stronger at day 21 and by positive Von Kossa staining. After induction of adipogenic differentiation, the cell morphology changed within 24 hours from elongated fibroblastic cells to oval-shaped cells. After 4 days, vacuoles in the cytoplasm of the oval-cells were observed. At the day 6, it was observed an increased number of these cells by positive Oil Red O staining. Chondrogenic differentiation was observed 21 days after induction, visualized by the staining of the extracellular cartilage matrix proteoglicans. The dog was treated with five applications of 4 x 106 alogenic DPSCs. The first 4 applications were conducted via the cephalic vein and the last by intramedullary route. The applications of DPSCs resulted in stability of the bone marrow response and increased percentage of the hematocrit. Six months after the last injection of DPSCs it was initiated the gradual reduction of the conventional medication. Currently, the female dog maintains the hematological values below the reference values, presenting mucosal membranes with normal color, feeding normally and performing normal physical activities.
Our findings reveal that stem cell therapy combined with conventional treatment can improve the dog's condition, as well as, increase her life expectancy in the case of myeloid aplasia.

9 - THERAPEUTIC USE OF MESENCHYMAL STEM CELLS IN THE TREATMENT OF MEDULLAR APLASIA SECONDARY TO CHRONIC KIDNEY DISEASE IN THE CAT.

Caroline Winck (CELLTROVET); Enrico Santos (CELLTROVET); Camila Braga (RenalVet)

The renal cortex is responsible for approximately 90% of erythropoietin production in the body. In advanced stages of kidney disease animals have reduced ability to synthesize erythropoietin, consequently, there is a deficiency of erythropoietin, resulting in erythroid hypoplastic anemia. In such cases the animals are being treated with synthetic erythropoietin. Prolonged treatment with synthetic erythropoietin results in the development of resistance leading to the medullar aplasia. We studied a female cat (indeterminate breed), 4 years old, presenting the stage 2 of chronic kidney disease. After 2 years of conventional treatment the animal developed aplastic anemia responsive to symptomatic treatment with blood transfusions. The cat made 5 blood transfusions in the period and no improvement was observed.
To investigate the effect of the applications of alogenic adipose stem cells (ADSCs) in a cat affected by aplastic anemia as a consequence of kidney disease.
Methods and Results: The adipose tissue collected was isolated and evaluate the proliferative potential of the stem cells. It was also assessed their ability to differentiate into osteogenic, chondrogenic or adipogenic. The data showed that after stem cells from adipose tissue melt, they remained as "fibroblast-like" morphology. Osteogenic differentiation was evidenced by the mineralization of extracellular matrix at day 11, which became stronger at day 21 and by positive Von Kossa staining. After induction of adipogenic differentiation, the cells morphology changed within 24 hours from elongated fibroblastic cells to oval-shaped cells. After 4 days, vacuoles in the cytoplasm of the oval-cells were observed. At the day 6, it was observed an increased number of these cells by positive Oil Red O staining. Chondrogenic differentiation was observed 21 days after induction, visualized by the staining of the extracellular cartilage matrix proteoglicans. The cat was treated with three applications of 4×10^6 ADSCs through the cephalic vein and one intramedullary. The applications of ADSCs resulted in stability of the bone marrow response and increased percentage of hematocrit (45%). Currently, the female cat is not subjected to any treatment and remains stable in her vital functions.
This study demonstrated that, in the case of medullar aplasia/chronic kidney disease, stem cell therapy combined with conventional treatment can improve the cat's condition, as well as, increase her life expectancy.

Note: The owner was aware and according to the treatment.

10 - APPLICATION OF ALLOGENIC STEM CELLS IN THE TREATMENT OF TENDON INJURIES. CLINICAL CASE REPORTS OF CELL THERAPY IN HORSES.

Camila do A. M. Alves (CELLTROVET); Caroline P. Winck (CELLTROVET); Enrico J. C. Santos (CELLTROVET)

In horses, stem cell therapies are a promising tool to the treatment of many injuries, which are common consequences of athletic animals. Tendon injuries are the most common morbidity that often compromising the performance in all types of sport horses and a return to the same level of activity. Although tendon injuries occur spontaneously during exercise, are preceded by progressive degeneration of the tendon matrix brought about by cumulative loading cycles, accelerated by competitive sport. Clinical injury results in a variable disruption of the tendon matrix, which induces an inflammatory response. This response is often short lived. Very soon after the injury, fibroplasia is initiated resulting in the formation of scar tissue within the tendon. Because of the poor functionality of scar tissue, new treatments should aim, at regenerating the tendon tissue.
To investigate the effect of the applications of allogenic adipose stem cells (ADSCs) in sixteen horses affected by tendon injuries.
Methods: The adipose tissue collected was isolated and evaluate the proliferative potential of the stem cells. It was also assessed their ability to differentiate into osteogenic, chondrogenic or adipogenic. All animals with tendonitis received 1 x 107 ADSCs into the injured tissue under local anesthetic and ultrasonographic control. After one month, ultrasonographic control was performed again. All procedures were approved by horse owners under signature of a veterinary service contract.
The data showed that after stem cells from adipose tissue melt, they remained as "fibroblast-like" morphology. Osteogenic differentiation was evidenced by the mineralization of extracellular matrix at day 11, which became stronger at day 21 and by positive Von Kossa staining. After induction of adipogenic differentiation, the cells morphology changed within 24 hours from elongated fibroblastic cells to oval-shaped cells. After 4 days, vacuoles in the cytoplasm of the oval-cells were observed. At the day 6, it was observed an increased number of these cells by positive Oil Red O staining. Chondrogenic differentiation was observed 21 days after induction, visualized by the staining of the extracellular cartilage matrix proteoglicans. Our study was based on clinical cases, the animals were heterogenous for age, weight and sex, but all of them were athletic horses. One month after ADSCs application into the lesion, the formation of healthy tissue has been observed. All treated horses showed a functional recovery and were able to return to their normal activity, without lesion recurred.
This study demonstrated that, in the case of tendon injury, the application of stem cell therapy in horses provided functional recovery of damaged tendons and treated animals were capable to return to their normal activity.

11 - THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELLS FROM EQUINE BONE MARROW

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Mesenchymal stem cells (MSCs) are characterized by extensive proliferative capacity and potential to differentiate into various mesenchymal lineages such as bone, cartilage and adipose tissue. In equine species, bone marrow (BM) is one of the most studied and used sources for obtaining adult stem cells. Studies with MSCs are increasing due to their immunomodulatory, anti-inflammatory and tissue regenerative properties. Therefore, MSCs are a promising tool for treatment of different types of injuries because of their secretion of numerous bioactive molecules leading to tissue regeneration.
Evaluate the therapeutic potential of mesenchymal stem cells from equine bone marrow (BM-MSC) through the positive expression of VEGF, VEGF-R, PDGF, PDGF-R.
Methods: Five clinically healthy crossbred horses of both sexes were used for the harvest of bone marrow. The samples were processed according to the procedures of the Laboratory of Advanced Reproduction and Cellular therapy (LANÇA) in FMVZ – UNESP- Botucatu. To confirm mesenchymal lineage of the cells, immunophenotypic analysis of BM-MSCs was performed by flow cytometry using CD90, CD34, CD105, CD44 and MHC class II markers. Additionally, BM-MSC were induced to differentiate into osteogenic, adipogencis and chondrogenic lineages. To evaluate the therapeutic potential the cells were plated in 24-well plates containing a culture medium consisting of 80% DMEM low glucose/F12 (1:1), 20% fetal bovine serum, 1% penicillin/streptomycin and 1.2% amphotericin B at 37℃ in a humidified atmosphere containing 95% air and 5% CO2 until second passage, at this time immunocytochemistry of VEGF, VEGF-R, PDGF and PDGF-R was performed.
Results: Immunophenotypic analysis of BM-MSCs by flow cytometry revealed MSCs with high expression of CD90, CD105 and CD44 markers and low or absent expression of CD34 and MHC II markers. Adipogenic differentiation was confirmed after 8 days by the deposition of lipid droplets in the cytoplasm using 0.5% Oil Red O staining. The osteogenic differentiation was confirmed after 10 days by positive staining of the extracellular calcium matrix using 2% Alizarin Red S staining. To confirm chondrogenic differentiation, after 21 days pellets were stained with Alcian Blue (pH=2.5) and toluidine blue (pH=1) to identify proteoglycans. All samples of BM-MSCs showed positive immunostaining of PDGF, PDGF-R, VEGF and VEGF-R as assessed by immunocytochemistry techniques.
This results confirm that mesenchymal stem cells have therapeutic potential by expressing platelet-derived growth factor (PDGF) and its receptor (PDGF-R), and vascular endothelial growth factor (VEGF) and its receptor (VEGF-R).
Support: FAPESP, CNPq.

12 - COMPARATIVE STUDY BETWEEN DOUBLE SPIN AND USE OF E-PET FILTER (EQUINE PLATELET ENHANCEMENT THERAPY) TO OBTAIN PLATELET RICH PLASMA IN HORSES – PRELIMINARY RESULTS

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The Platelet Rich Plasma (PRP) is a biotechnology technique that consists of high concentration of autologous platelets in a small volume of plasma in order to obtain better results in the treatment of various diseases. The use of PRP is justified by growth factors present in platelet's alpha granules. There are several protocols to obtain PRP in horses described in the literature, among which stands out double spin, automated and the filters.
This study aimed to compare the use of PRP obtained by double centrifugation protocol and obtained by the E-PET (Equine Platelet Enhancement Therapy), taking into consideration the final platelet and leukocyte concentration and cost-effective among such methods.
Four healthy horses were studied and they presented their complete blood count values within the normal range for the specie. It was used two samples of each animal to compare the two methods of achievement PRP. E-PET filter: each horse had 55mL of blood collected into a 60mL syringe containing 5mL of anticoagulant solution. This blood was transferred to the kit and had been filtered using a gravitation system in accordance with the manufacture instructions. The manufacture claims that the platelet concentration obtained by the filter is approximately seven times higher than the blood concentration. Double spin protocol: each horse had 20mL of blood collected in tubes containing sodium citrate (anticoagulant). Two centrifugations were done (300g for 5 minutes and 700g for 17 minutes) intercalated within rests of approximately 35 minutes in which the aim was to separate the plasma. After the second spin, it was discarded the 75% upper, being the remainder (25% lower) the PRP. The platelet and the leukocyte were counted using the Neubauer's chamber.
Double spin: A1: 936.775 platelets/µl of blood and 16.985 leukocyte/µl of blood; A2: 939.300 platelets/µl of blood e 17.587,5 leukocytes/µl of blood; A3: 1.121.000 platelets/µl of blood and 15.225 leukocytes/µl of blood; A4: 1.179.175 platelets/µl of blood and 13.440 leukocytes/µl of blood. Standard deviation: 124.713,7 platelets e 1.870,76 leukocytes.
E-PET: A1: 1.262.500 platelets/µl of blood and 12.810 leukocytes/µl of blood; A2: 1.595.200 platelets/µl of blood and 22.257,50 leukocytes/µl of blood; A3: 808.000 platelets/µl of blood and 23.307,50 leukocytes/µl of blood; A4: 1.315.525 platelets/µl of blood and 19.897,50 leukocytes/µl of blood. Standard deviation: 326.031 platelets e 4.725,68 leukocytes.
The amount of platelets and leukocytes obtained by the double spin and by the E-PET filter were similar, but they differed in relation to the use of a laboratory (not necessary on the filter), time (better at the filter), to be a close system (filter) and in relation to cost (higher in the filter). However, it's necessary to study the differency beetween the concentration of growth factors in these two methods.

Ethics Committee: Conselho de Comissão de Ética da FMVZ (Faculdade de Medicina Veterinária e Zootecnia - UNESP, Botucatu). Protocolo: 064/2013

13 - EFFICACY OF ULTRASOUND TO IDENTIFY MESENCHYMAL STEM CELL TRANSPLANTATION IN EQUINE MUSCLE

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Prospects of the studies with mesenchymal stem cells (MSCs) and tissue engineering have shown that MSCs may benefit the health of both humans and domestic animals. However, in horses, many of its biological characteristics remain unknown and scarce, like the residence time at the site of transplantation. In this context, the use of cell markers associated with methods of getting images in vivo are essential for tracking these cells.
This study aimed to compare images obtained through ultrasound (US) of MSCs marked and unmarked with Nanocrystal Qtracker 655 ® transplanted into Brachiocephalicus muscle of horses, with the aim of the potential traceability of the method employed.
Bone marrow (BM) was obtained from one healthy equine and sent to the laboratory (LANÇA) for isolation and cultivation to create a cell bank for allogeneic transplantation. On reaching a confluence of approximately 80%, MSCs were labeled with the nanocrystal and visualized with a fluorescence microscope. The amount of 1x106 labeled BM-MSCs were transplanted guided by ultrasound into the Brachiocephalicus muscle on the right side of the neck, and the same amount of unlabeled cells were transplanted on the left side of the neck of 12 healthy horses. The transplantation of BM-MSCs was performed at an average depth of 1 cm. Monitoring with ultrasound was performed at 0 and 24, 48, 72, 96 hours to evaluate dispersion of labeled MSCs into adjacent muscle tissue and echogenic differences.
Muscle with labeled MSCs showed regular, defined and hyperechoic images surrounded by a slight reverberation forming area at 0, 24 and 48 hours. A higher dispersion and an increase in heterogeneous echotexture along the muscular region were visualized at 72 and 96 hours. The muscle with unlabeled MSCs showed scattered and hyperechoic images, forming reverberation effect and moderate acoustic shadowing at 0 hours. A mildly heterogeneous muscle echotexture, suggestive of a healing process after inoculation was seen at 96 hour. No changes in the muscular topography were visualized at all other times.
Ultrasound monitoring of transplanted labeled MSCs proved to be of great value in clarifying the behavior of dispersion of MSCs in muscle tissue in vivo, so being able to contribute and encourage research on traceability of MSCs.
Support: FAPESP, CAPES.

Ethics Committee: CEUA (Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da UNESP - Campus Botucatu – SP). Protocolo 209/2012

14 - ISOLATION OF STEM CELLS FROM PETS USING GOOD MANUFACTURING PROCEEDINGS

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For nearly half a century, companion animals have played a key role in advancing stem cell therapies because they are recognized as critical translational models of human diseases. Nowadays primary care veterinarians, particularly for orthopedic conditions, are using adult stem cell therapies. However, veterinary stem cell therapies are still deficiency in good manufacturing proceedings to isolation of stem cells with substantial quantities and excellent quality.
Thus this study provide isolation of multipotent stem cell from dental pulp (DP) and umbilical cord (UC) that are noninvasive sources and have pluripotential niches according to requirements for human.
In order to isolation multipotent stem cells from DP were use clinically healthy pets with ranged age from 6 months to 3 years and UC from new born kittens and puppies. Herein, DP and UC cultured generating was performed by explant also multiples mechanical transfers into a new culture dish. The mesenchymal stem cells (MSC) from DP and UC were cultivated in DMEM-F12, supplemented with 15% SFB-hyclone. These cells were characterized using following antibodies: canine anti-Oct3/4, canine anti-SOX2, canine anti-NANOG, canine anti-CD44 antibody and canine anti-CD146, CD105 vimentin, alfa-actnina and fibronectina by immunofluorescence assay.
Firstly fibroblast-like cells appeared after five or six days. A total of four mechanical transfer of DP and UC was performed each 5-6 days without using enzymatic treatment. No changes, in both early transfer (T0) and later transfer (T4), were observed in morphology and expression of stem cells markers. DP and UC expressed filaments intermediary vimentin, fibronectin and alfa-actnin. The DP and UC cells expressed to CD44 and CD149 proteins. A few DP cells react positively to pluripotent stem cells markers, such as Oct3/4 and Sox2, while UC expressed Sox2 and nanog.
The DP and UC are phenotypic similar and even isolated by mechanical transfers these cells expressed proteins associated to MSC. Furthermore ours finding are of importante for the future of pet stem cell therapies, providing scaling-up of stem cells with minimum risk of losing their ‘‘stemness’’.

15 - ISOLATION, CHARACTERIZATION AND DIFFERENTIATION OF MESENCHYMAL STEM CELLS OBTAINED FROM BOVINE UMBILICAL CORD BLOOD

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Bone marrow and adipose tissue are the most common sources of mesenchymal stem cells, although their frequency and differentiating capacity decrease with age. Therefore, extra-embryonic tissues, including umbilical cord blood, amniotic membrane, amniotic fluid and umbilical cord matrix are alternative sources in stem cell therapy. Furthermore, the umbilical cord blood (UCB) cells show low levels of HLA antigens and no evidence of teratoma formation after transplantation, and have a high in vitro proliferative capacity. In cattle, there is little data concerning UCB cells, although this tissue can be considered a promising alternative for bovine regenerative medicine.

The aim of this study is to isolate, characterize and differentiate the mesenchymal stem cells obtained from bovine UCB cells.

For the UCB isolation, three samples were obtained from slaughterhouse, in the half of the gestational period. The samples were collected in sterile syringe containing heparin, and maintained cooled until been processed in the laboratory. Then, they were centrifuged, the supernatant was removed and was added culture medium containing DMEM/F12 (Gibco®) and 20% of FCS (Gibco), 100 IU/ml penicillin and 100 µg/ml streptomycin (Sigma) and 3 µg/ml amphotericin B (Sigma). The blood was diluted with the same amount of medium and added to the same proportion of Ficoll Histopaque before centrifugation to remove the mononuclear cell layer. The resulting mononuclear cells were centrifuged and then submitted to culture, in incubator with 5% CO_2 at 37,5℃. At the third passage, the UCB samples (each aliquot contained 1 x 10^6 cells) were reserved to flow cytometry. The antibodies used to detect cell surface antigens were: MHC II, vimentin and CD34. The other aliquot was reserved for in vitro differentiation into osteogenic and adipogenic lineage.

The UCB samples were capable of differentiating into osteogenic and condrogenic lineages in 10 days. When submitted to flow cytometry, 37% of the cells were positive for vimentin, indicating its mesenchymal origin. However, 16% showed positive staining for CD34, confirming its hematopoietic origin. The expression of MHC II was considered negative (< 2%).

The UCB cells are an alternative source of stem cells, since they can be obtained noninvasively, have the ability to proliferate and differentiate in vitro and thereby could be used to develop a stem cell bank. However, more information is necessary to improve the characterization of bovine mesenchymal stem cells and their immunomodulatory and remodeling ability.

Ethics Committee: Protocolo 30/2013

Veterinary Medicine

16 - PLACENTA AS A SOURCE OF ECM FOR TISSUE ENGINEERING APPLICATIONS

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Extracellular matrices (ECM) from decellularized tissues and organs have been widely applied as scaffolds for tissue regeneration due to its bioactivity, integration to the host site, degradability, reduction of scar and capacity to facilitate nutrients` exchanges. Placentas may represent a rich and important source of ECM as they have abundant stroma with a rich network of blood supply and are commonly discarded after birth. Placenta`s matrix is known to contain antioxidants, anti-clotting and other bioactive molecules such as collagen, elastin, laminin, proteoglycans and growth factors, and have immunoregulatory and anti-inflammatory properties. This study aimed at comparing different detergents in order to establish an effective protocol for decellularization of canine placentas.
Canine placentas were harvested and their maternal and fetal components were processed either separately or in conjunction. Samples having 5mm thickness were washed in ultra-pure water for 3 days and immersed in 1% SDS or 1% SDS/10mM Tris for 48 hours (group 1) and 72 hours (group 2) at 4℃. The protocols continued with incubation of the samples in 1% Triton X-100 at 4℃ for 2 days and wash in ultra-pure water. The processed tissues were then fixed in 4% paraformaldehyde and analyzed through histology and scanning electron microscopy (SEM).
Preliminary results demonstrated that the solution containing 1% SDS+10mM Tris is more effective than 1% SDS only. Although both experimental conditions allowed decellularization of the maternal and fetal components of the placenta while maintaining their tridimensional structure, as shown by SEM, the ECM tended to be more disorganized in group 2 (incubation for 72 hours) than in group 1 (48 hours).
Placentas can be successfully decellularized and represent an interesting source of ECM for tissue engineering strategies. Analyses of protein content of both placental components – maternal and fetal – as well as potential differences on their bioactivity have been investigated.

17 - EFFECTS OF VARIOUS DECELLULARIZATION PROCESSES ON THE ECM OF SKELETAL MUSCLES: IMPACT ON TISSUE ENGINEERING APPLICATIONS

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Decellularization of organs and tissues has been widely applied to produce bioactive ECM (extracellular matrix) scaffolds for tissue engineering applications. The natural protein content and tridimensional organization of these scaffolds represent important advantages over synthetic ones. Several decellularization protocols have been described. However, finding an optimal procedure that provides a cell-free matrix whereas preserves the composition, bioactivity and tridimensional structure of the ECM is crucial for future in vivo and in vitro applications. Here we analyze the influence of freezing temperatures and distinct detergents on the maintenance of 3D architecture of decellularized skeletal muscles of rats.

Biceps femoris, tibialis anterior, medial gastrocnemius and rectus abdominis muscles were harvested from adult male Sprague Dawley rats. Samples having 5mm thickness were washed in ultra-pure water, frozen at -20℃ and -150℃ and co mpared to samples that were kept at 4℃, for 24 hours. This was followed by static immersion in either 1% SDS or 1% SDS/10mM Tris solution for 48 hours. Samples were then subjected to incubation in 1% Triton X-100 at 4℃ for 2 days and again washed in ultra-pure water. Decellularized muscles were fixed in 4% paraformaldehyde and processed for histological and scanning electron microscopic (SEM) analyses.

Preliminary results indicate that freezing muscle samples at -20℃ prior to incubation with detergents may enhance and favor decellularization over storage at 4℃ or at -150℃. Lower temperature (-150℃) may lead to a more disorganize d ECM structure. Immersion of samples in SDS/Tris solution for at least 48 hours favors muscle decellularization over incubation in SDS, at the same period of time.

Optimal decellularization process is tissue-dependent. Freezing skeletal muscles prior to decellularization procedures optimize the effects of detergents. Studies have been conducted in order to analyze and compare the bioactivity of these scaffolds prepared by distinct protocols.

18 - EFFECT OF CRYOPRESERVATION ON IMMUNOPHENOTYPIC PROFILE AND CROMOSSOMIC STABILITY OF MESENCHYMAL STEM CELLS FROM INTERVASCULAR MATRIX OF EQUINE UMBILICAL CORD

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Umbilical cord intervascular stroma is composed of mesenchymal connective tissue; know as Wharton's jelly (WJ). The WJ and umbilical cord blood are sources of primitive stem cells and can be collected, stored and used for therapeutic uses or biotechnology.
The objective of this work was to evaluate the effect of cryopreservation process at controlled freezing system on immunophenotypic profile (CD44, CD90, CD34, MHCII and Oct-4) and cromossomic stability of mesenchymal stem cells from intervascular matrix of equine umbilical cord (MSC-UCMI) using different cryopreservation medium.
Samples of umbilical cord (n=5) were collected at delivery, processed and cultured in DMEM high glucose supplemented with 20% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1.2% amphotericin B at 37.5ºC in a humidified atmosphere, containing 5% CO_2 in air. At the end of second passage MSCs were trypsinized and submitted to immunophenotypic analysis by flow cytometry (FC) for the markers CD44, CD90, MHCII, CD34 conjugated to fluorescein isothiocyanate and for Oct-4 conjugated with alexa fluor 488. Furthermore, MSCs-UCMI were subjected to osteogenic (n=5) and adipogenic (n=4) differentiation assay and karyotyping. MSC were cryopreserved in a controlled freezing system using Mr Frosty (-1ºC/minute until -80 ºC, 24 hours, Nalgene®) with the following medium: Medium 1 (M1): DMEM high glucose, with 20% FBS and 10% DMSO; Medium 2 (M2) (free from FBS): DMEM high glucose, with 10% PVA and 10% DMSO; Medium 3 (M3): 90% FBS and 10% DMSO; Medium 4 (M4): 90% conditioned medium (DMEM high glucose + 20% FBS) and 10% DMSO. After three months storage in liquid nitrogen, samples from M1, M2, M3, and M4 were thawed, analyzed on FC and cultured for karyotype and differentiation assay. Data regarding immunophenotypic analysis before and after the cryopreservation with M1, M2, M3 and M4 were analyzed and compared using the tests Kruskal-Wallis One Way analysis of variance on ranks and All Pairwise Multiple Comparison Procedures (Dunn's Method) taking $P< 0.05$ as significant.
The samples were able to differentiate into osteogenic and adipogenic lineages. After thawing M2 group showed exceptional low viability, and samples were not used for karyotype analysis and differentiation assay. Changes on immunophenotypic profile were not detected ($P>0,05$) for none of studied markers independently of cryopreservation medium. Additionally, cryopreserveded MSCs-UCMI with M1, M3 and M4 medium showed cromossomic stability after cryopreservation presenting normal karyotype (2n=64) for equine specie without aneuploidy.
The process of cryopreservation on controlled freezing system does not promote changes on immunophenotypic profile and cromossomic stability of MSC-UCMI. However, the presence of FBS seems to be important for cryosurvival of the cells.
Acknowledgements: FAPESP.

Ethics Committee: CEUA 34/2013

19 - EXPRESSION OF OCT-4 AND NANOG IN MESENCHYMAL STEM CELLS FROM THE PERIVASCULAR PORTION OF MATRIX OF EQUINE UMBILICAL CORD

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Umbilical cord has been proposed as an alternative source of mesenchymal stem cells (MSCs) because of its pluripotency characteristic, non-invasive nature of isolation and low ethical implications (1). According to the literature the factors as Nanog, Oct-4 and Sox-2 are important in embryonic development and maintenance of pluripotency of embryonic stem cells (2).
The objective of this study was to evaluate the expression of pluripotency markers Nanog and Oct-4 in MSCs from the perivascular portion of the matrix of equine umbilical cord (UCMP-MSCs) in horses using the technique of flow cytometry.
Phenotypic analysis of UCMP-MSCs (n = 3 samples) was performed using the primary antibody anti OCT-4 (Millipore® Corporation, USA) and anti Nanog (Abcam®, USA) followed by labeling with secondary antibodies rabbit antimouse PE (abD Serotec®, UK) and donkey antirabbit FITC (Millipore® Corporation, USA). Prior to incubation with the pluripotency markers, cells were fixed and permeabilized with Cytofix/Cytofix/CytopermTM (Becton Dickinson® and Company, USA) to facilitate the diffusion of the antibodies used. Then, the antibodies were added and the cells incubated in the dark initially for one hour to primary antibodies, followed by the same time of incubation for the secondary antibody. The analysis was performed on LSR Fortessa flow cytometer (Becton Dickinson and Company®, USA), being recorded 10.000 events and the autofluorescence controls and secondary antibodies. It was adopted as positive staining expression above 2%. Data regarding the immunophenotypic analysis of pluripotency markers were shown as mean and SEM.
UCMP-MSCs showed positive expression for both markers of pluripotency, with higher proportions for Nanog (51.5 ± 16.4%) and lowest for Oct-4 (10.0 ± 3.1).
The results of immunophenotyping analysis based particularly on the Nanog protein demonstrates that umbilical cord perivascular portion of equine has population of progenitor cells with primitive characteristics that can be used to perform biotechnologies by example the production of pluripontency induced cells (iPS).
Acknowledgements to CAPES.

Ethics Committee: CEUA 34/2013

20 - IMMUNOPHENOTYPIC PROFILE AND VIABILITY OF BONE MARROW MONONUCLEAR FRACTION OF HORSES

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Horses are commonly affected by musculoskeletal, neurological and reproductive injuries. In order to treat these injuries, studies involving therapy with mesenchymal stem cells (MSCs) from bone marrow (BM) as well as the derived mononuclear fraction (MF) are increasing. The use of this uncultivated fraction from BM for therapeutic use has resulted in satisfactory improves, similar to the ones observed with cultured cells. The advantages of using the MF include practicality, low cost and no need for cultivation.
The aim of this study was to characterize immunophenotypically bone marrow MF of horses by flow cytometry using a panel of clusters of differentiations (CD44, CD90, CD105, and MHC-II CD18/CD11a) containing markers commonly used to characterize cultured equine MSCs. Furthermore, we also aimed to determine the viability of fresh equine MF for therapeutic use.
Bone marrow samples from 4 horses were collected by aspiration of the 5th sternebra after sedation and local anesthesia. After collection, all samples were filtered and centrifuged at 250g. At the remaining material was added low glucose DMEN in ratio 1:1, and the mixture subsequently added the Histopaque 1077 (Sigma® USA) slowly. After centrifugation at 350g for 30 minutes MF was aspirated and washed twice with low glucose DMEN. After the last wash the pellet of mononuclear cells was reserved to perform the viability analysis (n = 4) and immunophenotypic characterization (n = 3). For immunophenotypic characterization of MF were used monoclonal antibodies mouse anti-rat CD90 with FITC, mouse anti-horse CD44 with FITC, mouse anti-human CD105 with FITC, mouse anti-dog CD18 with alexa fluor 647 and mouse anti-horse MHC classe II (abD serotec®, UK). The analysis was performed by flow cytometer LSR FORTESSA (Becton Dickinson® and Company, USA), being counted 10.000 events and control of autofluorescence. It was adopted as positive markers expression above 2%. The viability test was performed by staining with 0.4% trypan blue to estimate the integrity of the cell membrane by the ratio between the number of unstained cells to the total cell number. Data regarding immunophenotypic analysis and viability are expressed as mean and SEM.
Immunophenotypic analysis revealed positive expression of markers CD18 (67 ±12,3%), CD44 (61,6 ±8,1%), CD90 (29,3 ±8,4%), MHC-II (22,5 ±7,2%) and negative for CD105 (1,7 ±0.4%). The analysis of cell viability showed excellent outcome for fresh MF (97,0±2,1%).
In experimental conditions, the cells of BM mononuclear fractions have showed excellent viability and this is an important feature for cryopreservation and therapeutic use. It is worth mentioning that, despite it has not been possible to predict the proportion of progenitor cells in the MF, since many of these CD are also expressed in leukocytes. Thus, it is important to identify markers that are expressed exclusively on stromal progenitor cells.

Ethics Committee: Protocolo n°91/2014-CEUA

21 - DIFFERENTIATION POTENTIAL OF AGOUTI (DASYPROCTA PRYMNOLOPHA) BONE MARROW MESENCHYMAL STEM CELLS BEFORE AND AFTER THWANING

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The agouti (Dasyprocta prymnolopha) is a rodent that live in South America. The isolation of mesenchymal stem cells from this species is a novelty in the field of wild animal medicine. The process of conservation and cryopreservation of mesenchymal stem cells is still a matter of study.
To isolate, expand and evaluate the differentiation potential of mesenchymal stem cells from agouti bone marrow mesenchymal stem cells (ag-BMMSC), before and after cryopreservation.
Four adult agoutis were used for this purpose. These animals were maintained in captivity in the Nucleus for Studies and Wild Animal Preservation (NEPAS) from Federal University of Piauí (UFPI). Bone marrow aspirates from the femur were collected and delivered for the Stem Cell Laboratory (LABCelt) of the Integrated Nucleus of Morphology and Stem Cell Research (NUPCelt) for isolation and expansion of undifferentiated cells. Samples of cryopreserved cells for 36 months were expanded to achieve $1x10^6$ cells. The protocols for adipogenic and osteogenic differentiation was performed for 21 days in non-cryopreserved and cryopreserved cells and subsequently were fixed with paraformaldehyde for the performance of specific staining protocols using oil red (Sigma-Aldrich®) for adipogenic (Sigma-Aldrich®) and alizarin red for evaluate the cell differentiation. The medium used and protocols for differentiation as the same of this manufacturer (StemPro®). The cells were observed in inverted light microscope and the images were recorded for evaluation.
Cells with fibroblastoid morphology and colony forming units (CFU) were observed, furthermore, the cell proliferation in cryopreserved cells was slower than non-cryopreserved cells. The adipogenic differentiation of cryopreserved and non-cryopreserved cells showed several lipid vacuoles stained with Oil Red staining (Sigma-Aldrich®). From osteogenic differentiation were observed calcium deposits stained in brown-red color, and formation of nodule-like structures. The same characteristics were observed in cryopreserved cells, besides in small number. Morphological alterations also was observed, at 15 days. Some cell cultures was maintained the fibroblastoid morphology even after differentiation.
The isolation and expansion of ag-BMMSC was possible even after crypreservation for 36 months. The morphology comparative study of non-cryopreserved and cryopreserved after adipogenic and osteogenic differentiation showed positive results in both cases, however, with more cells stained in non-cryopreserved cells.
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Ethics Committee: CEEA UFPI 018/14
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22 - CRYOPRESERVATION OF STEM CELLS DERIVED FROM EQUINE BONE MARROW

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To optimize the use of stem cells, the formation of cell banks is essential. Thus, the development of efficient cryopreservation techniques are important to allow cellular storage for prolonged period. DMSO despite being a good cryoprotector is toxic and therefore has some limitations for clinical use. Thus, several techniques have been studied in order to minimize the damage caused by the use of DMSO in cryopreservation; among them is the addition of FBS and disaccharides such as trehalose and sucrose to the culture media in order to decrease the concentration of DMSO. Disaccharides interact with membranes, liposomes and protein stabilizing these biomaterials during the process of freezing and thawing. While the beneficial effect of the FBS occurs through its interaction with cellular macromolecules during the cryopreservation process, stabilizing and protecting cell membranes.

The objectives of this study were to compare the use of traditional freezing media, based on low glicose DMEM/F12 media (1:1) suplemented with DMSO as cryoprotector, with the use of fetal bovine serum (FBS) based media for the cryopreservation of equine mesenchymal stem cells (MSCs). Moreover, the benefit of using trehalose as an additive to the cryopreservation medium was studied.

The MSCs culture (n= 3 animals) was performed at the Laboratory of Cell Therapy, Department of Veterinary Surgery and Anesthesiology, FMVZ- UNESP, Botucatu. After bone marrow aspiration and cell culture, five freezing media were tested, and cellular growth and viability was analyzed. The media used were: media 1 (control)- DMEM low glicose/F12 1:1 + 20% FBS, and 10% of the cryoprotector DMSO; media 2 (trehalose) - DMEM low glicose/F12 1:1 + 20% FBS, 0.5 M trehalose and 5% DMSO; media 3 (FBS) – 90% FBS and 10% DMSO; media 4 (FBS + trehalose) – 90% FBS + 0.5 M trehalose and 5% DMSO; and media 5 - commercial media Bambanker ®. For the freezing curve, the samples were placed in properly identified 1 ml ampoules, and brought to the -80 º C freezer for the period of 60 days. The cellular viability test was performed before cryopreservation with staining with trypan blue, used to estimate the integrity of the cell membrane; and after cryopreservation with staining with annexin V, used to evaluate the process of programmed cell death (apoptosis).

In the pre-freezing analysis with trypan blue, the samples presented 84.48% of viability. After thawing, the analysis was performed by flow cytometry with Annexin V. The media 1 showed 84.2% of viability; media 2: 71.2%; media 3: 87.4%; media 4: 57.7% and media 5: 84.9%.

Our results showed the most effective media for cryopreservation was FBS + DMSO. However, commercial media such as Bambanker ®, and the control media + Trehalose presented similar maintenance of viability. It was possible to conclude that both FBS and trehalose had a significant role in the maintenance of cell viability after thawing.

23 - MESENCHYMAL STEM CELLS AND MICROFRACTURE IN THE EQUINE CHONDRAL LESIONS TREATMENT- EXPERIMENTAL MODEL

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Articular cartilage defects represent a challenge for veterinary medicine due to the limited intrinsic potential for repair. Over the past decade, tissue engineering approaches have been developed. Mesenchymal stem cells (MSCs) have been used to repair cartilage, promote chondrogenic differentiation, act in an immunomodulatory capacity, and to decrease the degradation of articular cartilage and subchondral sclerosis.

This work aims to study the use of intralesional implantation of adipose MSCs (AD-MSCs) and microfracture in the treatment of articular chondral injuries in horses, noting the benefits and challenges of this regenerative therapy.

For this purpose the patellofemoral joints of 12 adults and health horses were approached by arthroscopy surgery to perform a 15 mm cartilage defect, on medial femoral trochlea. The experiment was designed with 2 groups of 6 animals (group A and B). The treatment with 6 microfracture perfurations and 107 autologous AD-MSCs (GA) and control group treated with only microfracture (GB). Arthroscopy for the induction of cartilage lesions was defined as zero time of the experiment (D-0). Evaluations of the synovial fluid of all groups were performed in several moments up to day 150 (D-150). The animals were submitted to magnetic ressonance on D150. Macroscopic analysis of the joint was done by arthroscopic surgery as well as biopsy of cartilage sample for histopathological analysis; both procedures were made at the beginning and at the end of the experiment (D-0 and D-150). Treatment was intralesionally, for all animals in D-30 through arthroscopic surgery.

Arthroscopy examination revealed a newly formed tissue, which was white in appearance, mechanically soft and adhered firmly and filled the chondral lesion in all groups. However, GA had an optimum filling of the lesion with repair tissue, proliferation, with an intense grip on the borders of the lesion and the subchondral bone. In the Histopathological exam both groups showed the formation of fibrocartilage, with different levels of fibrous tissue, at the site where the lesion was induced. GA showed more formation of cellular alignment, a larger number of cells similar to chondrocytes into the fibrocartilage and more type II collagen expression, comparing with GB.

The use of stem cells and microfracture together in the treatment of chondral injuries resulted in repair tissue with characteristics similar to a cartilage tissue, verified by gross examination and histopathology. Treatment with AD-MSCs minimized joint inflammation and provided better macroscopic aspect of the chondral lesion suggesting that AD-MSCs were beneficial in the treatment of chondral lesions.

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24 - TREATMENT OF EQUINE TENDONITIS WITH MESENCHYMAL STEM CELLS ASSOCIATED WITH PHYSICAL THERAPY: CASE REPORT

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Tendinitis of the superficial digital flexor tendon (SDFT) is one of the most common injuries in athletic horses. The healing process is long and has a repair, and not a regenerative nature. This feature results in loss of strength and elasticity, reducing the function of this structure. Despite the variety of therapies, the treatment for tendonitis remains a challenge. Beneficial results are expected in the use of mesenchymal stem cells (MSCs) in the treatment of tendonitis. That because, MSC have the capacity for self-renewal, chemotactic and immunoregulatory properties, and the ability of cell differentiation.

Our objective is to present a case report of a horse with important lesion of acute tendonitis submitted to the treatment with intralesional application of autologous mesenchymal stem cells.

A 6 years old quarter horse, used for cutting competitions, was attendant with lameness at the Large Animal Surgery Service of UNESP, Botucatu- SP, Brazil. After clinical evaluation, it was noted grade 3 (AAEP) lameness of the right forelimb, swelling in the palmar aspect of the metacarpal bone and sensibility to touch in soft tissues suggesting tendonitis. The ultrasound examination revealed peripheral and intra tendon edema, with 41% hypoechoic area in the 2B zone of the SDFT, thus it was diagnosed acute tendonitis of the SDFT. The treatment instituted was based in the use of nonsteroidal anti-inflammatory for 5 days, single intralesional application of CTM and physical therapy. Treatment with MSC was held after 30 days of cell culture, obtained by aspiration of autologous bone marrow from the sternal bone. The application was done using 3 ml of a solution containing 10^7 MSC resuspended in autologous blood plasma, distributed in three intralesional points. After cell application, the animal was submitted to a 15 days rest period and then instituted physiotherapy.

After 4 months of cellular application, it was noted reduction of edema, and absence of pain and lameness. In ultrasonography examination, it was visualized cell filling of the lesion with improvement of 90% and final phase of cellular organization. The animal was then led to a specific exercise protocol during 60 days. After 6 months of cellular implantation, the ultrasonography examination revealed complete cellular padding and organization of tendon fibers, being the animal released for normal training and competition. After 30 days of discharge the horse wan a prize in an open category competition.

In our experience, the use of MSC together with physical therapy has provided the best results against isolates treatment protocols. The MSC application was effective, promoting harmonious and consistent tendon repair. This case report is of crucial importance due to the success of the techniques applied, since the animal returned to its previous level of activity, ranking in first place in a competition of great importance.

25 - APPLICATION OF MESENCHYMAL STEM CELLS IN A DOG WITH BRAIN INJURY: CASE REPORT

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Mesenchymal stem cells (MSCs) are undifferentiated stromal cells that can be isolated from different tissues, being the most commonly sources the bone marrow and the adipose tissue. These cells have the potential to differentiate into bone, cartilage, fat, tendon, vascular endothelium and hematopoietic tissues. Recent studies have also demonstrated the therapeutic potential of MSCs to treat nervous system diseases, especially due to its regenerative, anti-inflammatory and immunomodulatory properties. Aiming better results and an efficient actuation on the nervous system, perineural application have being used because of its practicality, and low level of difficulty and risks.
The objective of this study was to report the effects of the application of mesenchymal stem cells derived from adipose tissue in a 8 years old dog, attended at a private practice for treatment of distemper, and later directed to the service of Veterinary Neurology of UNESP-Botucatu.
After additional tests to rule out infectious and neoplastic diseases, the animal was driven to the Acupuncture and Chronic Pain service in the same unit, for rehabilitation purposes. Physical examination revealed tetraparesis, decreased reflexes, intention tremor, with a clinical condition compatible with the one caused by the canine distemper virus. Acupuncture and physiotherapy treatment was performed, with partial improvement of the animal. Thus, we opted for the complementary treatment with epidural application of mesenchymal stem cells. For such, it was performed one application of 10 million cells every 20 days, in four applications.
From the second application, the animal regained the ability to walk.
We conclude that the cell therapy with mesenchymal stem cells from adipose tissue was effective in helping to reverse the brain condition caused by the canine distemper virus.

26 - HARVESTING, ISOLATION AND CHARACTERIZATION OF *Saimiri sciureus* ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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The Saimiri sciureus is one of the species of neotropical primates most used as a biological model due to its relatively small size and ease containment.
Establish technique of harvest, isolation and differentiation of adipose-derived mesenchymal stem cells (ASCs) of S. sciureus, targeting future applications in regenerative medicine and cell therapy in future experimental studies.
Upon approval of the Ethics Committee on Animal Use (CEUA) of the Evandro Chagas Institute (IEC), protocol N031/2013, was used two animals, adult, male, belonging to National Primate Center (CENP/IEC/SVS/MS) - Ananindeua - PA. The specimens were physically restrained, tranquilized with Tiletamine and Zolazepam Hydrochloride (6mg/kg/IM), and made local anesthetic with Lidocaine Hydrochloride. With the skin incision in the linea alba, dissecting the subcutaneous space for extracting adipose tissue fragment. The animals were given ketoprofen (2mg/kg), IM every 8h for 3 days and dressing with healing ointment. The process of enzyme digestion was performed collagenase type IV (4.5mg/mL), at 37℃ f or 1 h, and then inactivated by adding 5 mL complete medium (DMEM:F12 with 20% FBS) and centrifugation for 10 min at 1000 rpm, and the pellet resuspended and centrifuged again under the same conditions. The cell stroma was cultured at 37℃/ 5% CO2 and 56% relative humidity, with a medium change every 48h and cell viability analized each pass through exclusion test by trypan blue dye. By cytogenetic techniques for obtaining of chromosomes and G-banding, cells were analyzed in P4, 6 and 8, in order to verify chromosomal abnormalities. P8 cells were cultured with specific commercial means (StemPro, Gibco) for differentiation osteogenic, adipogenic and chondrogenic, following manufacturer's recommendations, and proven with tissue-specific dyes. Was performed characterization immunocytochemical with overnigth incubation in primary antibodies (CD105 - 1:100, CD90 - 1:100, CD73 - 1:50, CD34 - 1:50 e CD79 - 1:100) and secondary antibodies bound to biotin by 30 min/37℃ then streptavidin and revealed with diami nobenzidine.
With 24 h there were low amount cells adhered to the plastic surface, but obtained confluence, of approximately 90%, with 8 days of cultivation. After P1, the ASCs have spread rapidly, forming a homogeneous population of cells with fibroblastic morphology, keeping karyotype stable 2n 44 chromosomes, an average of viability above 80% until P8 and exponential growth. The S. sciureus ASCs differentiated into osteogenic, adipogenic and chondrogenic lineages, and had positive markers for CD105, CD90, CD73 and negative for CD34 and CD79.
S. sciureus ASCs may be isolated by a simple procedure and expanded in vitro without loss of their potential for proliferation, multilineage differentiation and expression of specific cell surface markers, keeping karyotype stable, enabling their use in therapeutic trials.

27 - VETERINARY CLINICAL INVESTIGATIONS: USE OF HETEROLOGOUS MESENCHYMAL STEM CELLS IN DOGS WITH KERATOCONJUNCTIVITIS SICCA

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Keratoconjunctivitis sicca is a common disease in dogs can be due to quantitative or qualitative deficiency of the tear film, or a combination of both. Current treatment is based on the use of topically applied artificial tear lubricants, stimulation of tear secretion and using anti-inflammatory drugs. Even advanced therapies like punctual plugs, cyclosporine B administration, and salivary gland auto-transplantation have led to a limited success. Thus, an attractive alternative stem cell treatment needs to be explored to provide better and long term relief to these patients.
To evaluate the use of heterologous mesenchymal stem cells (MSC) derived from adipose tissue in dogs with keratoconjunctivitis sicca (KSC).
MSC were obtained under good manufacturing practice conditions and fully characterized. KSC was defined by quantitative Schirmer tear test (STT) below 15mm/min. Eleven dogs of different gender, age and race were enrolled in present study. One eye of each dog was treated with 106 MSCs which were administrated in a total dose of 0.5ml of physiologic solution. Each dose was applied into two sites: 0.3 ml of MSC solution was administered directly to the main lacrimal gland and 0.2 ml to the third eyelid gland. The eyes were evaluated weekly during 8 weeks using STT, fluorescein test and slit-lamp biomicroscopy. The severity of eye score (SES) was evaluated according to conjunctival hyperemia, ocular discharge, corneal opacity or irregularity and neovascularization. Dogs, which did not show significant improvement five weeks after MSCs application, were submitted to a second application following the same protocol and scheme of evaluation.
After 3 weeks the dogs showed increased STT values when compared at baseline levels and were statistically significant ($p=0.0023$) This increase of STT values remained significant until the 5th week of MSM application and 55% of the dogs showed improvement in tear production with STT measurements above 15 mm/min. The remaining animals, which needed to receive two applications of MSC, reached their peak of tear production at the 7th week, demonstrating statistically significant results as well ($p=0.003$). After 8 weeks the dogs showed STT values increased, when compared with STT at the beginning of treatment ($p=0.0228$). The clinical improvements of corneal opacity ($p=0.006$) and conjunctival secretion ($p=0.0376$) in eyes were also observed. However, the data obtained regarding the degree of conjunctival hyperemia and corneal vascularization were not statistically significant.
MSC used in present study suggested their safety, once none of the animals demonstrated any type of rejection, allergic reaction or tumor formation. These cells demonstrated a clear clinical benefit in the treatment of KSC, thus improving the function of the lacrimal glands and of several other parameters. This study provides a basis for future clinical studies in humans with KSC.

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28 - USE OF MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE FUNCTIONAL RECOVERY OF DOG WITH CHRONIC SPINAL CORD INJURY BY DISC HERNIATION: A CASE REPORT

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The spinal cord is one of the most importante and sensitive organ systems in the body. If it is damaged, the nerve cells do not regenerate but are replaced with fibrous or scar tissue. Spinal cord injuries usually result in permanente, irreversible damage.The clinical incidence of intervertebral disc disease has been reported to be higher in the chondrodystrophoid breeds of dogs although disc degeneration can occurs in all breeds.
Describe the successful treatment and functional recovery of a dog with chronic spinal cord injury and herniated disc treated with ADMSC application via epidural catheter.
A 3 year old male dog, breed Lhasa Apso, was admitted showing signs of chronic spinal cord injury caused by an old herniated disc and with the following clinical state: pelvic limb paraparesis with presence of deep pain, increase spinal reflexes patellar, sciatic and tibia-cranial, small voluntary movement, but the absence of proprioception and pain sensation surface. The toraxical limbs worked fine while the pelvic were dragged when the dog tried to walk. The urinary bladder could only be emptied with manual compression. The dog owner said that eight months before the animal admittance it suffered a sudden paralysis of hind limbs without apparent cause. The dog was treated at an emergency service, where he received analgesics and nonsteroidal anti-inflammatory. A tomography showed severe medullar compression with a great portion of medullar material extrusion at the thoracolumbar (T12-T13) region. Back then, the dog was showing pelvic limb paralysis without deep pain sensation. Surgical correction was performed 3 days after the injury through hemilaminectomy associated with fenestration to remove the herniated disc. Postoperatively the dog received analgesics, anti-inflammatory and antibiotic prophylaxis, and physical therapy. Such treatment resulted in return of deep pain and slight voluntary movement, but the dog remained unable to stand and walk although during eight months having undergone physiotherapy and acupuncture.
The application was direct on the lesion using an epidural catheter (BD®Perisafe 20G) introduced with a lumbosacral (L7-S1) punction and its end was positioned in the epidural space at the T12 vertebra. A total of $2x10^6$ ADMSC were used mixed with a saline solution. After the procedure the animal continued with physical therapy rehabilitation program to enhance neurological stimulation.
In the first two weeks of the postoperative intensification of voluntary movement and return of superficial pain was observed. In 21 days the animals had return of proprioception with consequent locomotor function restored with only mild ataxia, but urinary function remained compromised.
According with the results shown we can conclude that adipose mesenchymal stem cells were capable of revert the paralysis and recover function.

29 - HETEROLOGOUS MESENCHYMAL STEM CELLS USED IN THE TREATMENT OF SEQUELA OF ENCEPHALOMYELITIS CAUSED BY CANINE DISTEMPER VIRUS (CDV) IN DOGS NATURALLY INFECTED

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Canine distemper is a highly contagious infectious disease caused by a virus, which affects domestic dogs. Manifestations on epithelium, optic nerves and central nervous system may be presented. The neurological infection may affect the brain and spinal cord, leading to encephalomyelitis. There is no effective treatment has been proven yet. Depending on the immune response created by the infection, the clinically affected dog may ultimately die or develop neurological sequel of encephalitis. Sequel such as paraplegia or quadriplegia, paraparesis or tetraparesis, hyperesthesia, compulsive movements, tremors, vestibular signs, focal and generalized seizures, myoclonus and more.
We aim to develop an alternative treatment to dogs, which developed neurologic encephalomyelitis caused by canine distemper virus. The treatment consists in the transplantation of heterologous mesenchymal stem cells (MSC) collected from adipose tissue of healthy young dogs.
To evaluate the treatment efficacy, 20 dogs naturally infected with CDV presenting neurological sequel were used. They were divided into 3 groups, according to their age and time of sequel onset. In group A, animals were up to 18 months old and had sequel for up to 12 months (n=6). In group B, animals were older than 18 months old and had sequel of up to 12 months (n=7). In group C, all animals were over 18 months old and had sequel for more than 12 months (n=7). MSC were obtained by enzymatic digestion of an adipose tissue fragment taken from a donor, a healthy young dog, during surgical castration procedure. The adipose tissue was brought to the laboratory to collect the MSC and then cultured in vitro. MSC were applied intravenously in 2 to 4 doses, with an interval of 30 days between the applications.
By the end of the application cycle, the animals were evaluated for the presence or absence of normal gait recovery. The results showed that in group A, the gait was recovery in 83.33% of the animals. In group B, the gait recovery rate was 85.71%, while in group C was 14.29%. The results suggest that when the neurological sequel is treated before it completes 12 months onset, the treatment with MSC demonstrates to be very effective. Either in young animals (group A) as in adult animals (group B), the gait recovery rate was remarkable. However, if the neurological sequel is treated with more than 12 months onset, the treatment efficiency is significantly lower (group C) compared to early developed sequel.
Additional studies still undergoing, in order to increase the number of animals evaluated and an efficient induction protocol. Although, the procedure have demonstrated the safety of the heterologous transplantation of MSC. As any dog had presented any form of reaction from the applications or tumor formation, even after a year of treatment. The results also demonstrated high efficiency of MSC in the treatment of neurological sequel of distemper.

30 - HUMAN ADIPOSE-DERIVED STEM CELL TRANSPLANTATION IN RATS SUBJECTED TO SPINAL CORD INJURY WITH OR WITHOUT CORTICOID TREATMENT

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Trauma is a major cause of mortality and often leads to disabling sequelae. Conventional treatment after trauma is the use of corticosteroids. Stem cells have been used in several preclinical trials for spinal cord injury and are considered a promising alternative in the treatment of trauma.
The aim of this study was to evaluate the effects of human adipose-derived stem cell (hADSC) transplantation in rats with spinal cord compression.
Spinal cord injury (SCI) in rats after laminectomy was performed at T10 and introduction of the Fogarty catheter nº3 in the epidural space, located on the cuff in T8 was inflated with 80µL of saline for 5 minutes. The animals were randomly divided into three groups: Group A, two injections of culture medium (50µL) (control group); group B, receiving two applications of $1.2x10^6$ hADSC, seven and 14 days post-lesion; group C, animals treated with 30mg/kg of methylprednisolone sodium succinate (MPSS) three hours after injury and two applications of $1.2x10^6$ hADSC, seven and 14 days post-injury. The location of hADSC and survival were assessed by in vivo bioluminescence images of animals in groups B and C (IVIS Lumina II). The emptying of the bladder was performed daily on average every 6h for 3 months. Assessments motor started 24h after SCI, and repeated daily until 3 months post-injury using the Basso-Beattie-Bresneham scale. After this period the animals were euthanized then samples were taken from the spinal cord and urinary bladder for histopathological analysis.
Bioluminescence analysis revealed large number of hADSC at the site of spinal cord injury, no cumulative effect from the first to the second transplant. Regarding urinary incontinence and motility, all animals in group A remained the state of enuresis and paraplegia after spinal cord compression, while part of the animals in groups B and C partially recovered urinary continence and motor function. Groups B and C showed statistical differences in urinary continence and mobility when compared to group A. The urinary bladder samples from groups A, B and C showed normal histological appearance with statistical difference in the degree of collagen, which is higher in groups B and C. Histological analysis revealed a higher percentage of medullary tissue preserved and largest concentration of neuropils in the spinal cords of animals in groups B and C when compared with group A.
The use of hADSC with or without the MPSS contributed positively to clinical improvement and preservation of nervous tissue after spinal cord compression in rats. The manual emptying prevented histological changes in the urinary bladder. No statistically significant differences occurred between the groups B and C. The use of corticosteroids (MPSS) not influenced results.

Ethics Committee: CEUA: 601 / CEP: CAAE: 04257912.6.0000.0020

Veterinary Medicine

31 - ASSOCIATION OF ADIPOSE DERIVED MESENCHYMAL STEM CELLS TO IMPROVE THE INTEGRATION OF FULL-THICKNESS SKIN GRAFT IN MURINE MODEL

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Evaluation of adipose derived stem cells (ADSC) effect on full-thickness skin graft (FTSG) as a wound healing model in ischemic conditions.
Two 12 mm diameter FTSGs were harvested and placed onto dorsal recipient beds of twenty-four Wistar rats, in two anatomic regions: cranial and caudal. Rats were randomized into five groups. Before grafting, group E FTSGs received subfascial injection of 1X106 ADSCs diluted in 200 µL of physiologic saline. Group EC FTSGs received only physiologic saline. Group B received ADSCs in the recipient bed edges; group C received physiologic saline in the edges. Group EB received the same ADSCs number and volume, half in the graft and half in the edges. Using planimetry, grafts were analyzed for graft´s contraction rate (d0, d5, d14), occurrence of epidermolysis and failure rate (d14). FTSGs samples were obtained on the d14 to hematoxylin-eosin and Masson's Trichrome staining for epidermal analysis (hairless, epidermal thickening, keratosis, acanthosis, hydropic degeneration) and dermal analysis (granulation tissue, inflammatory infiltrate). The obtained results were expressed by mean (n=5). Statistical significance ($p<0,05$) was calculated using Generalized Estimating Equations.
ADSCs treated groups had lower means for failure rate (B=0%, EB=2.81%, E=3.95%) than control (C=5.02%, EC=8.86%), but there were no statistically significant difference. The E group showed epidermolysis only in 30% of grafts while other groups showed 70-90%. Between d5 and d14, the contraction rate of EB (48.47%) was lower than EC (69.01%) ($p=0.05$). However, the contraction rate between d0 and d5 and d14 were homogeneous. Only the control groups, EC and C showed hairless (30%). There was no thinning of the epidermis in any rat. Considering thickening of the epidermis, the EB group (4.40) had greater mean than EC (2.80), C (2.60) and E (2.20) groups ($p=0.002$, $p=0.000$ and $p=0.000$ respectively). When acanthosis was evaluated, group E (2.00) showed a lower mean than EB (4.30) ($p=0.005$), followed by group C (2.60) ($p=0.000$). Analyzing inflammatory infiltrate in epidermis, group E (0.90) had a lower mean than EC (2.20) ($p=0.037$), while B (1.20) and C (1.20) had the same values, lower than EB (1.80). In hydropic degeneration, group E (2.00) showed lower mean than EC (3.40) ($p=0.021$). Considering granulation tissue in dermis, EB (4.50) had a greater mean than C (3.20) ($p=0.017$) and EC (3.50) ($p=0.039$). There was no statistically significant difference for keratosis in epidermis or inflammation and collagen deposition in dermis.
In conclusion, our results suggest that the application of ADSCs in subfacial FTSGs decreased the deleterious effects of ischemic injury by decreasing epithelial thickness, acanthosis, hydropic degeneration and inflammatory infiltrate.

Ethics Committee: Hospital de Clínicas de Porto Alegre. Protocolo 13-0414

32 - MSCs AND ANIMAL REPRODUCTION: USE OF ADIPOSE DERIVED MSCs IN A CO-CULTUTRE SYSTEM OF IN VITRO PRODUCED BOVINE EMBRYOS

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In vitro embryo production (IVEP) is a biotechnology used to multiply genetically superior animals in a short time interval. In bovines it is very common cultivate fertilized embryos on top of a granulosa cell monolayer derived from the oocyte (called “co-culture”) with the objective of reducing toxic metabolites and protect embryos against oxidative stress. Adipose derived MSCs (AMSCs) are multipotent cells, secrete growth factors and cytokines, and can be easily obtained.
Compare bovine AMSCs (b-AMSCs) and granulosa cells in a co-culture of bovine IVEP. We hypothesized that b-AMSCs could replace efficiently granulosa cells in co-culture of bovine IVEP.
Bovine-ADMSCs were isolated from adult male’s fat with collagenase type I (1µg/mL) and cultured in IMDM supplemented with 10% FBS and antibiotics. Stemness of b-ADMSCs was evaluated at passage 3 (p3) by immunophenotyping (CD73, CD90 and CD105) and in vitro differentiation (bone - stained with 2% Alizarin Red S; adipose - stained with 25% Oil Red; cartilage - stained with Alcian Blue 1%) using STEMPro Kit. For IVEP, bovine oocytes were obtained in abattoir and in vitro matured in TCM-199 supplemented with 10% FBS, FSH and LH for 20 hours. Mature oocytes were fertilized with semen obtained from only one bull for 24h and the resulting embryos were cultivated in 100 µL droplets of SOF medium supplemented with 5% FBS, 6 mg/mL BSA for 7 days. IVEP was undertaken at 38.5 ℃ in 5% CO_2 incubators. Experimental groups were based on cell type used in the co-culture system: Graulosa cells (group GRAN) or b-ADMSCs (1,000 and 10,000 cells, in between passage p3-p6). A group of embryos were cultivated without cells (CTRL). Blastocyst rate was evaluated on 7^{th} day after fertilization and total cell number per blastocysts was estimated by nuclear staining with DAPI (10µg/mL).
There was an combined effect of cell passage and cell amount of b-ADMSCs on the IVEP outcome (Two-way ANOVA; $p<0.05$) with the group 1,000 b-ADMSCs at earlier passages (p3-p4) rendering better IVEP results in comparison to 10,000 b-ADMSCs unregardless of cell passage. When compared to CTRL and GRAN groups, the 1,000 b-ADMSCs (p3-p4) group showed higher blastocyst rate (33.53%, 36.07% vs. 48.49%, respectively; $p<0.05$) and total cell number per blastocyst (125.68±52.06, 148.18±42.54 vs. 173.11±50.88, respectively; $p<0.05$).
Bovine-ADMSCs up to passage 4 are better than granulosa cells when utilized in a co-culture system in bovine IVEP, increasing the amount of blastocysts obtained as well as quality of them (measured in terms of total cell number).

33 - LABELING AND TRACKING GOAT ADIPOSE STEM CELLS (g-ASC) WITH QDOT® NANOCRISTALS

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The use of cell therapy is an alternative for the treatment of various chronic and degenerative diseases unresponsive to conventional treatments.
Evaluate the labeling of subcutaneous goat adipose stem cell (g-ASC) and tracking these cells after infusion in the mammary glands of goats affected by chronic mastitis.
The subcutaneous fat has collected by chest of discarded animals. The g-ASC was isolated by mechanic and enzymatic dissociation with collagenase type I (1 mg/mL). The medium used in culture was DMEM-F12 (Gibco®). The g-ASC differentiation in osteogenic, adipogenic and chondrogenic was performed using standardized medium of differentiation (StemPro®) according to the manufacturer's instructions. Flow cytometry was performed using CD 90, CD 45 and CD 105. For labeling cells was used Qdot Fluorescence Nanocrystals (Qtracker® 655) according manufacturer protocols. After intracytoplasmic Qdot® inclusion in g-ASC, these cells were prepared for infusion at a concentration of 4×10^6 g-ASC with nanocrystals (g-ASC-Nac) per microliter direct in left mammary gland. A control was performed by injecting phosphate buffered saline (PBS) in right mammary gland. Biopsies were performed 30 days after infusion.
Primary cultures showed high cellularity after 72 hours in culture and 95 per cent of variability. The fibroblastoid morphology was present in 20 days of culture. The study of plasticity present positive results in all cell types studied. Osteogenic essay demonstrated calcified matrix labeled by alizarin red. Adipogenic study showed lipid granules labeled by Oil Red. The chondrogenic essay was performed in monolayer, and showed cell aggregate nodule-like, although Alcian Blue did not stain it. Flow cytometry assay demonstrated only 30% positive for CD45 and CD 90, and negative for CD105. These inconclusive results need to be repeated. The g-ASC-Nac was applied in goat mammary gland at 4×10^6/mL. Histological biopsies were prepared in paraffin blocks. The processing in paraffin did not prevent the tracking of cells applied in the mammary gland.The g-ASC-Nac injected were tracked by fluorescence microscopy.
The process of Qtracker® cell inclusion in the g-ASC is easy to perform, not requiring adjustments in the protocol provided by the manufacturer. The tracing of g-ASC-Nac injected in mammary gland was possible even after 30 days of application, demonstrating a long-term labeling. It is not necessary to perform frozen cuts for tracking cells containing intracytoplasmic Qdots®.

Ethics Committee: UFPI/ CEAC nº 041/11; SISBIO nº 33058/ CEEA-UFPI nº 037/2012

34 - MESENCHYMAL PROGENITOR CELLS FROM COLLARED PECCARY SUBCUTANEOUS ADIPOSE TISSUE

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The understanding of cell biology and the isolation of mesenchymal stem cells in wild animals show prospects for conducting pre-clinical trials in these unconventional animals.
To evaluate the collared peccary (Tayassu tajacu) as a potential animal model for the isolation of mesenchymal progenitor cells, cell culture and cell differentiation protocols.
To perform this research we used four collared peccaries (Tayassu tajacu) from the Nucleus of Study and Preservation of Wild Animals (IBAMA / PI No. 02/08-618) from Federal University of Piauí (UFPI). Adipose tissue fragments were collected from the dorsocervical region and dissociated mechanically in laboratory. The material was placed in an incubator containing CO_2 - 95% at 37℃ and the cultures were expanded to fifth passage, evalluating cell concentration and viability. The culture medium alfa-MEM (LGC ® Biotechnology, Cat 170.83A) supplemented was changed every three days. The cell kinetics was evaluated in triplicate using growth curve performed during ten days, plating the initial concentration of $5x10^4$ cells / ml per well in P3 six-well culture plate. For cell differentiation in osteoblasts, adipocytes and chondrocytes were plated $5x10^4$ cells/mL in P3 cells culture in six wells with the respective medium for inducing differentiation (StemPro Differentiation Kit ®) plates.
The first cells with fusiform adherent morphology were visualized after 5 days of cultivation. On the eleventh day the first colony forming units (CFU), and adherent fibroblastoid morphology were observed. The isolated cells cultured to P5 have always presented characteristic fibroblastoid morphology with basophilic cytoplasm and spherical nuclei proliferation in monolayer with a mean viability of 93.8%. The growth curve showed the lag, log and plateau phases, reaching a maximum value of $14x10^4$ cells / mL. The osteogenic differentiation showed cytoplasmic calcium deposit and osteoblasts intensely marked by Alizarin Red. After 21 days, the adipogenic differentiation presented cytoplasmic lipid droplets with variable size, stained with Oil Red O. The chondrogenic differentiation performed in monolayer demonstrated the formation of aggregates (nodule-like), confirming its potential for chondrogenic plasticity.
Mesenchymal stem cells from adipose tissue of collared peccaries are a valuable tool for future scientific investigations. We suggest the use of this wild species as an alternative model for preclinical studies in cell therapy.

Ethics Committee: UFPI/CEEA nº018/13; SISBIO nº 33058-1

35 - STEM CELLS DERIVED FROM BONE MARROW CULTURED ON A MULTILAYER FILM AND SUBSEQUENTLY ANALYZED BY SCANNING ELECTRON MICROSCOPY (SEM)

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Mesenchymalstem cells (MSC) have been widely studied due to their broad potential of multi-differentiation and self-renewal. It is known that the microenvironment is highly correlated to their biological behavior. Thus, several in vitro protocols aim to mimic the tissue microenvironment and complexity. Therefore, in this experiment we choosetwo biopolymers to create a support environment to the MSC. The cells used were mesenchymal cell obtained from bone marrow due to their rapid development in vitro, and their healing potential.
The main objective of this protocol was to test a multilayer biofilm to mimic the tissue microenvironment for MSC, maintaining physiological pH and temperature. During the experiment the growth and behavior of these cells in this environment, as well as their ultrastructure were analyzed.
As base for the culture we chose a paper sheet with a pore size of 0.40 microns, negatively charged on their surface, which was covered by a multilayer biofilm. Biopolymers of choice were chitosan (material monitoring in biotechnological studies, widely found in nature, biodegradable and biologically active at physiological pH, presenting also a bactericidal action) and hyaluronic acid (mimics the tissue environment, since it is widely found in human and animals tissues as well as being biodegradable). The preparation of the material was done trough a layer-by-layer technique developed by DESCHER et al. 1992. The cells were plated on this surface and cultured for five days with medium change every 48 hours. The material was fixed with 2,5% glutaraldehyde for 24 hours and processed through standard procedure for scanning electron microscopy.
Five samples were analyzed and all presented continued growth, with the cell intertwining between the paper fibers, forming colonies in the material. A relevant fact notice in this protocol was that the cells remained round, with no morphological changes to fibroblastoid conformation. The ultrastructural analyzes showed the presence of round cells adhered to the paper fibers. Those cells were morphologically different form red blood cells since their format was round and not flattened.
It could be observed that the createdmicroenvironment gave full condition for MSC to grow, multiply and adhere on the paper surface, maintaining the characteristic of colony forming. However, the cells remained a round shape with no fibroblastoidmorphology as usually observed in plastic. This particular morphology may be due to the pores of the material. Although the cultured cells did not resemble blood red cells, more tests need to be made to discard the presence of other hematopoietic cells.
Acknowledgment: CAPES e FAPESP

36 - GOAT ADIPOSE DERIVED STEM CELLS (G-ADSC) FOR THERAPY OF INJURIES CAUSED BY CHRONIC MASTITIS: PRELIMINARY RESULTS

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The use of mesenchymal stem cells in regenerative medicine is not a novelty, however, stem cell therapy for fibrosis occasioned by chronic mastitis was not studied yet.
Evaluate the goat adipose derived stem cells (g-ADSC) for goat chronic mastitis treatment.
For cell culture of g-ADSC fat was collected by subcutaneous goat chest, and isolated by mechanic dissociation and enzymatic digestion with collagenase type I (1 mg/mL). Cells was characterized as an mesenchymal cells by differentiation, unit forming colonies (UFC) and flow citometry with CD 90, CD 105 and CD45. The cells were expanded to a concentration of 4 x 10 $4x10^6$ per animal, labeled with Qdot® fluorescent nanocrystals (Qtracker®), and transplanted by direct injection in left mammary gland. The contralateral mammary gland was used as control with PBS injection. Before infusion was performed microbiological tests, antibiotic therapy, ultrasonography examination and histopathological analysis. The histological slides was stained with Hematoxylin-Eosin and Masson's Trichromy. Images was obtained by software Image Pro Plus®. The same procedures were repeated after 30 days.
The presence of fibrous tissue in the parenchyma of the mammary gland prior to cell infusion on histopathological examination and ultrasound was identified. The ultrasonographic findings are suggestive of inflammation, since different intensities of determining brightness of images heterogeneous echogenic by the presence of fat, glandular fluid (milk), however this echogenicity is altered by the presence of fibrous tissue resulting from healing of lesions caused by inflammation. There was greater involvement of the peripheral tissues of the udder cistern. The interior of the tank showed up hypoechoic, indicating the presence of milk. The tissue changes are in agreement with the results of the histopathological analysis, which showed intense proliferation of connective tissue, aggregates of macrophages, epithelioid and giant cells and absence of normal morphology of mammary alveoli. After g-ADSC infusion was observed in ultrasonografic more homogeneous pattern of echogenicity, with less indication of fibrous tissue. And by histopathology also was no difference, with reduction of the fibrous tissue.
The therapy with g-ADSC was performed to repair the damage in the mammary gland of goats for a long time with chronic mastitis and regain function. However, although preliminary results are suggestive that there has been progress in the repair of lesions in the gland, it is not yet possible to make further conclusions. It is also necessary to investigate the functionality of the mammary gland in relation to the quantity and quality of milk production.

Ethics Committee: UFPI/ EAEC nº037/12

37 - EVALUATION OF THE MULTIDIFFERENTIATION OF CANINE ADIPOSE-DERIVED STEM CELLS (C-ASC) EARLY PASSAGE EX VIVO FROM DIFFERENT ANATOMIC REGIONS (PRELIMINARY RESULTS)

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Adipose-derived mesenchymal stem cells (ASC) exhibit three characteristics: multipotentiality, immunomodulation and angiogenesis, that makes them very attractive and suitable for tissue engineering. Those cells could be used as an alternative to traditional treatment in animal models and veterinary medicine. Domestic animal models offer a relevant contribution in advancing stem cell therapies for both human and clinical veterinary applications. Specifically in orthopedic medicine the usage of mesenchymal stem cell for cell therapy as alternative treatment has been indicated because it could be in vitro differentiated into osteocyte and in vivo promoted osteogenesis. Furthermore, several studies adopting cell therapy with ASC in human and canine bone defects was able to accelerate and improve tissue regeneration. But there are not enough basic studies on in vitro multipotentiality and passages effect of canine ASC obtained from different anatomic regions upon cell therapy efficacy.
The objective of the present study is to compare two different sources of ASC – subcutaneous fat (Sc) and visceral fat (Vs) and passage effect in relation to its multipotentiality.
During ovarectomy surgery, the ASC were obtained from the adipose tissue of 5 young female canines (4 – 11 months) from two anatomic regions: abdominal (from Sc) and periovaric (from Vs). The Sc and Vs ASC in passages 2 and 4 (P2 and P4) were induced to in vitro differentiation into adipocytes, chondrocytes and osteocytes. The osteogenic differentiation was certified by specific staining with Alizarin Red and microscopic images were randomic captured (9 fields/well) for analysis with ImageJ software.
The isolated cells were confirmed as ASC when was possible to detected fibroblastic morphology, adhesiveness to plastic and ability to differentiate into adypocites, chondrocytes and osteocytes. The isolated cell population from ASC-Sc showed 7.84 ± 1.89 % area osteogenic differentiation and ASC-Vs 2.51 ± 0.15 %. These data demonstrated that ASC obtained from Sc had a higher capacity to in vitro differentiate into osteogenic lineage than Vs in both passages P2 and P4 ($p < 0.01$). Besides, when ASC from passages P2 and P4 were compared was possible to observe no interference in potential osteogenic differentiation. The adipogenic and condrogenic in vitro differentiation was confirmed in both anatomic ASC sources. The other tests to evaluate senescence, chromosomic stability and immunomodulation capacity will be performed to complete ASC in vitro characterization.
These finding suggests that the osteogenic differentiation potential can be influenced by the anatomic region of the tissue and ASC-Sc showed higher osteogenic differentiation capacity than ASC-Vs.
These studies are still undergoing, with the purpose to better understanding the differences about mesenchymal cells obtained from different regions in order to optimize cellular therapy.

Ethics Committee: HCPA-POA. Protocolo 130510

38 - EQUINE MESENCHYMAL STEM CELLS FROM UMBILICAL CORD MATRIX: ISOLATION, CULTURE AND PHENOTYPIC CHARACTERIZATION

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In equine species, bone marrow (BM) is one of the most studied and used sources for obtaining mesenchymal stem cells (MSCs). However, adipose tissue (AT) is also an abundant and accessible source of MSCs that can provide a large number of cells required for use in cell therapy. Additionally, cells from the amniotic membrane and umbilical cord (UC) are a promising source of MSCs because they are less immunogenic and their collection is noninvasive.
In the present study, we aimed to evaluate cell culture, immunophenotypic characteristics and the differentiation potential into mesenchymal lineages of MSCs from umbilical cord tissue (UC-MSCs).
To obtain the UC (n = 6), two samples were collected from two births and four samples from the slaughter of horses. Approximately 10 cm of umbilical cord from the fetal portion was collected and stored in a sterile 50 mL conical tube containing HBSS plus 2% penicillin/streptomycin. Then, UC samples were washed three times in HBSS/penicillin and submitted to mechanical dissection to separate vein and arteries, which were discarded. The UC tissue was fragmented using a scalpel and anatomical forceps to approximately 0,2-0,3 cm size and then placed in a 0.04% solution of type 1 collagenase at 37℃ for 60 minutes. After th is period, the solution was filtered through a 70-micrometer filter, DMEM high glucose (1:1) was added, and the solution was centrifuged twice at 340 × g for 15 minutes. Cells were cultured at a density of approximately $10x10^3$ cells/cm2 in 25-cm2 flasks containing a culture medium consisting of 80% DMEM high glucose/F12 (1:1), 20% fetal bovine serum, 1% penicillin/streptomycin and 1.2% amphotericin B at 37℃ in a humidified atmosphere containing 95% air and 5% CO_2. MSCs were cultured until third passage (P3) and evaluated in vitro for their osteogenic, adipogenic and chondrogenic differentiation potential, immunophenotypic characterization with CD44, CD90, CD105, CD34 and MHC-II markers by flow cytometry, and MHC-II was also assessed by immunocytochemistry.
UC-MSCs were able to differentiate into osteogenic (after 15 days), adipogenic (after 15 days) and chondrogenic (after 21 days) lineages, confirmed by Alizarin Red S, Oil Red O and Alcian Blue and toluidine blue staining, respectively. Immunophenotypic analysis revealed UC- MSCs with high expression of CD105 (94.2±2.1), CD44 (95.7±1.5) and CD90 (67.7±6.5) markers, negative expression of CD34 (0.20±0.3) and low expression of MHC-II (5.9±1.8) markers. The MHC-II was not detected by immunocytochemistry techniques.
Equine UC is viable source for obtaining MSCs, confirmed by the immunophenotypic and multipotentiality characteristics of these cells. Due to the low expression of MHC-II by UC-MSCs, this source could be used in clinical trials involving allogeneic therapy in horses.
Support: FAPESP, CNPq.

39 - REGENERATIVE THERAPY USING PLATELET RICH PLASMA AND FIBRIN PATCHES ON SKIN LESIONS OF EQUINES

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Sport horses suffer very often injuries in the distal region of their legs. The healing with traditional treatment usually takes long time and often might develop in fibrotic or exuberant granulous tissue scars. Lately regenerative medicine, has been improving really fast. This therapy is based on the principles that regulate cell organization and regeneration. Platelet Rich Plasma (PRP) is one of the most popular products of regenerative medicine used in treatment of soft tissue in horse medicine.
Evaluate the repair produced by regenerative treatments using PRP and fibrine patches of skin wounds on horses.
Three female thoroughbreds mares (one 4 years old and two 1 and half years old) with skin injuries after unsuccessful traditional treatments, were submitted to regenerative treatment with intradermal perilesional applications of Platelet Rich Plasma (PRP) and topics of fibrin patches (FP). The PRP was obtained by double centrifugation and the FP after simple centrifugation of blood with anticoagulant. The FPs of 1ml of plasma gelified with 10% of $CaCl_2$ (average $150x10^3$ platelets/ul), were applied on clean wounds, for 3 days at 1 week intervals (3 to 6 FP each horse). The PRP was applied intradermally perilesional, (two to six, 1 ml applications / per horse, in 2 sites with an average of 460 $x10^3$ platelets/ul). The evaluation of the treatments above described were performed up to 2 months.
In all the cases, 1 month after starting the regenerative treatment the wounds were better, with epithelization and granulous tissue and were 50% smaller. After 2 months, the wounds shrank from 70% or to complete resolution. No exuberant granulous tissues appeared, nor adverse reactions were observed in any of the cases studied.
Even though this work studied very few cases, the regenerative treatment applied, accelerated the healing and had less scar tissue, when compared to the traditional treatment.
In order to establish a regenerative medicine protocol of skin treatment using PRP and FP, more studies will be carried out.

Ethics Committee: CEUAFVET-PI-22/14 -Exp. 111130-000934-14

40 - STANDARDIZATION OF USING MONONUCLEAR CELLS IN ASSOCIATION WITH ALVEOLAR MACROPHAGES FOR IN VITRO STUDY

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The alveolar machophage is the resident cell lineage in lungs and it is responsible for phagocytosis in respiratory diseases, such as asthma and a similar disease in horses, the Recurrent Airway Obstruction (RAO). Since the treatment with corticosteroids presents various side effects, the cell therapy is an option, but there are no studies in horses with RAO.
Standardize the optimal concentration and moment of addition of bone marrow-derived mononuclear cells (BMMCs) of horses for the in vitro study of alveolar macrophage phagocytosis.
In order to obtain the alveolar macrophages, three samples of bronchoalveolar lavage fluid of horses were collected, centrifuged at 340 g for 10 minutes at 4℃ and the cell concentration was adjusted to 5x106 cells/mL. The BMMCs were obtained from the fifth sternebra in previous collections and adjusted to concentrations of $1x10^3$, $1x10^4$, $1x10^5$ and $1x10^6$ cells/mL. The association of alveolar macrophages with BMMCs was analyzed by phagocytosis assay in a 96-well plate, stimulated by zymosan, at the four concentrations of BMMCs and in two different moments, each sample in triplicate. On moment 1 (M1), 100uL of BMMCs were allowed to adhere for one hour along with 100uL of alveolar macrophages and on moment 2 (M2) the BMMCs were added after stimulation of macrophages by zymosan. A triplicate with only alveolar macrophages and a triplicate for each BMMCS concentration, with no association with macrophages, were used as the control group. The result was obtained by absorbance at 550nm. Statistical analysis was performed by Kruskal-Wallis test followed by Dunn's test.
The results for M1 and M2 were not statistically significant (P>0,05), probably due to the small number of samples, but it was possible to observe a variation between the moments. M1 caused an increase in phagocytic activity and M2 caused its decline, which shows promise for a reduction in the inflammatory response. It was observed that the absorbance in controls for only alveolar macrophages and only BMMCs was similar, being the result of M1 the sum of alveolar macrophages and BMMCs in controls. For M2 the absorbance values decreased whereas the BMMCs alone already generate response. Also related to the M2, two concentrations of BMMCs are more efficient, they are $1x10^3$, quoted in the literature for similar studies in human asthmatics, and $1x10^6$, which was the most significant outcome for this study.
It can be observed promising results for cell therapy in respiratory diseases by its influence on the activity of alveolar macrophages after stimulation.

Ethics Committee: CEUA-PUCPR n°788

41 - EFFECTS OF THE APPLICATION OF MESENCHYMAL STEM CELLS ON MOVEMENT AND URINATION OF RATS SUBJECTED TO SPINAL CORD INJURY

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Cell therapy has been reported as a possible treatment for spinal trauma, frequently in people and animals, and that does not exhibit pharmacological cure for damages arising out of the primary lesion.
Were evaluated the effects of the application of human adipose derived stem cells (hADSC) in rats after spinal cord injury.
The hADSC were cultured, used between the third and fifth passages and part of them was transduced for screening in vivo after transplantation. Spinal cord injury was performed with a Fogarty catheter nº. 3 inserted into the epidural space with cuff located at T8 and filled with 80 µL of saline for 5 minutes. The control group A (n=12) received culture medium (50 µL) and group B (n=12) received two applications hADSC ($1.2x10^6$), seven and 14 days post-injury, both the tail vein. The bladder emptying by massage was performed daily for three months. Evaluations motors were repeated daily until three months post-injury using the Basso-Beattie-Bresneham scale. After this period the animals were euthanized and histological analysis of urinary bladder and spinal cord were performed.
The bioluminescence analysis revealed hADSC application site and lungs. There was improvement of the function of the urinary bladder in 83.3% of the animals in group B and 16.66% of the animals in group A. The analysis of motor and histological evaluations of the spinal cords and urinary bladders no demonstrated significant differences between groups A and B.
The results indicate that transplanted hADSC influenced urination, beneficially, by a mechanism of action at a distance, telecrine.

Ethics Committee: CEUA-PUCPR: 637 / CEP: CAAE: 04260412.0.0000.0100

42 - EFFECT OF CRYOPRESERVATION ON CD34+/CD45- CELL COUNT IN CANINE BONE MARROW FROM DOGS WITH LYMPHOMA THAT UNDERWENT AUTOLOGOUS BONE MARROW TRANSPLANTATION

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Autologous transplantation involves temporary removal of the hematopoietic stem cells (HSC) from the receptor itself. The HSC are derived from bone marrow or peripheral blood and can be reinfused in the receptor after induced myelosuppression by chemotherapy or radiation. However, cryopreservation of bone marrow or peripheral blood bags may reduce the number of nucleated cells. Therefore, it is important to quantify these cells after freezing, prior to infusion into the patient.
The aim of this study was quantify CD34+/CD45- stem cells by means of flow cytometry in dogs with lymphoma that underwent autologous bone marrow transplantation, before and after cryopreservation.
Seven dogs diagnosed with lymphoma were included in this study. They were 3-9 years old and weighed 12 to 39Kg. Three dogs were male and four dogs were females. There were two Rottweilers, two mongrel dogs, one Labrador Retriever, one Cocker Spaniel and one Golden Retriever. All patients were treated with Madison-Wisconsin protocol. After induction phase, dogs that achieved complete remission had the bone marrow collected from iliac crest. The transplantation protocol consisted of bone marrow harvesting (10 mL/kg), followed by administration of cyclophosphamide (400 or 500 mg/m^2) and intravenous reinfusion of bone marrow. Bags containing bone marrow were processed in steps including red cell depletion, plasma depletion and cryoprotectors addition. After homogenizing the contents of the freezing bag, samples of 1ml were separated to quantify stem cells before the freezing process. Bags were stored at -80℃ for four days. After the freezing p rocess, the cells viability was evaluated. We used the "International Society of Hematotherapy and Graft Engineering" (ISHAGE) protocol for quantitation of CD34+ stem cells. Analyses were performed in duplicate with monoclonal antibody anti-CD45+ conjugate to isothiocyanate of fluorescein (FITC) (Rat Anti-Dog CD45: FITC, SEROTEC) and anti-CD34+ conjugated with phycoerythrin (PE) (PE Mouse Anti-Dog CD34, BD Pharmingen) analyzed with FACSDiva software FACSCANTO on the flow cytometer (Becton Dickinson, San Jose, CA, USA) for identification and quantification of CD34+CD45- cells. T-test was used to detect difference between moments. P
The mean values and standard deviations of HSC (CD34+/CD45-), before and after freezing were 0,145 (± 0,171) x 10^6/kg and 0,09 (±0,108) x 10^6/kg, respectively. The difference between these periods was not significant (p> 0,05). We did not find any description that showed the stem cells quantification in bone marrow bags of dogs in similar conditions to our methodology.
Cryopreservation did not influence the count of HSC (CD34+/CD45-) in bone marrow bags stored at -80^{o}C for four days, reinfused in dogs with lymphoma.

Comitê de Ética: Estudo foi aprovado pela Comissão de Ética e Bem-estar Animal da FCAV/UNESP; protocolo n°17265-06

43 - COMPARATIVE GENERATION OF INDUCED PLURIPOTENT CELLS (IPS) DERIVED FROM HUMAN AND ANIMAL MODELS

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Re-acquisition of cellular pluripotency is routinely accomplished in animal models after nuclear transfer (NT) of somatic cells, however isolation and in vitro maintenance of pluripotent embryonic stem cells is challenging in animals due to the lack of knowledge on specific pluripotency mechanisms. The advent of iPS cells generation may represent a new possibility of using pluripotent cells derived from large animals for either enhancing animal production trough NT or else for its use as suitable models in translational medicine.
The present study aimed to generate bovine and equine induced pluripotent stem cells (biPS and eiPS) using murine and human pluripotency induction protocols (miPS and hiPS) as controls.
Bovine, equine, human and mouse iPS cells were produced trough lentiviral transduction of mesenchymal or fibroblasts cells with a polycistronic excisable vector containing human or mouse OCT4, SOX2, C-MYC and KLF4 pluripotency-related transcription factors (h or m OSMK). Cells were transduced overnight, cultured in vitro for 5 days and transferred to MEFs in iPS culture media (KO DMEM/F12 supplemented with 20% KSR, 1% glutamine, 1% neaa, 1% antibiotics and 10ng/ml bFGF with LIF and/or 2i when needed) for at least 21 days. Clonal iPS lineages were produced and a minimum of 3 different clonal lines for each species was characterized regarding morphology, protein expression of pluripotency factors by immunofluorescence, alkaline phosphatase detection, embryoid body formation, in vitro differentiation, in vivo teratoma formation and required supplementation.
Bovine cells could be reprogrammed with mouse factors but not with human OSMK; however, equine cells could be better reprogrammed with human OSMK. Human and equine colonies were formed more rapidly when compared to bovine and murine iPS colonies and presented a flat shaped morphology, whereas biPS and miPS presented dome-shaped colony morphology. All lineages studied were positive for alkaline phosphatase, OCT4, SOX2 and NANOG protein detection, they formed embryioid bodies; however, teratomas were observed only when biPS or miPS were injected in nude balb/c mice. hiPS could not be enzymatic dissociated for passaging. LIF was used for mIPS culture, and 2i was not essential for iPS generation in any species.
In summary, iPS cells from bovine, equine, human and mouse models were generated in similar conditions, however biPS resembled miPS whereas eiPS resembled hiPS cells. A better understanding of nuclei reprogramming mechanisms in different species should enhance the efficiency of reproductive biotechnologies and allow the use of pluripotent cells in cellular therapies.
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Ethics Committee: Fundação Hemocentro CONEP n°. 250 /2010 / Comitê de ética em Experimentação Animal da FZEA/USP

Basic Research

44 - EVALUATION OF THE PROTECTIVE EFFECT OF MESENCHYMAL STEM CELLS-DERIVED MICROVESICLES IN MODELS OF CHRONIC KIDNEY INJURY

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Chronic kidney disease (CDK) has a great impact on clinical practice, with options of treatment restricted to dialysis and kidney transplantation. However, the reduced number of donors for transplantation and complications during dialysis turn the search for new therapeutic alternatives an immediate necessity. In this context, stem cell-based therapy has been considered an innovative strategy for the contention of chronic kidney injuries progression, and the use of mesenchymal stem cells (MSCs) has showed efficient to promote both fibrosis reduction and functional recover in experimental models of kidney injuries. Recently, have been demonstrated that MSCs-derived microvesicles (MSCs-MVs) play an important role in transferring information by cell-to-cell intercommunication, being as effective as MSCs to promote kidney repair.
This study aims to assess the protective properties of MSCs-MVs using in vivo and in vitro experimental models of chronic kidney injury.
Mice were infused with MSCs-MVs at a dose of 100µg/infusion via tail at days 0, 1, 3 and 6 after UUO induction. Mice were euthanized on day 7 after UUO and blood, urine and renal tissue were collected for further analysis. To in vitro analysis, the renal epithelial cells (MM55K) were distributed in the following groups: 1) Control, without any stimulus, 2) Stimulated with 10 ng/mL of TGF- β1 and 0,5 ng/mL of TNF- α, and 3) Stimulated with 10 ng/mL of TGF- β1 and 0,5 ng/mL of TNF- α and MSCs-MVs during 48 hours (5 infusions of 50 µg each 8 hours).
The mice treated with MSCs-MVs demonstrated significant reduction of pelvis proteinuria, and consequently reduced proteinuria/creatinuria rate than non-treated animals. Histological analysis showed lower Collagen IV and Fibronectin expression. Furthermore, we observed by PCR analysis an elevated level of IL-6 and IL-10 transcripts in renal tissue treated with MSCs-MVs, considering that potentials marker of fibrosis and inflammation such as α-SMA, TGF-β and TNF-α had its expressions reduced. Interestingly after MSCs-MVs infusion, it was observed in renal tissues an increase of BMP-7 expression, an important renoprotective molecule related to kidney fibrosis. In addition, in vitro assays demonstrated that renal epithelial cells stimulated with TGF-β1 and TNF-α obtained a spindle-like phenotype, losing its cell-to-cell adhesion abilities and cubic morphology. On the other hand, epithelial cells co-cultured with MSCs-MVs showed a reduction of this phenomena and lower expression of α-SMA and TGF-β1 mRNAs, demonstrating the protective role on fibrosis and epithelial-to-mesenchymal transition (EMT).
Our partial conclusion suggests that the infusion of MSCs-MVs is able to prevent fibrosis by EMT and inflammation modulation, a key events involved in CDK progression, promoting renal functional recover. However, more robust analysis, both in vivo and in vitro, are required to elucidate the global protective mechanisms of MSCs-MVs in renal fibrosis context.

Basic Research

45 - ANTIINFLAMMATORY ACTION OF DIFFERENT EXTRACTS OF *Cordia ecalyculata* IN CULTURES OF HUMAN MESENCHYMAL STEM CELLS AND FIBROBLASTS

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Cordia ecalyculata, a member of the Boraginaceae family, is used in Brazil as a healing agent. Due to the fact that many patients do not achieve remission from chronic wounds, the Blood Bank Biotechnology Department of São Paulo State University, UNESP, Botucatu campus, has established a protocol for the production of bioactive dressings which interact with the wound and stimulate re-epithelialization by inducing the stem cells.
The aim of this study is to compare the apoptosis rate (AR) and cell viability (CV) between the cultures of human mesenchymal stem cells (MSCs) and fibroblasts and to measure the levels of inflammatory cytokines released.
Tests were performed from different plant purifications: crude extract (CE), aqueous phase (AP), hexane phase (HP), and ethereal phase (EP) in three concentrations – 25, 50, and 100µg/ml – which were applied in cultures of human MSCs and fibroblasts. The AR was analyzed through the caspase-3 method, while the cytokines were analyzed through CBA. The MSCs suffered more apoptosis when submitted to the EP in concentrations of 50µg/ml and 100µg/ml.
The fibroblasts were susceptible, both at HP (concentration of 100) and at EP in the concentrations of 50 and 100µg/ml. CV was compatible with the apoptosis; during the EP in the 100 µg/ml concentration, there was 100% apoptosis in fibroblasts and 90% in MSCs. When analyzing the cytokines, the production of IL-6 and IL-8 by fibroblasts and MSCs was observed.
Therefore, it is possible to conclude that Cordia had a greater inflammatory action in the fibroblasts than in the MSCs, in controlling the production of inflammatory cytokines, which clearly shows the cell differentiation between fibroblasts and MSCs.

Basic Research

46 - DIFFERENTIATION POTENTIAL OF MESENCHYMAL STEM CELLS DERIVED WHARTON'S JELLY OF CANINE UMBILICAL CORD, BEFORE AND AFTER CRYOPRESERVATION

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Study of mesenchymal stem cells (MSCs) are in evidence generating much anticipation, such as their use in cell therapy for the cure of degenerative diseases, like Parkinson's and Alzheimer's. The fetal membranes in particular, are increasingly being studied, but for that, the uses of banks with cryopreserved cells are necessary. Studies in dogs are important for the future of these experimental tests aiming their possible use in human medicine.
Thus, the goal of this study was to assess differentiation potential of cells from canine umbilical cord extravascular matrix before and after cryopreservation.
For this, four samples of umbilical cord (n = 8), obtained from cesarean sections were used, the samples were processed immediately, by isolating the cellular fraction of the tissue, and then subjecting to culture in basal medium containing low glucose DMEM (Gibco ®) and 20% Fetal Bovine Serum (Gibco ®) supplemented with antibiotics and antimycotics (Gibco ®). The cultures were grown in an atmosphere of 5% CO_2 at 37.5℃. At the moment of the first passage a pa rt of the cells were cryopreserved in a mixture of 10% vol. dimethylsulfoxide (DMSO) and 90% vol. Fetal Bovine Serum. The other part of the cells was cultured until third passage. At this moment differentiation was induced using Stem Pro kits (Gibco®) for osteogenic and adipogenic differentiation. Media were prepared according to the manufacturer's instructions, and changed every 3 days.
After thaw the cells were cultured until third passage and the same analysis were performed. Before cryopreservation 62,5% of cell positively differentiate for osteogenic and adipogênica lineage, and after cryopreservation the differentiation rate was 75%.
With these results we suggest that the cryopreservation did not change the characteristics of the canine umbilical cord cells. The mesenchymal stem cells of umbilical cord extravascular matrix retained their differentiation potential, indicating a possible use to assembly a cell bank.
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Basic Research

47 - IMPAIRED MITOCHONDRIAL FUNCTION AND REDUCED VIABILITY IN BONE MARROW CELLS OF OBESE MICE

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Bone marrow cells (BMCs) are the main type of cells used for transplantation therapies. Obesity, a major world health problem, has been demonstrated to affect various tissues, including bone marrow. This could compromise the success of such therapies. One of the main mechanisms underlying the pathogenesis of obesity is mitochondrial dysfunction, and recent data have suggested an important role for mitochondrial metabolism in the regulation of stem cell proliferation and differentiation.
Since the potential use of BMCs for clinical therapies depends on their viability and capacity to proliferate and/or differentiate properly, the analysis of mitochondrial function and cell viability could be important approaches for evaluating BMC quality in the context of obesity.
We therefore compared BMCs from a control group (CG) and an obese group (OG) of mice and evaluated their mitochondrial function, proliferation capacity, apoptosis, and levels of proteins involved in energy metabolism.
BMCs from OG had increased apoptosis and decreased proliferation rates compared with CG. Mitochondrial respiratory capacity, biogenesis, and the coupling between oxidative phosphorylation and ATP synthesis were significantly decreased in OG compared with CG, in correlation with increased levels of uncoupling protein 2 and reduced peroxisome proliferator-activated receptor-coactivator 1α content. OG also had decreased amounts of the glucose transporter GLUT-1 and insulin receptor (IRβ).
Thus, Western-diet-induced obesity leads to mitochondrial dysfunction and reduced proliferative capacity in BMCs, changes that, in turn, might compromise the success of therapies utilizing these cells.

Basic Research

48 - ESTABLISHMENT OF EFFICIENT CONDITIONS FOR EMBRYO DEVELOPMENT USING MESENCHYMAL STEM CELLS AS FEEDER LAYERS

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Despite the advances in assisted reproduction techniques, the poor quality and failures in in vitro embryo development remain as a drawback resulting in low pregnancy rate. Bone marrow mesenchymal cells (MSCs) have emerged as a novel therapeutic option due to their unique properties of releasing bioactive factors and supporting others cell growth. In addition, murine embryonic fibroblasts (MEFs) have been widely used as a feeder layer to support embryonic stem cells due to their release of growth factors.
In the present study we have compared the role of MSCs and MEFs in supporting C57Black6 mouse early embryo development.
MSCs and MEFs were isolated from mice and cultured in DMEM-F12 with 10% fetal bovine serum up to the third passage. All the embryos were obtained ~42 hours (2^{nd} day) after mating and were randomly distributed in the following groups: CTRL - cultured in control culture medium; iMSC - co-cultured with MSCs inactivated to arrest proliferation; and iMEF - co-cultured with MEFs inactivated to arrest proliferation. Inactivation was performed using mitomycin C. Embryo development was evaluated daily for 5 days (7^{th} day after mating). Immunocitochemistry, diameter and total cell number of blastocysts were measure at the 3rd day after culture. The statistical analysis was performed by non-parametric Kruskal-Wallis test with Dunns post-test and $p<0.05$ was considered as statistically significant.
We observed at 2^{nd} day after mating (day of embryo acquisition) the proportion of following development stages: 87.0% at 2-cell, 6.5% at 3-cell, 3.7% at 4-cell and 2.8% at 5-8-cell. After the 3rd day in culture, the embryos co-cultured with iMSC or iMEF showed a greater development when compared with the CTRL group. On the 5th day in culture the rate of hatched blastocysts in iMSC (84.1±5.8%) and iMEF (90.3±4.2%) groups were higher than CTRL group (49.2±8.8%). We did not observed any difference in the proliferation or apoptosis among the groups, however, the blastocysts co-cultured with iMSC presented a significant higher number of inner cell mass (26.1±1.6%) and a lower number of trophoblast cells (73.9±1.6%) when compared to the CTRL group (20.4±1.5% and 79.6±1.5%, respectively). The iMSC and iMEF groups presented a higher cell number (70.9±2.5 and 74.5±2.7, respectively) and diameter (133.7±2.53 and 139±2.3 µm, respectively) when compared to the CTRL group (cell number: 60.3±2.14; and diameter: 123.8±2 µm).
In summary, our data suggests that co-culture with inactivated MSCs or MEFs greatly support and improve the early embryonic development in vitro.
Supported by FAPEMIG, FAPERJ, CNPq, CAPES and DECIT/MS.

Basic Research

49 - EFFECT OF BONE MARROW MONONUCLEAR CELLS TRANSPLANTATION ON HEPATIC MITOCHONDRIAL BIONERGETICS IN RATS WITH LIVER FIBROSIS INDUCED BY BILE DUCT LIGATION

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Mitochondrial dysfunction has been associated with several diseases, including liver cholestatis, which is characterized by activation of Kupffer cells and fibrogenic cells that produce excessive extracellular matrix. Toxic bile salt accumulation in liver parenchyma leads to chronic injury with mitochondrial damage, ATP synthesis reduction, ROS increase and apoptosis, resulting in liver function impairment. Our previous works showed the positive effect of bone marrow mononuclear cells (BMMNC) transplantation on liver fibrosis resolution and hepatic function recover in cholestatic rats.
This study aimed to analyze mitochondrial physiology and biogenesis, oxidative stress, and collagen expression in the liver of cholestatic rats transplanted with BMMNC.
Wistar male rats were divided into four groups: normal animals, animals with cholestasis after 14 and 21 days of bile duct ligation (BDL), and animals with cholestasis after 14 days of BDL that received $1x10^7$ BMMNC, via jugular vein, and were killed after 7 days. The livers were analyzed by high-resolution respirometry and western blotting.
Our data demonstrated increased collagen type I content in livers of 21d BDL group compared to normal group, indicative of fibrosis, and its decrease after BMMNC transplantation. Liver mitochondrial physiology analysis showed that 14d BDL and 21d BDL groups have significantly reduced maximum ADP-stimulated respiratory rates (State 3), indicating reduced carbohydrates and fatty acids oxidation capacity. In addition, respiratory control ratio (RCR), indicative oxidative phosphorilation coupling to ATP production, was significantly decreased in 14d BDL and 21d BDL groups, suggesting mitochondrial uncoupling. However, BMMNC transplantation significantly increased both State 3 respiration and RCR to levels similar to those of normal group, recovering hepatic mitochondrial function. These results were confirmed by WB analysis, which showed that 21d BDL group had a significantly increase in liver mitochondrial uncoupling protein content, UCP-2, and reduced PGC-1α content, a mitochondrial biogenesis co-factor. However, after BMMNC transplantation both proteins returned to levels similar to normal group. In addition, WB analysis showed that 14d BDL had a significantly increase in 4-HNE content compared to normal group, indicative of oxidative stress, but after BMMNC transplantation 4-HNE content significantly reduced, compared to 14d BDL and 21d BDL groups, suggesting oxidative stress reduction.
BMMNC transplantation had a positive effect on hepatic mitochondrial bioenergetics of cholestatic rats, increasing oxidative capacity and reducing oxidative stress, which, in turn, contribute to liver function recover.

Basic Research

50 - MIGRATION OF BONE MARROW GFP+ CELLS IS ASSOCIATED WITH STROMAL CELL-DERIVED FACTOR-1 SECRETED BY THE FIBROTIC LIVER

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Liver fibrosis is characterized by persistent parenchymal injury and deposition of extracellular matrix resulting in loss of liver function.Our previous studies have shown that transplantation of bone marrow cells (BMC) contributes for organ regeneration through multiple pathways, including extracellular matrix and apoptosis balancing. Thus it is important to analyze the migration of these cells to the injury site, and to identify the molecules implied.
This study aimed to analyze the percentage of GFP+ cells in the liver of healthy mice and of mice with liver fibrosis induced by bile duct ligation (BDL), after transplantation of BMC from C57BL/6 eGFP+ healthy donors.
Animals were divided in four experimental groups as follows: (1) normal, (2) normal that received BMC being sacrificed 7 days later, (3) 14 days of fibrosis and (4) 7 days of fibrosis that received BMC transplantation and were sacrificed after 7 days. The migration of GFP+ cells was analyzed qualitatively by confocal microscopy, along with immunohistochemistry using anti-SDF-1 (Stromal cell-derived factor 1) primary antibody, and quantitatively by flow cytometry, after digestion of livers with type IA collagenase.
Flow cytometry analysis showed that the fibrosis transplanted group had a significantly higher number of GFP+ cells (13.62%± 2.92%) compared to the normal transplanted group (4.92%±0.63%).This difference was also observed in confocal microscopy, where GFP+cells were found near sites of high SDF-1 expression in the fibrotic liver, indicating that these molecules may be important to BMC migration and establishment in the injured liver. It is known that chronic injuries increase SDF-1 expression in the liver as seen in this study, thus implicating this factor in the higher amount of BMC GFP+ cells found in fibrotic livers, compared to normal livers transplanted with these cells.
The present findings confirm that the regenerative effect observed in cell therapy for liver fibrosis is linked to a massive migration of transplanted cells to inflammation sites, and demonstrate SDF-1 role as a protective molecule secreted by the chronically injured liver
Acknowledgements: CAPES, FAPERJ and CNPq.

Basic Research

51 - EFFECT OF GESTATION ON THE OSTEOGENIC POTENTIAL OF ADIPOSE TISSUE STEM CELLS OF RATS

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The objective of this study was to verify the effect of gestation under osteogenic potential of adipose tissue stem cells (AT-SC) of female rats.
Twelve Wistar rats were distributed among the control and gestation groups. The experiment was approved by the ethics committee on animal experimentation. AT-SCs were grown in osteogenic medium. At 7, 14, and 21 days of osteogenic differentiation of AT-SCs, 3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) conversion, gene expression for collagen I, osteocalcin, osteopontin, BMP-2, alkaline phosphatase were analyzed. The number of mineralized nodules and number of cells per field were analyzed at 21 days.
The gestation increased reduction of MTT in formazan crystals at 14 day and alkaline phosphatase activity at 21 day. The gestation group showed significantly increased the expression of gene transcripts for alkaline phosphatase and osteopontin in all experimental periods. However, the gestation reduced the expression of osteocalcin at 14 day compared to the control group. The expression of BMP-2 and collagen I did not differ between gestation and control groups. The number of cells per field was significantly higher in the gestation group compared to the control group at 7 and 21 days. However, the number of mineralized nodules per field did not differ between groups.
It was concluded that the AT-SCs of pregnant rats exhibit increase in some parameters of osteogenic differentiation.
Acknowledgment: CNPq and FAPEMIG

Basic Research

52 - ESTABLISHMENT OF A CELL LIBRARY FROM THE BRAZILIAN POPULATION AS A TOOL FOR DRUG SCREENING USING INDUCED PLURIPOTENT STEM CELLS.

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The development of a new drug is a long and costly process. Even when it is approved and commercialized, adverse drug reactions and efficacy of the drug are important health issues to be addressed. Among the factors that modulate individual drug response, genetics is known to be more important than age, sex, type of disease and interactions with other drugs. Hence, pharmacogenetics accounts for adverse drug reactions and ineffective therapy. At present, drugs tested and approved in Europe or North America are sold in developing countries like Brazil without knowing how effective or safe they are, so these drugs will not necessarily be effective in the Brazilian population due to genetic factors. We are building up a library of human induced pluripotent stem cells (hiPSCs) from samples of the Estudo Longitudinal de Saúde do Adulto (ELSA) blood bank, which represents the genetic heterogeneity of the Brazilian population. We will use this cell library for in vitro studies of toxicity and response to drugs. To assess them, specific differentiated cell types must be produced from the hiPSCs, in particular functional hepatocytes. The liver plays a major role in metabolism, drug response and toxicity. The hepatocyte models currently used are generated from pluripotent stem cells by directed differentiation, which is based on the application of extracellular signals in two dimensional cultures, employing extracellular matrixes, growth factors, cytokines and hormones mimicking the physiological metabolic networks required during human liver development.
In order to eventually evaluate drug response and toxicity in vitro, we used a directed differentiation protocol to obtain hepatocyte-like cells (HLCs), derived from both human embryonic stem cells (hESCs) and hiPSCs. Methods: hESCs and hiPSCs were derived into HLCs following a well-defined protocol (Hay et al. 2011). Briefly, pluripotent stem cells were cultured in three successive steps of priming into definitive endoderm, hepatic differentiation and hepatic maturation, each step comprising a set medium and factors. Total time of the differentiation protocol was 21 days. On the final day of differentiation, cultures were immunostained for human liver markers albumin, E-cadherin and CYP3A4.
Both hESCs and hiPSCs exhibited a series of profound morphological changes, leading to hepatocyte morphology of polygonal appearance. HLCs stained positive for human liver markers. Negative controls omitting the primary antibody showed no labeling. Conclusions: We have set up in our lab a highly reproducible in vitro model to generate human HLCs derived from hiPSCs, reflecting the genetic diversity of the Brazilian population. The characterization of the HLCs is a first step to determine hepatic functionality and use for in vitro drug screening.

Basic Research

53 - THERAPEUTIC POTENTIAL OF COMBINED TREATMENT WITH STEM CELLS AND PHOSPHODIESTERASE-5 INHIBITOR IN MONOCROTALINE-INDUCED PULMONARY ARTERIAL HYPERTENSION IN RATS

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Pulmonary arterial hypertension (PAH) is a disease characterized by adverse pulmonary vascular remodeling leading to increased arterial resistance, hypertension and right heart failure. PAH can be experimentally induced by monocrotaline (MCT) administration. The use of phosphodiesterase-5 inhibitors (IPDE5) is one of the current therapies for HAP, but has limited beneficial effects for the patients. Stem cell therapy is currently a promising strategy for regeneration of injured tissues. Thus, our objective is to evaluate the therapeutic effect of combined treatment with IPDE5 and bone marrow-derived mononuclear stem cells (BMMC) in the PAH induced by MCT in rats.
The study was approved by institutional ethics committee (CCS/IBCCF 010). 25 male Wistar rats (200g), randomized in 5 groups: control (CTL), monocrotaline (MCT), MCT plus IPDE5 (IPDE5), MCT plus BMMC (BMMC) and MCT plus IPDE5 plus BMMC (IPDE5+BMMC). PAH was induced by one dose of MCT (60 mg/kg, i.p.). 2 weeks after MCT injection, BMMC (1x10^7, i.v.) were injected and IPDE5 (25mg/kg/day) was administered orally for 14 days. At end of treatment, were evaluated: echocardiogram parameters (TAc: flow acceleration time, VTI: velocity time integral, and area of the RV and LV in diastole); right ventricular systolic pressure (RVSP), body and heart weights. Results are expressed as mean ±SEM, with $p<0.05$ as significative.
Survival rate was decreased in MCT (60% survival; p<005 vs. CTL). Treatments improved the survival of IPDE5, BMMC and IPDE5+BMMC (80%, 75% and 75% survival vs. CTL, respectively). The heart weight normalized by body weight was increased in MCT (4.47±0.29 mg/kg) compared to CTL group (3.13±0.28; p<0,05), but not in treated groups(IPDE5: 3.43±0.15; BMMC:3.50±0.15; and IPDE5+BMMC: 3.55±0.13). The echocardiographic analysis of the 4th week post MCT showed decreased values of TAc in MCT when compared to CTL group (16.2±2.0ms vs. 50.9±1.8ms, p<0.001), but it was increased in IPDE5 (33.33±2.19ms; p<0.001 vs. MCT) and IPDE5+BMMC (39.6±6.4ms; p<0.01 vs. MCT) groups. The VTI was decreased in the MCT (2.7±0.2 cm) compared to CTL (5.6± 0.2cm, p<0.01). However, treatment with BMMC (5.5±2.2 cm; p<0.01) or IPDE5+BMMC (5.5±0.5; p<0.01) increased VTI in relation to MCT group (5.5±2.2cm; p<0.01 and 5.5±0.5; p<0.01, respectively). RV area was increased in all groups (MCT: 34.0±3.1mm2, IPDE5: 33.5±2.3mm2, IPDE5+BMMC: 28.0±2.0mm2) compared to CTL (17.7±1.4mm2; p<0.01); and the LV area was reduced only in MCT (27.49±0.43mm2) compared to CTL group (43.8±2.6 mm2, p<0,001). RVSP (mmHg) was increased in MCT (44.4±2.8) compared to CTL (25.3±2.6; p<0.001), however IPDE5(28.3±1.7; p<0,01) and IPDE5+BMMC(35.0±2.8; p<0,05) groups showed decreased RVSP compared to MCT.
The combined treatment with IPDE5 plus BMMC attenuated the pulmonary arterial resistance and decreased hypertrophy of rats with MCT induced HAP.

Basic Research

54 - VAGINAL PRIMARY CULTURE CELLS OBTAINING AND CHARACTERIZATION FOR USE IN TISSUE BIOENGINEERING

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The uterine and vaginal agenesis, also known as Syndrome Mayer-Rokitansky-Küster-Hauser (SMRKH) is a congenital malformation that, although rare, carries important clinical and psychological repercussions, due to the impairment of sexual activity and reproductive life. The treatment for this disease is divided into bloodless and surgical methods and consists in the construction of a neovagina, allowing the patient to have a normal sexual life. However, there is no consensus on best approach of treatment because of the advantages and disadvantages present in both cases.

Our aim is to use the patient's own cells to produce an autologous vaginal tissue in the laboratory to be used in the near future in neovaginoplasty.

We performed 05 full-thickness biopsies from vaginal vestibule of ~ 1cm^2. After several washes in PBS with antibiotics, the tissue was minced into small pieces which were then put in culture plates with DMEM/F-12 supplemented with 10% of fetal bovine serum to allow cells to migrate from the explants. After cells reached 70% of confluence, the explants were removed so cells could grown to remaining spaces, at this moment the plates were harvested and cells were frozen in this first passage at density of 10^6 cells/vial, if we could not obtain this cell density, cells were further expanded and frozen at second passage. Using flow citometry, 03 samples were characterized using the following antibodies: CD90, CD73, CD105 and STRO-I for mesenchymal stem cells as well as pan-citokeratyn, CD133, CD9, CD227 and CD1d for epithelial cells, being the last two specifically used for vaginal epithelial cells. Finally we used CD34 for hematopoietic progenitor cells and vimentin for fibroblasts. The data was acquired using FACS Canto II –BD Bioscience and analyzed with FlowJo 7.6.5 software.

Using explant technique we were able to obtain a mixed cell population of viable cells. These cells were characterized by 67% of mesenchymal stem cells (CD90+ CD73+ CD105+; SD=10,18); we find high positivity of STRO-I cells (75,2%, SD=8,77) and vimetin (69,4%, SD=4,59). For most epithelial markers used we also find high values: 71,9% of CD133, 73,7% of CD9 and 80,3% of CD227 (SD= 6,7, 5,74 and 8,77 respectively). Curiously, we found citokeratyn variation among the samples (50,3%, 13,9% and 31,2%) and a weak positivity of CD34 cells (14,7%, SD=4,98).

Using explant technique we were able to establish primary cell cultures from vaginal biopsies. Those cultures are characterized by having a mixed population of cells, namely, stem cells, epithelial cells and fibroblasts, showing that this approach could be used to obtain autologous vaginal tissue. Further experiments should be conducted to verify physiological parameters of those cells.

Basic Research

55 - MODULATION OF BONE MARROW-DERIVED MESENCHYMAL STEM CELL BEHAVIOR BY TISSUE MICROENVIRONMENT

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Mesenchymal stem cells (MSCs) are self-renewing, unspecialized cells that can give rise to multiple cell types. The use of MSCs has shown great promise for regeneration in failing heart. The behavior of these cells depends on the balance of complex signals in their microenvironment, represented by a subset of cells associated with extracellular matrix and soluble factors.

Mimic tissue microenvironment using the conditioned medium (CM) and the extracellular matrix (ECM), obtained from human heart and umbilical cord tissues, to evaluate its potential to modulate MSCs behavior and differentiation.

We obtained the CM from cardiac and umbilical cord explant culture and the ECM through tissue decellularization. For decellularization, the tissue fragments were treated with 1% SDS and homogenized. The resultant pellet was digested with pepsin, and then treated with nuclease. For the assays, culture plates were coated with 100 µg/mL of each of the matrices. Characterization of ECMs and CM were done by mass spectrometry and immunolabeling.

Different protein profile patterns were identified between ECM and CM. The CMs showed a number of different cytokines, chemokines and growth factors, related to immune response, regeneration and wound healing. Several types of collagen, fibrillin 1, filamin-A, fibronectin and laminin were identified in the matrices. They were not cytotoxic for MSCs and increased the levels of adhesion and migration of these cells in vitro. Furthermore, MSCs showed a higher rate of proliferation when cultured with ECM and/or CM of umbilical cord in comparison to the other conditions analyzed. Regarding cardiomyogenic differentiation, no differences in the staining for cardiac troponin I, connexin 43, GATA-4 or alpha smooth muscle actin between control and cardiac ECM/CM cultures were shown.

Although the culture protocol used in this study was unable to induce cardiomyogenic differentiation of MSCs, we demonstrate the functionality of tissue environment on MSCs influencing cell adhesion, migration and proliferation, reinforcing its importance in cellular interactions.

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Basic Research

56 - NEURODIFFERENTIATE ADULT STEM CELL TISSUE OF HUMAN UMBILICAL CORD

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Mesenchymal stem cells (MSC) have the potential to form various tissues. They can be obtained from several sources and those derived from the umbilical cord has raise the interest of researchers due to its simplicity of collecting, no risk to donors, in addition to their potential for proliferation and differentiation. Also, the umbilical cord tissue is richer than the umbilical cord blood. Several studies have investigated the potential for tissue regeneration in the central nervous system, but few employ MSC derived from umbilical cord tissue in the investigation of its neurogenic potential.
Isolate, characterize and neurodifferentiate adult MSC from human umbilical cord (HUC) tissue.
This study was approved by CEP-PUCRS (CEP 1285/11) and stem cells from HUC were obtained from one patient admitted to the Obstetric Center of Hospital São Lucas after signing the consent form. The arteries and veins were isolated, cut into small pieces and cultured in DMEM supplemented with 10% FBS, 100 U/ml of penicillin, 100 U/ml of streptomycin, and 100 g/ml of gentamicin. When the cells had fibroblast morphology and confluence between 80% and 90% they were subcultured. After five passages, MSCs were characterized according to the criteria recommended by the ISCT. The potential of MSCs has been defined by osteocytes and adipocytes differentiation when cultured in specific media and confirmed by Oil Red (adipocytes) and Alizarin Red (calcium) staining. The neurodifferentiation (adapted from Song et al. 2008) was obtained by culturing the MSC in DMEM supplemented with 0.001% β-mercaptoethanol for three days, followed by DMEM/F12 medium for three days, and finally in N5 medium containing 20 ng/ml brain-derived neurotrophic factor (BDNF) and 20 ng/ml of growth of granulocyte colony stimulating factor (GCSF) for seven days.
The adipogenic and osteogenic differentiation was confirmed by staining with Oil Red and Alizarin red, respectively. The profile of surface antigens expression was: CD34=0.33%; CD19=0.08%; CD45=0.21%; CD90=0. 36%; CD14=0.03%; CD105=11.15%; CD11b=0.02%; CD73=70.86%; CD117=0.10%; HLA=0.13%. The neurodiferentiation was confirmed with a solution containing anti-NeuN (neuronal nucleus), anti-NFL (neuronal cytoskeleton), anti-MAP2 (neuronal microtubule) and anti-β-tubulin III (neuronal microtubule) antibodies to characterize the neuronal polarization and cytoarchitecture.
The potential of adult stem cells derived from umbilical cord tissue to differentiate into mesenchymal lineage was confirmed by surface antigens expression profile, and the presence of specific protein of neural cells confirming its ability to neurodifferentiate, corroborating its potential application in tissue regeneration and cell therapy.

Basic Research

57 - VASCULARIZATION POTENTIAL OF POLYCAPROLACTONE NANOFIBER MESHES ASSOCIATED WITH ENDOTHELIAL-DIFFERENTIATED CELLS FOR BONE TISSUE ENGINEERING IMPLANT

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Tissue engineering is an emerging biomedical technology based on the association of cell culture in structural matrices and the incorporation of signaling molecules, targeting tissue regeneration. Despite its enormous potential, this strategy faces a major challenge concerning the maintenance of cell viability after the implantation of the constructs. The lack of a functional vasculature within the implant compromises the nutrition and removal of metabolites by cells, which can lead to implant failure. In this context, our research group is trying to create a new strategy for enhancing vascularization in bone tissue engineering constructs.
The present study aimed to use polycaprolactone (PCL) nanofiber meshes associated with endothelial-differentiated cells to improve the vascularization of bone implants in three-dimensional polyhydroxybutyrate and polybutylene succinate (PHB/PBS) blends matrices.
To this end, adipose-derived stem cells (ASCs) isolated from Lewis rats were cultured in both types of scaffolds: in PHB/PBS matrices in which cells were cultured with osteogenic medium and in PCL nanofiber meshes in which cells were cultured with endothelial differentiation medium. The PHB/PBS matrices only or associated with PCL nanofiber meshes colonized or not by cells were subcutaneous implanted into the backs of rats and after 30 days histological analyses were developed (Ethics Committee on Animal Experiments - N0242/2008).
Analysis by SEM revealed that the scaffolds have structures that allow cell adhesion. The endothelial and osteogenic differentiation of ASCs seeded on PCL nanofiber meshes or PHB/PBS matrices, respectively, was confirmed by immunofluorescence assay and polymerase chain reaction assay which revealed expression of endothelial and osteogenic markers. Histological analysis demonstrated that when implanted in vivo the scaffolds did not induce a high inflammatory response and did not affect the function of tissues in the region of the implant. Histological and immunohistochemical analysis suggests that the PCL nanofiber meshes associated with endothelial-differentiated cells promoted vascularization within the implants.
These results showed that the combination of scaffolds and endothelial-differentiated cells could be a valid alternative to improve vascularization and success of implants in bone tissue engineering application.

Basic Research

58 - EXOSOMES (EXOs) DERIVED FROM MESENCHYMAL STEM CELLS (MSCs) MINIMIZED THE LPS ACUTE KIDNEY INJURY BY RENAL PLURIPOTENT CELLS ACTIVATION (rPCs).

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Since acute kidney injury (AKI) has high morbidity and mortality, it is of relevance to search for alternative therapeutics.
We investigated the effects of MSCs, your conditioned medium (CM) or EXOs in a LPS-induced nephrotoxicity model and the effects rPCs in this model.
Rats received i.v.: LPS (10 mg/B.W.) or PBS (CTL) with MSCs ($1x10^6$), CM (500 µl) or its EXOs (100 µg/ml) from MSCs incubated or not for 12 hours with cytochalasin B (CB; 1 µM) or actinomycin D (AD; 2.6 µM) and given in 1 or 3 doses and sacrificed after 72 hours. Blood and urine samples were collected for creatinine (sCr), urea (sU) and FENa. Kidneys were analyzed for HE, KI67, caspase 3, BrDU markers of RPCs as Wnt1, PAX2 and CD24. Also markers of EXOs as CD63, presence of Y chromosome, IL6, TNF-α, INF-γ and IL10 were also evaluated.
As expected, it was observed increases in sCr, sU, FENa, caspase 3 marking, proinflammatory cytokines and reduction of KI67 with lesions in proximal tubules induced by LPS. However, these parameters were ameliorated with MSCs, CM or EXOs treatments. EXOs increased BrDU, Wnt1, PAX2, CD24 and CD63 expressions indicating activation of RPCs. CB and AD inhibited the protective effect of EXOs. It was impressive the effect of 3 times administration of MSCs or CM or EXOs decreasing the mortality in LPS group.
Therefore, results support that the MSCs and its CM and EXOs protected from AKI induced by LPS. It is reasonable to suggest that the mediation of by EXOs is, at least in part, by stimulating rPCs evaluated by PAX2, Wnt1 and CD24 positive staining. Also, suggest at least in part, participation of rPCs in this cascade of events and those EXOs alone could be employed in order to ameliorate LPS nephrotoxicity.

Basic Research

59 - EXPRESSION OF 9-O-ACETYL GD3 GANGLIOSIDE IN NEURAL STEM CELLS/PROGENITORS OF THE SUBVENTRICULAR ZONE OF ADULT MICE

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The neural stem cells (NSCs) are a hope for the treatment of neurodegenerative diseases. In culture, the NSCs are characterized by the ability to form neurospheres in the presence of factors EGF and FGF-2, but in vivo there are no effective markers that can distinguish and isolate these cells.
In this study, we propose the analysis of ganglioside 9-O-acetyl GD3 (9acGD3) as a possible marker of NSCs. The 9acGD3 ganglioside is present in the central nervous system (CNS) and peripheral system of mammalian during development and has been described as a molecule associated with cell migration and axonal extension events during this period. In adults, it ceases to be expressed in most CNS and is observed only in the subventricular zone (SVZ), rostral migratory stream (RMS), cerebellum and retina. As the SVZ is a neurogenic region in adult mammals, the aim of this study was to analyze the possible association of 9acGD3 ganglioside with NSCs.
For this, we analyzed through the test of neurospheres formation the presence of NSCs in the cell population that express the ganglioside. The ganglioside-positive cells were isolated by the technique of MACS (magnetic activated cell sorting), from the brain of rats in embryonic age (E16) and the SVZ of rats in postnatal age 21 (P21) and adult rats.
We observed a higher number of neurospheres in positive population for 9acGD3 ganglioside isolated from SVZ of mice at all ages studied. We were able to obtain secondary neurospheres, demonstrating the capacity for self-renewal of these cells, and obtained different cell types such as neurons, astrocytes and oligodendrocytes, demonstrating the multipotentiality of these cells. Through immunohistochemical analysis of the location of that ganglioside in the SVZ of the animals, we observed that this molecule is expressed in both neural progenitors and immature neurons that will migrate to the olfactory bulb via the RMS, so it could be a marker of undifferentiated cells in the CNS.

Basic Research

60 - MIGRATION AND NEURODIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELL (IPS) FROM PATIENTS WITH CORTICAL DYSPLASIA.

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Cortical dysplasia is one of the most frequent forms of malformation during cortical development, due to an epilepsy refractory to treatment condition. Changes in cellular traffic are present in several neurological diseases, including epilepsy and cortical dysplasia. The reprogramming of adult somatic cells is an attractive and promising approach due to the possibility of in vitro studies of complex genetic diseases that are not well understood. Direct cell reprogramming by the addition of four genes (Oct4, SOX2, KLF4 e c-Myc) in somatic cells, generate the induced pluripotent stem cells (iPS), which are very similar to embryonic stem cells with the potential self-renewal and differentiation. The establishment of iPS using in vitro models have been described in several pathologies related to the central nervous system, including Parkinson's disease, Huntington's and Amyotrophic Lateral Sclerosis.
Develop a cellular model of cortical dysplasia by generating iPS
Fibroblasts were obtained from skin biopsies from patients enrolled in the Program Plastic Surgery (Hospital São Lucas da PUCRS), and cut into small fragments and cultured in supplemented DMEM media with bovine fetal serum and antibiotics. Skin fragments were removed when fibroblasts were growing followed by five passages. Cell migration test was carried out by cultivating fibroblasts over membrane inserts and migration was induced with 30% of FBS during 24, 48 and 72 hours. Neurodifferentiation was performed as described by Song at al 2008. Characterization of neuronal structures (NeuN, NFH, β-tubulina-3, and MAP2) were determine by confocal microscopy (Fluoropan Neuronal Marker – Chemicon). The iPS was generated through two transfections with viral vectors containing the pluripotency genes using STEMMCA kit (Chemicon). Six days later, the transfected cells were cultured over feeder layer of fibroblasts.
We were able to detect cell migration 48 hours after induction. There was no difference between the potential of cell migration among patients. The induction neurodifferentiation did not promote neuronal differentiation in fibroblasts. iPS clones could be observed 10 days after transfection. After the colonies with embryonic stem cell morphology being selected and transferred to new feeder layer of fibroblasts, three different clones were obtained.
The skin fibroblasts have the potential of cell migration after 48 hours after induction with 30% fetal bovine serum. Skin fibroblasts do not exhibit structural characteristics of mature neurons after induction neurodifferentiation. iPS colonies with morphological characteristics of embryonic cells can be generated using STEMMCA kit.

Basic Research

61 - STUDY OF TOXIC EFFECTS OF GLYPHOSATE ON STEM CELLS DERIVED FROM HUMAN ADIPOSE TISSUE

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Glyphosate is the most commercialized herbicide in the world, known commercially as Roundup®, and many studies was conducted to investigate the potential damage caused by it. To carry out this work we chose stem cells derived from human adipose tissue (hASC). These cells are able to differentiate into adipogenic and osteogenic lineages, which enables an investigation of the toxic effects of Roundup® in undifferentiated and differentiated state of these cells. Furthermore, in vitro studies with human embryonic umbilical and placental cells have shown that the formulations of glyphosate are capable of generating damage to the genetic material, which reinforce the need to test this compound in other types of human cells.
The main goal of this work is to investigate the citotoxicity and genotoxicity of Roundup® on hASC in short and long term exposure.
Stem cells were evaluated for the presence of surface markers to defining multipotent mesenchymal stromal cells. Immunophenotyping was performed using the technique of flow cytometry. Viability assays were performed periodically using the MTT method, in order to assess the toxicity of glyphosate throughout the days of culture. The alkaline phosphatase activity was evaluated by BCIP-NBT assay. This assay is used to detect activity of bone cells, which are derived from hASC in the differentiation process. Another test was conducted to detect the difference necrosis from apoptosis in cells exposed to Roundup® for short and long time. For this assay, we used flow cytometry with Annexin V-FITC kit. hASC were grown in medium supplemented with differentiation factors for osteogenic and adipogenic lineages. The ability of differentiation of hASC exposed to glyphosate formula was also verified by observation of morphological changes and the expression of genes related to the differentiation.
We observed that cells exposed to Roundup® die through apoptosis mechanism and also undergo morphological changes when subjected to a mixture of Roundup® within adipogenic and osteogenic differentiation medium. Moreover, these cells exhibit a shift of MTT metabolism and production of alkaline phosphatase when exposed to herbicide for long term. Gene expression is also affected, with inhibition of some important that are expressed when differentiation occurs.
In conclusion, Roundup® is cytotoxic and genotoxic for adult stem cells derived from adipose tissue.

Basic Research

62 - INVESTIGATION OF THE NEUROPROTECTIVE POTENTIAL OF BONE MARROW MESENCHYMAL STEM CELLS IN AN IN VITRO MODEL OF ALZHEIMER´S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disease with high prevalence and morbidity, for which there are no effective therapies. Soluble oligomers of the amyloid-β peptide (Aβ) or ADDLs (Aβ-derived diffusible ligands) are the main neurotoxins involved in the early synaptic dysfunction and oxidative stress associated with the disease. The therapeutic potential of bone marrow mesenchymal stem cells (MSCs) has been investigated in several models of neurological diseases and the main mechanism of action of these cells is based on paracrine signaling, through the release of trophic or neuroprotective factors.
The aim of the current study was to evaluate the neuroprotective actions of MSCs against the deleterious effects caused by exposure of rat hippocampal neurons to Aβ oligomers. We have also investigated the interaction of ADDLs with MSCs and possible mechanisms of neuroprotection.
We established a model of indirect coculture of neurons and MSCs and our results indicate that the ADDLs do not alter the viability (LIVE/DEAD), proliferation (Ki67 expression) and respiration of MSCs (Oroboros oxygraph) in vitro. In addition, MSCs were resistant to oxidative stress induced by exposure to ADDLs (500nM) for 24h or oxygen peroxide (1mM) for 10min. Presence of MSCs in coculture also protected neurons against oxidative stress generated by exposure to ADDLs (500nM) for 6h (preventing the increase of 3 times in the levels of reactive species) or hydrogen peroxide (100μM) for 10min (reducing by half the increase of ~8 x in the levels of reactive species).
Furthermore, MSCs were able to preserve the integrity of synapses, evaluated by the expression of pre and post synaptic proteins in hippocampal neurons after exposure to ADDLs (500nM) for 24h. We also found a reduction in the concentration of exogenously added ADDLs in the culture medium after increasing periods of incubation with MSCs (reducing about 10 times the ADDLs concentration after 24 hours), which could partially explain the neuroprotection actions of MSCs. In fact, we observed that MSCs were able to internalize ADDLs, that were found in lysossomes (by colocalization between 6E10 and LAMP1), indicating a degradation pathway. Interestingly, pre-incubation with E64d, an lysossomal protease inhibitor, prevented the degradation ability of ADDLs by MSCs. MSCs were also able to internalize 500nM latex beads, that also were found in lysossomes, suggesting a phagocytic ability of MSCs.
Finally, we found high levels of the antioxidant enzyme catalase in the culture medium of MSCs after 24h of incubation with ADDLs (500nM), that could be represent another mechanism related to the neuroprotection against the oxidative stress observed. These data suggest that mesenchymal stem cells may constitute a novel therapeutic alternative for the treatment of Alzheimer’s disease.

Basic Research

63 - CHARACTERIZATION AND NEUROPROTECTIVE EFFECT OF EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STEM CELLS

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Alzheimer's disease is the most common form of dementia in older people and its incidence tends to increase with the increase in life expectancy of the population yet there is no definitive treatment. Soluble oligomers of the amyloid-β peptide (Aß) or ADDLs (Aß-derived diffusible ligands) are the main neurotoxins involved in the early synaptic dysfunction and oxidative stress (OS) associated with the disease. Mesenchymal Stem Cells derived from Wharton jelly (WJMSC), have been extensively studied in many diseases and lesions in the central nervous system, including showing promising results in clinical trials. The main mechanism of action of these cells is their paracrine effect through the release of trophic factors or neuroprotective that could act in stimulating neurogenesis and neuroprotection. A novel method of cell-to-cell communication has recently emerged from groundbreaking discoveries in the past few years on content of extracellular membrane vesicles (EMVs). EMVs have been demonstrated to facilitate horizontal transfer of mRNAs, microRNAs (miRNAs), and proteins between cells without direct cell-to-cell contact. There are several categories EMV known to-date, which are included under the general terms exosomes, microvesicles.
Based on this background, the objective of this study was to characterize EMVs from WJMSC and the evaluate the neuroprotective actions of EMVs against the deleterious effects caused by exposure of hippocampal neurons to Aß oligomers. EMVs characterization was performed by Flow cytometry, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Neuroprotection against oxidative stress was verified by General Oxidative Stress Indicator - CM-H2DCFDA and EMVs enzyme content by Oroboros oxygraph.
Our results indicate that EMVs have markers of stem cells membrane (Flow cytometry), and its peak release between 3 and 5 hours after cell stress (SEM and TEM).
The presence of EMVs in culture protected neurons against oxidative stress generated by exposure to ADDLs to control levels (CM-H2DCFDA). Our hypothesis that this protection is based on the presence of catalase in content of EMVs, proven by Oroboros oxygraph. Obtained results show the potential of EMVs in treatment against damage by OS in Alzheimer's disease.

Basic Research

64 - THE PROTEIN ARGININE METHYLTRANSFERASE PRMT8 INCREASES EMBRYONIC STEM CELL APOPTOSIS AND MODULATES CELL CYCLE PROGRESSION

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Addition of methyl groups to arginine residues is catalyzed by a group of enzymes called Protein Arginine Methyltransferases (PRMT). Although PRMT1, the most studied member of the family, is essential for development, little it is known about its paralogue PRMT8. Despite regulation and cellular substrates of PRMT8 are not well understood, it was reported that its expression is restricted to adult brain neurons of mice and that it plays a critical role in embryonic and neural development in zebrafish.
Since the role of PRMTs in ESCs has not been studied in great detail and PRMT1 seems to be necessary for embryonic development but not for ESC survival, we decided to investigate if PRMT8 has a role in the maintenance of ESCs properties.
Ainv15 and R1 mouse Embryonic Stem Cell (ESC) lines, HC11 and induced pluripotent stem cells (iPSCs), previously obtained in our lab from mouse embryonic fibroblasts, were cultured under standard conditions. Pluripotency was evaluated by embryoid body and teratoma formation assays. Lentivirus were used for stable cell line generation and ectopic transcription factor expression. The doxycicling (dox) inducible pLKO-Tet-On system was used for short hairpin RNA (shRNA) silencing of PRMT8. Gene expression was analyzed by real-time quantitative RT-PCR and immunofluorescence. Cell proliferation was studied by Bromodeoxy Uridine (BrdU) incorporation and crystal violet assays. Cell cycle analysis of DNA content was performed by propidium iodide (PI) staining and flow cytometry analysis. Cell death was measured by Anexin-V/PI and TUNEL assays.
We found that PRMT8 expression was up-regulated in ESCs and in iPSCs, and that it decreased during differentiation. We showed that PRMT8 expression was induced by pluripotency transcription factors Oct4, Nanog and Sox2, since ectopic expression of these factors affected PRMT8 expression in a heterologous system (HC11, mammary epithelial cell line). Then we established a R1 derived ES cell line (R1 1036) that enables PRMT8 silencing in an inducible manner by a shRNA regulated by dox. PRMT8 sub-expressing ESCs were ableto self-renew and remained pluripotent. Remarkably, we found that these cells formed bigger colonies. Intriguingly, the percentage of PRMT8 sub-expressing cells that were in S phase diminished when assessed by BrdU incorporation and propidium iodide DNA staining. Finally, we found that PRMT8 down-regulation increased ES cell survival as dox-treated R1 1036 showed a diminution of cells undergoing early apoptosis and necrosis compared to control cells, analyzed by anexin-V and TUNEL assays.
Although PRMT8 down-regulation did not affect ESC self-renewal or pluripotency, we found that PRMT8 modulated ES cell cycle and apoptosis, showing to be relevant for stem cells' survival. These results suggest novel roles for PRMT8 in key cellular mechanisms in pluripotent stem cells.

Basic Research

65 - SOD2 GENE EXPRESSION IS INDUCED BY NANOG

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Pluripotent stem cells (PSCs) have two main properties: self-renewal and pluripotency. Oct4, Sox2 and Nanog are critical transcription factors (TF) to preserve these properties. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) possess a complex system that protects them from oxidative stress and ensures genomic stability, vital for their role in development. It has been reported that antioxidant activity diminishes along stem cell differentiation, but little is known about the transcriptional regulation of the involved genes.
With the hypothesis that some genes involved in antioxidant defense systems in PSCs could be regulated by Oct4, Sox2 and/or Nanog, we decided to study the expression profile of Catalase, Glutaredoxin, Glutathione peroxidase, Glutathione reductase, Peroxiredoxins, Superoxide dismutases (Sod), Thioredoxins, and Thioredoxin reductases.
Ainv15 and R1 mouse ESC lines, HC11 and iPSCs, previously obtained in our lab from mouse embryonic fibroblasts, were cultured under standard conditions. PSCs were differentiated using the in vitro embryoid body formation assay. In experiments where TF levels were modulated, R1 ESCs were cultured in the standard conditions or in the absence of LIF for 4 days. Gene expression was analyzed by real-time quantitative RT-PCR. We then generated a reporter vector, pSod2-Luc, cloning a 1142 kbp fragment of the promoter region of Sod2 into pGL3-Basic vector upstream of the Luciferase gene. A trans-activation assay was performed in HC11 cell line by co-transfection of pSod2-luc vector and an expression vector for Nanog. Statistical comparisons of data were performed using Student paired t-test or a randomized block design ANOVA for biological replicates using Infostat statistical software. When necessary, Tukey Test was used for comparisons between means.
We studied the gene expression pattern of some of the components of the oxidative stress defense system in ESCs and iPSCs in the undifferentiated state and during differentiation. We found a great diversity in their transcriptional profiles, some genes were found to be up-regulated along the process, others highly repressed, and some resulted unaffected. Nevertheless, Sod2 gene was highly repressed during differentiation and its expression pattern was similar to Nanog gene’s profile in both experiments: hanging drop differentiation protocol and LIF–depleted culture conditions. Moreover, Sod2 promoter activity was induced by Nanog when transactivation assay was performed.
Sod2 expression is repressed during differentiation. Its expression pattern under different culture conditions is similar to Nanog’s, one of the main stemness’ transcription factors in pluripotent stem cells. Performing a transactivation assay, we found that Nanog induced Sod2 gene promoter.

Basic Research

66 - EFFECT OF BONE MARROW MONONUCLEAR CELLS THERAPY IN A MODEL OF SPINAL CORD IN HEMITRANSECTION

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The aim of our study was to evaluate the potential of bone marrow mononuclear cells (BMMC) therapy in a rat model of unilateral T10 spinal cord injury (SCI).
Wistar rats were divided into 3 groups: false-operated group, underwent laminectomy, SCI + saline group and SCI+ BMMC group. The locomotor recovery was assessed by Basso Beattie Bresnahan scale (BBB) from 3 days after surgery. In histochemical analysis, immunostaining was performed using markers as ED-1 (CD68), Il-1β and Neu-N marking macrophages and microglia, pro inflammatory cytokine and neuron respectively.
Functional analysis showed that the treated group (SCI + BMMC) showed better locomotor recovery through the BBB scale compared with SCI + saline group. In the histochemical analysis of bone marrow mononuclear cells were found 3, 7 and 14 days after injury (DAI). The SCI + BMMC group showed decrease Il-1β in all time windows observed and activated microglia and macrophages at 7 and 14 DAI, compared with SCI + saline group.
Our data suggest that injection of bone marrow mononuclear cells after spinal cord injury provided better locomotor recovery associated with a higher presence of axons and reduction of reactive microglia in different time windows.

Basic Research

67 - CARDIAC STEM CELLS PROFILE IN PHYSIOLOGICAL CARDIAC HYPERTROPHY INDUCED BY AEROBIC EXERCISE TRAINING IN MICE

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The purpose of this study is to evaluate whether different types of cardiac stem cells (CSCs) are involved in the physiological cardiac hypertrophy that follows the aerobic exercise-training in mice.
For this, male C57Bl/6j mice were subjected to swimming training, lasting 90 minutes each, twice a day, 5 days in a week, during a period of 4 weeks, and compared with sedentary mice (control group). Physiological cardiac hypertrophy and associated features was assessed at the end of the training period by means of cardiac weight measurements and morphometrical studies including myocyte size calculation and fibrosis quantification as well as electrocardiogram. Resident CSCs were quantified after heart processing via enzymatic digestion by means of flow cytometry, in order to quantify c-kit+lin- or Sca-1+lin- CSCs or via low density cell culture in order to quantify the number of colony forming units of fibroblasts (CFU-F), which are derived from cardiac mesenchymal stem cells (cMSCs).
Aerobic exercise training by swimming was effective in producing physiological cardiac hypertrophy in mice confirmed by the increase in heart weight and in myocyte size as well as reduction in cardiac fibrosis and sinusal bradycardia. Regarding to counting of CSCs, we have found that the number of c-kit+lin- CSCs were increased in the hearts of trained animals, while the numbers of Sca-1+lin- CSCs and cMSCs were not changed with the physiological hypertrophic process.
Even though the functional properties of these CSCs had not been investigated, these results show that resident CSCs population are differently modulated during physiological cardiac hypertrophy, suggesting a role of augmented c-kit+lin- CSCs during the cardiac hypertrophy induced by aerobic exercise training in mice.

Basic Research

68 - ESTABLISHMENT OF A MURINE MODEL OF CHAGAS CHRONIC CARDIOMYOPATHY (CCC) IN DOUBLE-TRANSGENIC MERCREMER/ZEG MICE

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Cardiovascular diseases are still the leading cause of death worldwide. Among them, we highlight chronic cardiomyopathy caused by Chagas disease (CCC). In this context, studies have been conducted using mice as an animal model, since it is possible to investigate the physiological and pathological abnormalities seen in subjects with CCC.
The aim of this work was to establish a murine model of Chagas cardiomyopathy using double-transgenic MerCreMer/ZEG mice, in which will be possible to evaluate whether there is renewal of cardiomyocytes during illness and analyze important cellular and molecular questions.
To induce recombination mediated by Cre, double-transgenic MerCreMer/ZEG mice (n = 14, 8 weeks) generated by crossbreeding transgenic B6129-Tg(Myh6-cre/Esr1)1Jmk/J (MerCreMer) mice (Myh6 promoter drives expression of a tamoxifen-inducible Cre) with B6.Cg-Tg(ACTB-Bgeo/GFP)21Lbe/J (ZEG) reporter mice (constitutive b-galactosidase expression is replaced by the expression of GFP) received Tamoxifen (20mg/kg/day, Sigma) dissolved in peanut oil, intraperitoneally for 14 days. GFP labeling and efficiency of recombination was examined 5 d after the end of the pulses by immunohistochemistry. To analyze the experimental model of chagas cardiomyopathy, MerCreMer/ZEG mice (n = 20, 10 weeks) were divided into two groups: infected (n=15) with $3x10^4$ blood trypomastigotes (Brazil strain, intraperitoneal) and non-infected (n=5). The area and cardiac function were analyzed by echocardiography prior to infection, 4 and 6 months after. The parasitemia (number of parasites/ml of blood) was assessed for 40 days. After this period, the hearts were histologically stained with hematoxylin and eosin for evaluation of inflammatory infiltrate, and Sirius Red for detection of fibrous tissue.
Analysis by immunohistochemistry revealed a rate of Cre-mediated recombination of 77.0 ± 2.12 in the MerCreMer/ZEG mice. The parasitemia was low, with two peaks, one at 14 ° dpi (1.36 ± 1,04) and another at 28º dpi (2.02 ± 0,98) ($x10^5$ trypomastigotes/ml of blood) and a mortality rate of 30%. Echocardiographic analysis reveals an increase in right ventricular (AVD) area in mice after 4 and 6 months of infection, when compared to pre-infection period (Pre: 8.9 ± 0.27; 4 months: 11.24 ± 0.51; 6 months: 13.44 ± 0.63, P <0.01). However, no differences between the area of the left ventricle (AVE) and ejection fraction (EF) in the analyzed groups were observed. Histological analyzes reveal the presence of focal and diffuse inflammatory infiltration, although not a persistent interstitial fibrosis has been detected in the analyzed heart.
We observed that MerCreMer/ZEG mice achieved a satisfactory rate of recombination and develop dilated cardiomyopathy similar to CCC observed in other studies of murine models but have a lower mortality rate, enabling the use of this as experimental model of the disease.

Basic Research

69 - MODELING SCHIZOPHRENIA CEREBRAL CORTEX IN 3D USING HUMAN PLURIPOTENT STEM CELLS

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Pluripotent stem cells (PSCs) can differentiate into any cell type of an organism. Moreover, they have a remarkable capacity to self-organize and develop into three-dimensional structures resembling miniature organs. Lancaster et al. (2013) found that neural cells generated from human PSCs recreate early steps of the human cerebral cortex development.
Those cerebral organoids show great potential for human modeling studies, particularly for diseases with a developmental component. To investigate this, we build cerebral organoids from induced pluripotent stem cells (iPSCs) derived from a schizophrenic patient. Our methodology was an improvement on Lancaster´s protocol, with the entire differentiation process being conducted on a spinner flask to promote nutrient absorption. Embryoid bodies (EB) were embedded in MatrigelTM and grown in neural differentiation media containing retinoic acid.
After 15 days of differentiation, continuous neuroepithelia could be observed as well as budding outgrowth. After 30 days in medium containing retinoic acid, the expanded neuroepithelia gave rise to polarized cells: neuroprogenitor cells, expressing Pax6, BLBP, Tbr2 and PH3, surrounding cavities resembling lateral ventricles; and an outer layer of preplate, mature neurons expressing β tubulin III and MAP2. By this time, cerebral organoids reached about 800 µm of diameter with no sign of degeneration.
As cerebral cortex architecture is altered in schizophrenia, we aim to evaluate neural stem cell number, possible alterations in specific neuronal types and migration in comparison to control organoids, all those postulated to be involved in schizophrenia pathophysiology.
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Basic Research

70 - RADIATION-INDUCED LIVER DAMAGE: HEPATIC STELLATE CELLS ARE INVOLVED?

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Liver is a multifunctional and metabolic organ deeply affected by radiotherapy. Radiation-induced liver damage (RILD) development is an expected outcome with no treatment available. Elucidation of cellular mechanisms underlying fibrosis is mandatory. In normal liver, stellate cells are described as being in a quiescent state. When the liver is damaged, stellate cells can change into an activated state. It has been described that activated hepatic stellate cells (HSC) play an important role in fibrogenesis. Then, our goal was to evaluate whether the state of the HSC are impaired in a model of radiation-induced hepatic injury

C57/BL-6 mice, weighing 25-30 g, were divided into 3 groups: control (C, n=5) and irradiated with 15 Gy (IR15, n=5) and 20 Gy (IR20, n= 5). All mice were submitted to aspartate aminotransferase (ALT) and Albumin (ALB) evaluation of the peripheral blood before and 60 days after irradiation procedure. Then the animals were euthanized to perform liver immunohistochemistry to detect stellate cells in their activated state (alpha-smooth muscle actin) and hepatocytes (cytokeratin 18). Histomorphometry to quantify the number of activated HSC was performed using the STEPanizer web-based system (www.stepanizer.com). The study protocol was approved by the local ethical council (CEUA 162/13).

ALB analysis showed that liver function was impaired in both irradiated group (IR15=1.73±0.08 g/dl and IR20=1.275±0.085 g/dl) when compared to control (C= 3.117±0.076 g/dl). Also, ALT showed liver damage in IR15= 175.7±36.33 g/dl and IR20= 212.0±15.78 g/dl as compared to control 40±11.27 g/ dl. Only in IR20 group was observed an increased imunoreactivity for SMA (6.33 ± 1.72 cells/mm2) when compared to control (1.26 ± 1.38 cells/mm2). The SMA expression in IR15 (3.69 ± 1.58 cells/mm2) was similar to control. No alteration was observed in hepatocytes surrounding the activated HSC among the groups analyzed.

The decrease of ALB and the increase of ALT showed that both radiation doses (15 and 20 Gy) induced liver damage. However, the amount of hepatocyte was not reduced. It is important to highlight that only 20 Gy induced an increase of SMA which may explain the radiation-induced liver fibrosis.

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Basic Research

71 - IDENTIFICATION, ESTABLISHMENT, CULTURE, AND CHARACTERIZATION OF PROGENITORS CHOROID PLEXUS CELLS USING PORCINE AS ANIMAL MODEL

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This study aims to characterize the cells from choroid plexus region to a greater understanding of cerebral spinal fluid production and probably stem cell niche using porcine as an animal model in its fetal stage.
The choroid plexus region was collected and part was fixed in 4% paraformaldehyde for histological analysis protocols using histology and immunohistochemistry. And other part was followed by cell culture for performing the isolation and characterization the cell population using immunocytochemistry and flow cytometry techniques. The results were analyzed and compared between the animals studied for a better comprehension of the main differences with the goal of a greater understanding of present cell in this region in relation to their morphology and function.
Our results in cell culture, and studies of cell immunocompatibility were according to the few authors who have conducted similar studies. The cell culture performed with DMEM did not observe progress and the culture performed with medium DMEM F12 cells of the choroid plexus were progressive. Also analyzes showed the expression of the pluripotency marker Oct-4 and not expression of Nanog, which proves that there is a pluripotent cell niche in the cells studied. The immunocytochemistry and immunohistochemistry showed expression for Beta tubulin III and Nestin markers and negative for GFAP marker, showing the presence of certain differentiated cell niche and neuronal lineages. The flow cytometry analysis confirmed that the cells of the choroid plexus do not have mesenchymal immunophenotype and have to source positive for CD45 as hematopoietic cells.
Our finding in this study was that the choroid plexus is a unique tissue and incomparable with different cellular niches with pluripotent cells, hematopoietic cells and neuronal cells, which provide its functionality as complex and differentiated.

Basic Research

72 - miR-208a-3p IS DOWNREGULATED IN CARDIAC REMODELING AND POSSIBLY CONTROLS GATA4 EXPRESSION

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miR-208a-3p, a cardiac specific microRNA encoded by an intron of the α-myosin heavy chain (α-MHC) gene, is a predicted post-transcriptional repressor of Gata4.
Hence, the aim of this work was to correlate the temporal expression profile of miR-208a-3p and Gata4 in cardiac remodeling after myocardial infarction (MI).
Wistar rats were submitted to permanent occlusion (PO) of the left anterior descending artery and analyzes were performed in SHAM and MI groups with 2 (n=10 e n=7), 28 (n=8 e n=9) and 90 days post-PO (n=8 e n=9). Cardiac function was examined by echocardiography and Langendorff, while infarct area was obtained from histological analyzes. The relative expression of miR-208a-3p, α-MHC and Gata4 were obtained by RT-qPCR.
There were no differences in infarct areas between infarcted animals in all timepoints and functional data were consistent with dilated cardiomyopathy. The relative expression of miR-208a-3p was downregulated both 2 days post-PO (0.12 ± 1.52 $p<0.01$) and 90 days post-OP (0.18 ± 1.78, $p<0.01$) in the MI group when compared to SHAM. mRNA levels of α-MHC and Gata4 were also decreased in the MI group at the same times post-PO (α-MHC 2 days: 0.004 ± 6.98, 90 days: 0.05 ± 3.42; Gata4 2 days: 0.25 ±1.54, 90 days: 0.24 ± 3.01). Pearson's correlation coefficient revealed the existence of a linear correlation between the mRNA levels of Gata4 and α-MHC at 2 days ($p=0.0003$, $R2=0.78$) and 90 days post-PO ($p<0.0001$, $R2=0.86$). Moreover, the correlation between Gata4 expression and miR-208a-3p was also present at 2 days ($p=0.009$, $R2=0.54$) and 90 days post-PO ($p=0.04$, $R2=0.34$), as well between α-MHC e miR-208a-3p at 2 days post-PO ($p=0.01$, $R2=0.49$).
Since miR-208a-3p is coexpressed with α-MHC, which is a direct target of Gata4, we propose that miR-208a-3p decreases Gata4 expression through negative feedback loop. In this context, miR-208a-3p would function as a rheostat, regulating the levels of Gata4. Given that the heart after myocardial infarction remodeling progresses with a reduction in levels of GATA4 in cardiomyocytes and that it is harmful to the heart, it is possible that the decrease in negatively regulated by miR-208a, using antimiR, generate beneficial effects.

Basic Research

73 - QUANTITATIVE PROFILE OF CARDIAC STEM CELLS FROM SPONTANEOUSLY HYPERTENSIVE RAT

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In the last decade, cardiac stem cells (CSCs) such as c-kit+ cells, sca-1+ cells or cardiac mesenchymal stem cells were discovered into the mammalian hearts, which could contribute to heart regeneration in both physiological and pathophysiological states like myocardial infarction, aortic banding, etc.
The major aim of the present study was to evaluate the number counting of c-Kit+ and Sca-1+ cardiac stem cells, by means of flow cytometry, and of cardiac mesenchymal stem cells, by means of colony forming units-fibroblasts (CFU-F) assay, into the heart from spontaneously hypertensive rats (SHR), compared to normotensive Wistar-Kyoto (WKY) rats.
Briefly, four-, 16-22- or 25-30-old SHR and WKY rats were studied, having their hearts excised after euthanasia in order to proceed the harvesting of stem cells via mechanical tissue fragmentation, enzymatic digestion (with collagenase type I at 1%) and differential filtration (at 40µm). The resulting cardiac cell suspension was then analyzed by means of flow cytometry, in order to quantify the number of c-Kit+ and Sca-1+ cardiac stem cells or it was seed in low density in culture dishes, in order to quantify the number of CFU-Fs, which are clonally derived from individual cardiac mesenchymal stem cells.
The SHRs presented with hypertension and cardiac hypertrophy after 16^{th} week of age. The amount of c-Kit+ cardiac stem cells into the hearts of SHRs was higher at the 4^{th} week of age (0,74±0,22% versus 0,46±0,21% in WKY rats, $p<0,05$) and lower at the 16-22^{th} and 25-30^{th} week of age (16-22 weeks: 0,45±0,19% versus 0,78±0,23% in WKY rats $p<0,05$ and 25-30 weeks: 0,41±0,19% versus 0,77±0,26% in WKY rats, $p<0,05$). The number of Sca-1+ cardiac stem cells did not differ between rat strains in any studied age. On the other hand, the counting of CFU-F was markedly higher into the hearts of SHR at 4^{th} and 16-22^{th} or 25-30^{th} weeks of age, when compared with same aged control WKY rats.
The reduced number of c-Kit+ cardiac stem cells into the heart of SHR observed at the evolving phase (16-22 weeks) and at the established phase (25-30 weeks) of hypertension, with ongoing cardiac hypertrophy, suggests that this numeric reduction could be a consequence of hypertensive and cardiac hypertrophic process. On the contrary, the higher number of CFU-Fs, precociously detected in pre-hypertensive phase (4^{th} week) seems to suggest that this increase could participate in some way of the pathogenesis of cardiac hypertrophy associated with systemic arterial hypertension. Further studies are necessary to elucidate the real role played by these stem cells in the context of systemic arterial hypertension and cardiac hypertrophy.

Basic Research

74 - EFFECT OF ADIPOSE TISSUE-DERIVED STEM CELL TREATMENT IN AN ANIMAL MODEL OF TUBULE-INTERSTITIAL NEPHRITIS

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Adipose tissue-derived stem cells (ASCs) are an attractive source of stem cells with regenerative properties that are similar to those of bone marrow stem cells. Tubule-interstitial nephritis (TIN) can be defined by inflammatory involvement of the renal interstitium affecting tubules, with interstitial edema and acute tubular injury. If the offending agent persists there are the formation of interstitial fibrosis and tubular atrophy. Studies in animals show that intake of adenine mimics the long-term damage of TIN.
In this study we intend to evaluate the protective role of ASC in an animal model of tubule-interstitial nephritis induced by adenine.
The animals were divided into three groups wherein two groups received a diet containing 0.25% adenine for 10 days: sham group (n=5), which no received the diet; adenine group (n=6), which received only the diet and adenine + ASC group (n=6) that received the diet plus administration of ASC. After 10 days, the animals were sacrificed and kidney fragments and blood were collected for analysis.
The group of animals treated with ASC showed better renal function compared with the untreated group, with lower levels of creatinine and urea. Flow cytometric show us a minor inflammatory cell infiltration in animals treated with ASC. The decrease of the inflammation may also be seen by the decrease of renal expression of TGF-β, IL-6 and TNF-α in animal treated group. Following this, we also found less renal fibrosis in adenine + ASC group by analysis of picrosirius staining and immunohistochemical analysis of collagen type I and IV.
We conclude that treatment with ASC in TIN model can promote functional improvement as well as prevent the formation of tubule-interstitial fibrosis.
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Basic Research

75 - GENERATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELLS OVEREXPRESSING HG-CSF AND HIGF-1

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Mesenchymal stem cells (MSC) are a promising tool in the cellular therapy field. In addition to its differentiation potential into multiple cell types, MSC have the ability to secrete bioactive molecules and thus exert multiple biological effects, such as induction of regeneration of injured tissues, reduction of fibrosis and inflammation and neovascularization. Considering these properties, we aim to evaluate the therapeutic effects of MSC overexpressing growth factors known to play important roles in immunomodulation and tissue repair, such as granulocyte colony-stimulating factor (G-CSF) and insulin-like growth factor (IGF-1). Therefore, the objective of our work is to generate and characterize hG-CSF and hIGF-1 overexpressing-mouse mesenchymal stem cell lines.
To generate overexpressing stable cell lines we used a second generation lentiviral system. The coding sequence for hG-CSF and hIGF-1 were amplified by PCR, using specific designed primers and cloned into a lentiviral transfer vector with constitutive promoter. Lentivirus particles were produced through the co-transfection of HEK293FT with lentiviral system plasmids. MSC obtained from GFP transgenic mouse bone marrow were transduced with hG-CSF and hIGF-1 lentivirus infective particles. Overexpressing cells of monoclonal origin were obtained by limiting dilution. G-CSF and IGF-1 gene expression was quantified by qPCR, and protein production by ELISA. The generated cells were characterized by immunophenotyping and assessment of its differentiation potential.
We have successfully generated 4 hG-CSF and 8 hIGF-1 overexpressing mMSC lines. The expression of the respective genes was confirmed by qPCR and ELISA. All overexpressing clones showed higher expression of the factors compared to the empty vector or mock cells. No statistically significant difference was observed for either gene among the analyzed clones. The clones obtained showed fibroblast-like morphology, typical of MSC, and expressed MSC cell markers, as shown by flow cytometry analysis. IGF-1 superexpressing clones had increased proliferative rate when compared to mock-transfected cells. All of the G-CSF overexpressing cell lines were able to differentiate into osteocytes, condrocytes and adipocytes, demonstrating they kept their multipotent characteristics.
In this work we generate functional tools to evaluate the therapeutic effects of the growth-factor overexpressing cell lines in different animal lesion models, by comparing its effectiveness to that of non-transduced mMSCs. Additionally they can also be used as a tool for basic research studies, to better understand how the overexpression of G-CSF and IGF-1 may affect MSC biology.

Basic Research

76 - EVALUATION OF THE CORRELATION BETWEEN CD56, KDR AND PDGFRα EXPRESSION AND EMBRYONIC STEM CELL DIFFERENTIATION TO CARDIOMYOCYTES

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Differentiation of embryonic stem cell to cardiomyocytes is a valuable tool for drug discovery and cell therapy but the process is time consuming and expensive. There are a number of molecules known to be involved in mesordermal and cardiac differentiation that could predict the efficacy of differentiation.
The objective of this work was to correlate neural cell adhesion molecule (NCAM, CD56), vascular endothelial growth factor receptor 2 (VEGFR2, KDR) and Platelet-derived growth factor receptor, alpha (PDGFRα) expression to the efficacy of cardiac differentiation of hESC NKX2.5 GFP cell line.
After hESC NKX2.5 GFP cell line reached confluence cells were dissociated to small clusters which were cultured on non-adherent plates in StemPro-34 culture medium (Invitrogen) supplemented with penicillin/streptomycin, ascorbic acid, monothioglycerol and transferrin (differentiation medium) and BMP4. After 24 hours, the medium was replaced by differentiation medium supplemented with 10ng.ml-1 of BMP4 and 6ng.ml-1 of Activin A for 24, 48 or 72 hours. Cell aggregates were then cultivated in differentiation medium supplemented with 10µM of Wnt3A inhibitor XAV939 and VEGF during four days and after this step embryoid bodies were cultivated in differentiation medium supplemented with VEGF only. CD56, KDR and PDGFRα expression were evaluated by flow cytometry at the time points indicated and the efficacy of differentiation was accessed through quantification of troponin T positive cells by flow cytometry at the 15° day of the protocol.
CD56 and PDGFRα fluorescence increased over time, reaching a peak around 60-80 hours after BMP4 and Activin A incubation and then both fluorescences decline. We detected CD56 expression before PDGFRα and the gain of fluorescence of PDFGRα was smaller than that of CD56. Next, we evaluated if the expression of these molecules could indicate a time frame in which Wnt inhibition could drive cardiac differentiation. Neither CD56 nor PDGFRα absolute median intensity of fluorescence at the moment of Wnt inhibition could be correlated with the percentage of TnT+ cells at day 15 (p=0.378 and 0.500, respectively). The gain of PDGFRα fluorescence at the moment of Wnt inhibition did not correlate with cardiac differentiation (p=0.1623 R2=0,2972). However, there is a significant negative correlation between the gain of CD56 and the efficacy of the protocol (p=0.0088 R2=0,70). KDR analysis revealed that after Wnt inhibition three distinct populations can be observed: KDR negative, KDR dim and KDR high populations. KDR dim population percentage correlated with the efficacy of the protocol at day 15 (p=0.0004), although with a small R2= 0,39.
CD56 fluorescence gain can indicate when inhibition of canonical Wnt pathway can drive cardiac differentiation and KDR dim population correlate with the percentage of TnT+ cells but its predictive power is limited.

Basic Research

77 - BONE MARROW THERAPY AT PRE AND POST SYMPTOMATIC PHASES IN A MOUSE MODEL OF ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurological disease that affects selectively the motor neurons. The detail mechanisms of selective motor neuron death remain unknown and no effective therapy has been developed.
The aim of this work is to investigate the therapy with bone marrow mononuclear cells (BMMC) in a mouse model of ALS (SOD1-G93A mice). We injected 106 BMMC in the lumbar portion of the spinal cord of the SOD1-G93A mice at pre-symptomatic (9 weeks old) and symptomatic (14 weeks old) phases. In each condition, we analyzed the progression of disease and the lifespan of the animals.
We observed a delay of three weeks in the disease onset in the animals injected with BMMC at pre-symptomatic phase, although we did not observed increase in the lifespan. When we injected the BMMC at symptomatic phase, we did not observe difference in the animal's lifespan or in the functional outcome. The immuhistochemistry assay showed that there is a decrease in the number of motor neurons during the disease course, that is not affected by the treatment.
Using different strategies to track the BMMC, we noticed that few cells remain in the spinal cord after the transplant.
These results indicate that, although the treatment with BMMC in the spinal cord of a mouse model of ALS at pre-symptomatic phase delayed the progression of the symptoms, it did not increase the lifespan of the animals or increase the motor neurons survival, possibly because the BMMC did not remain in the injected site.

Basic Research

78 - CHARACTERIZATION OF A MOUSE INDUCED PLURIPOTENT STEM CELL LINE, CARDIAC DIFFERENTIATION AND TRANSPLANTATION INTO CHRONIC CHAGASIC MICE

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Chronic heart failure (CHF) is a major public health issue worldwide. Chagas disease is still a relevant etiology of CHF in Brazil. The generation of induced pluripotent stem cells (iPSC) has brought new perspectives for the investigation of heart diseases and development of new drugs or cell-based therapies. In this study we aimed to derive and characterize cell lines of mouse iPSC (miPSC), induce cardiac differentiation and test their potential use for cell replacement in an experimental model of chronic chagasic cardiomyopathy in mice.
Lines of miPSC were generated from murine embryonic fibroblasts (MEFs), reprogrammed with forced expression of Oct4, Sox2 and Klf4 by transduction with lentiviral vectors. The characterization of pluripotency was done by immunofluorescence and teratoma formation assay by injection into NOD/SCID mice. Standard chromosome analysis (g-banding) was performed. In order to induce cardiac differentiation, miPSCs were aggregated to form embryoid bodies (EBs) maintained in low-adherence plates for 2 days in medium lacking LIF. EBs were then transferred to 24-well plates coated with Matrigel, for 2 additional days of culture adhered to the plate, followed by 7 days of culture in the presence of 50 μM ascorbic acid (AA). Cardiac differentiation was detected by visualization of areas of spontaneous beatings, qRT-PCR for detection of GATA-4 and Nkx2.5 and immunofluorescence analysis. Selection of cardiomyocytes in AA-induced cultures was done using a glucose-free, lactate supplemented medium. The differentiated cells were dissociated, stained with fluorescente probe CellTracker Green and injected intramyocardially in mice with chronic Chagas disease, guided by ecography.
The miPSCs were positive for pluripotency markers (Oct-4, Nanog and SSEA-1) and formed teratomas when injected into immunodeficient mice. Kariotype analysis showed an overall normal number and morphology of chromosomes in the majority of the cells. The protocol for cardiac differentiation using AA reproducibly induced the expression of GATA-4 and Nkx2.5 and the appearance of spontaneous beating areas. Those cells were found to be positive for cardiomyocyte markers (cTNT and Cx43). Culture in glucose-free, lactate supplemented medium efficiently induced selection of cardiomyocytes. The intramyocardial transplantation was safe and feasible. Some transplanted cells could be detected in heart sections after injection.
AA-induced cardiac differentiation protocol is a simple and reproducible method to obtain cardiomyocytes derived from iPSC, which can be further selected by glucose deprivation. Furthermore, iPS-derived cardiomyocytes engraft when transplanted into the hearts of Chagasic mice. Further studies will address its safety and potential use as a new strategy for cell therapy in chronic chagasic cardiomyopathy.

Basic Research

79 - THE ROLE OF INFLAMMATORY MICROENVIRONMENT IN THE ACTIVATION AND BIOLOGY OF MESENCHYMAL STEM CELLS

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Currently, has been defined that Mesenchymal stem cells (MSC) have powerful immunoregulatory properties on several inflammatory cells (NK, B cell, T cell, macrophages, dendritic cells) and immune regulatory cells (Treg) being widely explored in a large numerous of experimental approaches. Although MSC-based therapies had presented positive results in phase I-II studies, double-blinded randomized studies did not have demonstrated the same efficacy to inflammatory disorders. Alternatively, have been investigated that MSC possess some key receptors (TLRs, TNFR, INFRs) and signaling pathways (PKR, STAT-1, NF-kB) that could be activated by inflammatory milieu, enhancing its immunosuppressive performance. However the precise mechanism behind of MSC activation remain yet little elucidated.
Hence, this present study aim to investigate the molecular signature involved during MSC activation by inflammatory microenvironment using distinct kinds of in vitro stimulus.
The MSCs were cultivated under normal conditions and under different inflammatory stimulus (LPS, 10 µg/mL; Zymosan, 10 µg/mL; TNF-α, 100 ng/mL and IFN-γ, 100 ng/mL) by 48 hours. Furthermore unstimulated and stimulated MSC were submitted a set of analysis to identify their molecular signature involved during MSC activation. Firstly, MSC cultures were properly characterized and submitted to cultures with or without inflammatory stimulus.
We verified neither morphological changes after MSC stimulus. Furthermore we observed that stimulated MSC did not alter its oxidative stress status (anion superoxide) and apoptosis index than normal MSC. Moreover we detected in MSC co-cultures with inflammatory conditions a slight increase in the G2+M cell cycle phase. In addition, we found in MSC stimulated with inflammatory stimulus an intense modulation of several molecules involved with activation (TLR-2, TNFR, iNOS and NFkB-p65), migration and engraftment (CCR3, CCR4, CCR5 and CCR7) and matrix remodeling (MMP-9 and TIMP-1). More important we identified that potent immunomodulatory molecules (IDO, PGE-2, SOC3, CTLA-4, PDL-1 and PDL-2) also were strongly modulated in activated MSC. Finally, we detected that some epigenetic parameters such as global DNA methylation, HDAC and HAT activity were altered after MSC inflammatory stimulus.
In conclusion our preliminary results suggest that according with each stimulus utilized, the MSC activation may lead to a distinct epigenetic status, which differently can control the expression of molecules related with activation, migration, remodeling and immunomodulation. Thus, we expect in the future evaluate the precise association between global trascriptome and epigenetic profile during MSC activation to identify specific mechanisms involved with MSC-based therapies.

Basic Research

80 - INFLUENCE OF CULTURE MEDIUM ON STEM CELLS MARKERS EXPRESSION IN HUMAN DENTAL APICAL PAPILLA

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Dental stem cell populations present properties similar to those of mesenchymal stem cells, such as the ability to self-renew and the potential for multilineage differentiation. They have the capacity to differentiate into all three germ line cells, proving that a population of pluripotent stem cells exists in the dental tissues. Consequently they do not only have applications in dentistry, but and other applications in the field of tissue regeneration. Recent methodologies for cell isolation and expansion of these cells have limitations concerning their use in cell therapy. These cells are usually cultured in Eagle's basal medium and incorporate animal serum.
Because that the medium can influence the cell population in cultures we investigate the effects of different media (MEM, RPMI 1640 and 199 media) supplemented with human serum or animal serum on stem cell surface marker expression in cells of dental apical papilla at the root of developing permanent teeth.
For this, third molars were obtained from tooth extractions from patients ranging from ages 15–19 years. From these teeth, cells of apical papilla was isolated immediately resuspended and following conditions: containing 10% Fetal Bovine Serum (FBS) + DMEM/F12, containing 10% human serum + DMEM/F12, containing 10% FBS + 199 media, containing 10% human serum + 199 media, or containing 10% FBS + RPMI 1640, containing 10% human serum + RPMI 1640. Flow cytometry was performed to determine the expression levels of the cell surface markers CD90, CD146, and p75 neurotrophin receptor (p75NTR), a typical neural crest marker.
All conditions resulted in cells with expressed high levels of CD 90 (>98%), CD 146 (>82%) and p75 (>78%). Additionally, between the conditions, there were no differences noted in the expression of these cell surface markers regardless of the different media or serum source.
The findings reported that stem cell of dental apical pappila can be isolated and expanded different media devoid of MEM and still maintain their phenotypic properties. In addition, these cells can be isolated and expanded without the incorporation of animal serum in the media but, as a substitute, throughout using human serum without changes in expression of stem cell markers.

Basic Research

81 - GALECTIN-3 INHIBITION IN CARDIAC MESENCHYMAL STEM CELLS REMARKABLY IMPAIR ITS PROLIFERATION, CELL CYCLE AND IMMUNOMODULATORY PROFILES

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Galectin-3 is a soluble lectin involved in a variety of processes, both physiological or pathological, including cell proliferation and apoptosis. The role of this molecule in the regulation of cardiac fibrosis and remodeling and its contribution to the development and progression of heart failure has been recently described. Galectin-3 gene is overexpressed in the hearts of mice with chronic Chagas disease and its expression correlates with inflammation and fibrosis. Cardiac mesenchymal stem cells (CMSC), among other cells, express galectin-3.
In view of this, we evaluated the effects of galectin-3 inhibition in CMSC.
Cardiac mesenchymal stem cells (CMSC) were obtained from cardiac tissue of adult mice by partially digestion with collagenase type I and cultured as explants. The cells obtained were characterized by flow cytometry. The knockdown for galectin-3 was achieved by letiviral transduction with shRNA, that was confirmed by qRT-PCR and immunofluorescence. The cell proliferation was determined by 3H-thymidine incorporation assay and FACS was performed to evaluate the cell cycle. The lymphoproliferation assay was performed to evaluate the immunomodulatory potencial of CMSC.
Cells migrated from the cardiac tissue and attached to the surface of the culture flask, displaying polymorphic and fibroblast like morphological characteristics. Characterization by flow cytometry revealed a low percentage of cells expressing hematopoietic cell markers and the majority of the cells displaying markers of mesenchymal stem cells. The knockdown for galectin-3 was confirmed by qRT-PCR as well as immunofluorescence that demonstrated a low expression of galectin-3 and collagen-1 in comparison with mock cells. Assessment of cell proliferation by 3H-thymidine incorporation showed a decreased proliferation in CMSC knockdown for galectin-3. Moreover, cell cycle analysis by FACS showed reduced division cell ratio. Analysis by qRT-PCR showed a reduction of cyclin D1 gene expression in CMSC knockdown for galectin-3. Importantly, these cells have a reduced inhibitory activity in lymphoproliferation assay when compared to mock cells. Galectin-3 knockdown also reduced the gene expression of TGF-β1 in CMSC.
Knockdown of galectin-3 decreased cell proliferation, altered the cell cycle and shifted the immunomodulatory profile of CMSC, indicating the importance of galectin-3 for mesenchymal cell functions.

Basic Research

82 - TRANSPLANTATION OF BONE MARROW MONONUCLEAR CELLS MODULATES EXPRESSION OF GROWTH FACTORS IN CHRONIC PILOCARPINE-TREATED EPILEPTIC RATS

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Epilepsy affects 1% of the world population and 30% of these patients are refractory to available medication. Stem cells host hope in the treatment of epilepsy since they can proliferate, differentiate and produce factors which may activate endogenous mechanisms to restore the injured brain.
Knowing that the administration of bone marrow mononuclear cells (BMMC) has therapeutic potential in an experimental model of epilepsy, the aim of this study was to investigate the mechanisms by which administered cells exert their beneficial effect. Thus, a comparative study was done to detect the expression of trophic factors as brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β), and their receptors, tyrosine receptor kinase B (TrkB), GDNF family receptor alpha 1 (GFR-α1), tyrosine receptor kinase A (TrkA), vascular endothelial growth factor receptor (VEGFR-2), and transforming growth factor β receptor 1 (TGF-βR1).
Chronic epilepsy was induced by pilocarpine injection (320 mg/kg; ip). Twenty-two days after status epilepticus (SE), rats were treated with either saline or BMMC and randomly assigned into groups: Control, Pilo, Pilo+BMMC. BMMC groups received cell transplantation (obtained from EGFP C57BL/6 mice) via tail vein (1x107 cells, 100mL). Pilocarpine-treated animals were monitored for the presence of spontaneous seizures for seven days prior to transplant. The protein and mRNA levels of trophic factors were analyzed 3, 7 and 14 days after transplant in the hippocampus of animals by ELISA and RT-PCR techniques.
Our data revealed increased protein expression of BDNF, GDNF, NGF, and VEGF and reduced levels of TGF-β1 in the hippocampus of transplanted animals. Additionally, an increase in the mRNA expression of BDNF, VEGF and TGF-β1 and a reduction in the mRNA levels of the receptors TrkA and VEGFR-2 were observed. Trophic factors that regulate development, maintenance and function of nervous system increased in a time dependent manner post-transplantation.
The gain provided by transplanted BMMCs in the epileptic brain may be related to the ability of these cells in modulating the network of neurotrophins and angiogenic signals.

Basic Research

83 - TRANSPLANTATION OF MONONUCLEAR CELLS FROM HUMAN UMBILICAL CORD BLOOD MODULATES INFLAMMATORY RESPONSE BY IL-1B IN THE MODEL OF GERMINAL MATRIX/INTRAVENTRICULAR HEMORRHAGE

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The germinal matrix/intraventricular hemorrhage (GMH/IV) is the most common neurologic disorder in preterm infants, in which the severity and incidence are inversely related to gestational age at the time of birth. This event is associated with mortality and morbidity (e.g. cerebral palsy, motor developmental delay and cognitive disabilities) in early childhood. Previous studies have shown that infants affected by GMH/IV have elevated levels of proinflammatory cytokines such as IL-1β, IL-6, IL-8, IL-18 and TNF-α, and may be related neurological sequelae. Cell therapy has been regarded as promising for the treatment of neurological diseases and injuries. The fraction of mononuclear cells from human umbilical cord blood (HUCB-MCs) has several types of stem cells and is considered an interesting alternative in the treatment of neonatal complications also associated modulation.
To elucidate the role of HUCB-MCs in the inflammatory response in the GMH/IV model the aim of this study was to measure the levels of proinflammatory cytokines IL-1β, IL-6, IL-8, IL-18 and TNF-α.
Induction of GMH/IV was performed by injecting 0.5 U of bacterial collagenase VII in the right periventricular region of Wistar rats neonates with six-day-old rats. The animals received intraperitoneal transplant of HUCB-MCs (107 cells; 500 µL) in 3h or 24h after GMH/IV induction. The measurement of IL-1β, IL-6, IL-8, IL-18 and TNF-α was performed from homogenates of the right hemisphere of animals submitted to GMH/IV model and transplanted with HUCB-MCs by sandwich ELISA. The animals were euthanized at 1, 3 and 7 days after induction of the model. Preliminary results indicate that the induction of GMH/IV increases brain levels of the proinflammatory cytokine IL-1β and treatment with HUCB-MCs at 24 hours after induction of the model, but not at 3h, decreased expression levels of IL -1β at 1 and 3 days after GMH/IV induction compared to the control group.
It is provable that the transplantation of HUCB-MCs have a role in modulating the anti-inflammatory response in the GMH/IV model, but surely this is just one of their actions. Other complementary studies should be conducted to fully elucidate the mechanisms of action of these cells.

Basic Research

84 - NEW APPROACHES FOR THE TREATMENT OF TYPE 1 DIABETES: IMMUNOPROTECTION OF DIFFERENTIATED INSULIN CELL CLUSTERS

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Type 1 Diabetes Mellitus (T1DM) is a syndrome characterized by autoimmune destruction of pancreatic beta-cells. An interesting alternative therapy for T1DM is the engraftment of insulin-producing cells (IPCs) clusters differentiated from murine embryonic stem cells (mESCs). Microencapsulation of these clusters using biocompatible materials creates an immunoprotected environment, eliminating the need for an immunosuppressive regimen.
We aim to: a) promote the final maturation of IPCs clusters in a functional and immunoprotected microenvironment of innovative microcapsules; b) analyse the process of pancreatic beta-cell differentiation, at the molecular level, through functional analysis of genes repressed during this process.
We promoted mESCs differentiation into IPCs and analysed the downregulation of some genes during this process through qRT-PCR. We also developed an innovative type of microcapsule by adding polymerized laminin to our previously developed biomaterial Bioprotect®. This biomaterial had its in vitro stability tested by different types of stress. Biocompatibility was also tested through co-incubation with RAW 264.7 macrophages.
We confirmed the downregulation of the TXNIP gene, related to regulation of the redox-status, during the beta-cell differentiation process. Since pancreatic beta-cells are known for their poor antioxidant defences, we hope to improve the differentiation of mESCs into IPCs by the modulation of TXNIP throughout the entire differentiation process. We were also able to produce a stable and biocompatible biomaterial containing polylaminin. Production of microcapsules with an extracellular membrane component (polylaminin) may improve the complete differentiation of encapsulated IPCs.
Both proposed strategies may lead to improvement in the efficiency of mESCs differentiation into IPCs, by regulating their redox status through modulation of TXNIP gene expression and by microencapsulating these clusters using a stable, biocompatible and functional biomaterial.

Basic Research

85 - PLATELET-RICH PLASMA (PRP) STIMULATES HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS PROLIFERATION AND MIGRATION IN VITRO

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Platelet-rich plasma (PRP) constitutes a rich source of growth factors and cytokines important for the healing process. Also, mesenchymal stem cells (MSC) possess immunomodulatory and anti-inflammatory properties, capable of producing cytokines that act at the lesion site resulting in tissue repair. Therefore, the use of MSC in combination with PRP could have an important therapeutic additive or synergistic effect in cell therapy procedures
Investigate the influence of PRP on the proliferation and migration of MSC of the adipose tissue in vitro.
PRP was obtained from a unit of whole blood centrifuged at 900 g for 5 minutes to separate the plasma and 1500 g for 15 minutes to concentrate the platelets. MSC were obtained from fragments of adipose tissue in the abdominal region. The fat was processed to obtain the stromal fraction and cell culture. It was performed immunophenotyping of MSC and induction of osteogenic, adipogenic and chondrogenic differentiation. Proliferation assay (n = 6) with CCK-8 kit was employed to assess the growth of MSC with 0%, 10%, 25%, 50% and 100% of PRP or 10% of fetal bovine serum (FBS). MSCs were subjected to migration assay with 0%, 10% of PRP or 10% FBS and the percentage of closure of scratch was obtained by Image J. Statistical analysis was performed using the Kruskal-Wallis test, being the significant result when $p<0.05$. The study is registered in the Platform Brazil (CAAE Number: 11214513.6.0000.5404).
After processing, the final concentration of platelets in PRP was 465.000/uL, the equivalent to 2x of the concentration in whole blood (232.000/uL). MSC showed positive expression (≥ 95%) of the markers CD 73/CD 90 (99.78%), CD 73/CD 105 (97.63%), CD 90 / CD 105 (97.09%) and negative (≤ 2%) of exclusion markers CD 34 (0.26%) CD 11b (0.04%) CD 19 (0.02%), HLA-DR (0.73%). After exposure to the medium of induction, MSC showed osteogenic, chondrogenic and adipogenic differentiation. After 24 hours of culture there was no stimulus for proliferation of MSC front of the addition of PRP. However, after 48 h of culture, MSC cultured with 10% PRP showed absorbance reading two fold higher (0.670 ± 0.023) compared to the group without PRP (0.320 ± 0.023), with $p <0.05$. The migration assay showed a statistically significant difference ($p <0.05$) on the percentage of scratch closure for MSC cultured with 10% and 0% PRP after 24h (17.07 ± 6.47 and 10.48 ± 3.63) and 48h (20.02 ± 2.30 and 11.89 ± 2.68).
Considering these results, it can be postulated that the use of PRP has the potential to stimulate, at low concentrations, the proliferation and migration of MSC in vitro. Furthermore, at high concentrations, PRP could exert a stimulatory role in the metabolic pathways such as PI3K/AKT, subject to activation mediated by growth factors, which would result in stimulation of the paracrine mechanisms of stem cells via protein synthesis regulated by mTOR signaling pathway.

Basic Research

86 - CONSTRUCTION AND EXPRESSION ANALYSIS OF A PLASMID VECTOR FOR ANGIOPOIETIN1

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Several clinical studies using growth factors to promote myocardial angiogenesis suggest that the therapeutic benefit can be obtained even using the most basic naked plasmid DNA vectors. Angiogenic growth factors such as vascular endothelial growth factor (VEGF) and angiopoietin 1 (Angpt1) are highly potent and readily secreted allowing limited delivery of the transgene and can produce the desired therapeutic effects.
The objective of this study was the creation of plasmid vector containing the human Angpt1 cDNA and the evaluation of their expression by lipofection in murine cells.
The expression vector pcDNA3.1/V5-His TOPO TA (Invitrogen) system was used to clone the Angpt1 cDNA, obtained by RT-PCR with specific primers for the gene (NM_001199859), for RNA isolated of a cell culture inducted with VEGF. For attesting Angpt1 expression and to test their influence on VEGF, two clones were transfected into murine cells (NIH3T3) for 24, 48 and 72 hours and assayed by ELISA immunoassay for both molecules.
The cDNA obtained by PCR showed 1530 bp of amplification, the size expected for Angpt1 complete sequence. The identity was confirmed by sequencing. After ELISA assay, it was found that in 24 hours, the vector (named pcDNA-Angpt1) provided induction of expression from Angpt1, which declined in the remaining time. After 72h, the exogenous expression of Angpt1 also induced the expression of endogenous VEGF.
The construction of pcDNA-Angpt1 vector was performed and their expression was achieved in and eukaryotic system. The vector constructed was able in vitro to stimulate VEGF and Angpt1 secretion in heterologous cell system. The use of VEGF in gene therapy is fairly well around the world, however, there are few studies in the literature using other angiogenic factors. The present study demonstrated that Angpt1, in addition to promoting the development of vessels, may have benefit as the induction of own VEGF expression, as seem in vitro.

Basic Research

87 - EVALUATION OF WAVE BIOREACTOR POTENTIAL AS ALTERNATIVE FOR PRODUCTION OF MESENCHYMAL STROMAL CELLS IN LARGE SCALE

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In the last 15 years, the use of mesenchymal stromal cells (MSCs) for tissue engineering therapies and clinical applications in regenerative medicine has passed the status of simple promising technology for concrete reality. The transfer of practical knowledge obtained in laboratory scale for clinical and commercial scale of MSCs necessarily involves the development of cultivation technologies in bioreactors that are reproducible, robust, secure and viable commercially. Many types of bioreactors have been evaluated for the expansion of MSCs, among the best options are those that use disposable technologies and approved by regulatory agencies, with the Wave Bioreactor being one of the most recent quoted candidates.

In this context, this work aims to evaluate the Wave Bioreactor potential for efficient, rapid, reproducible, economical and safe expansion of MSCs, preserving the therapeutic quality.

The research was performed in Wave bioreactor 2/10 (GE Healthcare), cultivating the hMSC-TERT cell line in the culture medium α-MEM supplemented with incubated nucleosides and 15% v/v fetal bovine serum at a temperature of 37 ℃ an d pH in inoculation of 7.20. The initial working volume in the bioreactor was 300 mL. For anchoring of hMSC-TERT cell line during the cultivation in suspension was used 2.5 g/L of microcarriers (adhesive particles) CultiSpher-S®, whose average diameter was 150 µm. Since the inoculum concentration is an important process parameter, in a first experiment of the study, two levels of inoculum $7.5x10^4$ and $1.0x10^5$ cell/mL were evaluated. In two subsequent experiments using inoculum of $1.0x10^5$ cell/mL, considered the most appropriate, the hMSC-TERT cell line was cultivated under fed batch operation, with exchange of culture medium every 24 hours during the cultivation progress. This strategy aimed to prevent premature depletion of nutrients and the drastic decrease in pH.

The results of the experiments show that inoculum above $1.0x10^5$ cell/mL improve culture adhesion and proliferation. They also show that the fed batch operation is crucial to prolong the culture time and thus increase cell expansion.

The collected data showed that the Wave bioreactor present a good potential for growing MSCs, getting a maximum specific growth rate of 0.027 h-1, which is equal to that obtained in other conventional systems used in our laboratories. On the other hand, cell growth expansion factor, despite achieving only 2.9X when compared to the 10X values routinely obtained in conventional laboratory scale bioreactors, the Wave Bioreactor showed the versatility to allow larger expansion of MSCs culture.

Basic Research

88 - EFFECT OF HUMAN ADIPOSE DERIVED STEM CELLS AND POLY-3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE SCAFFOLDS IN CRITICAL-SIZED CALVARIAL DEFECTS

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Human adipose-derived stem cells (hASCs) are multipotent cells that can be differentiated along an osteogenic lineage providing an excellent cell source for bone regeneration and repair. Several materials have been used to develop scaffolds that act as a temporary matrix for cell guidance and extracellular matrix deposition in order to use in tissue engineering applications. Among the materials, poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHB-HV) is an excellent biomaterial candidate for bone tissue engineering due its properties, such as natural origin, biodegradability, biocompatibility, nontoxicity, piezoelectricity, and thermoplasticity.
In this study, we evaluate the effect of hASCs with PHB-HV scaffolds in the regeneration of a non-healing mouse calvarial defect.
The scaffolds were produced by freeze-drying technique. The hASCs were harvested from female lipoaspirate. Critical-sized (4 mm) calvarial defects were created in the parietal bone of adult male nude mice. Defects were either left empty, treated with PHB-HV scaffold alone, or scaffold with hASCs for 4 or 12 weeks. Micro computed tomography scans were obtained postinjury to visualize the formation of new hard tissue. Histology (H&E staining) and immunohistochemistry for bone proteins (osteocalcin and osteopontin) were performed. Gene expression of bone markers were assessed by quantitative PCR. This study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais (nº. ETIC 11668613.7.0000.5149 and nº. 373/2012).
There were no significant differences in bone mineral density among the groups. The PHB-HV scaffolds, in association or not with hASC engrafted calvarial defects improved the repair of the bone defect in comparison to control groups that showed no healing. Implanted scaffolds alone or with cells developed a moderate inflammatory process and neo-angiogenesis. Immunohistochemistry of sections from the engrafted defects revealed the expression of osteocalcin and osteopontin proteins and the expression of bone markers genes were higher at 12 weeks of implantation than at 4 weeks. No repair was seen in the control animals without implants.
So PHB-HV scaffolds alone or in combination with hASCs seemed to mediate repair of critical sized mouse calvarial defects which make this strategy promising for bone tissue engineering applications.

Basic Research

89 - AMNIOTIC STEM CELLS THERAPY TO TREAT INDUCED CIRRHOSIS

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Liver cirrhosis is a chronic disease, characterized by presenting modifications on the basic histological units of liver, forming nodules of regeneration and causing diffuse increase in connective tissue, with disorganization of lobular and vascular architecture. Several studies using stem cells have been performed for the treatment of liver cirrhosis. The use of stem cells derived from amniotic membrane has been extensively studied and can also be an alternative for the treatment of hepatic fibrosis and cirrhosis.
The objective of this study has been the evaluation of the use of stem cells derived from amniotic membrane for the treatment of liver cirrhosis and fibrosis in rats by thioacetamide model.
Thirty (30) Wistar rats (Rattus norvegicus) were used, being submitted to intraperitoneal injection of thioacetamide 200 mg/kg three times per week, for 8 weeks to induce fibrosis (n = 12), and 14 weeks to induce cirrhosis (n = 10), besides control groups (n = 8). Animals were weighed weekly, and blood collected fortnightly. After this period, single application of 1×10^6 stem cells derived from rat amniotic membrane was performed intravenously, followed by observation of the animals by a 90-day period. After euthanasia, liver sections were fixed in 10% formalin and processed for light microscopy, for stainings: hematoxylin-eosin for morphological analysis through staging and grading of cirrhosis, and observation of cellular changes; picrosirius for collagen morphometry; periodic acid-Schiff for observation of neutral mucopolysaccharides and alcian blue for analysis of acid mucopolysaccharides. Serum biochemistry for AST, ALP, TP, ALB and GGT are being processed, such as immunohistochemistry for type 1 and 3 collagens, aSMA and PCNA3.
Experimental mortality was 10%. Through weighing, constant weight loss was observed during induction of cirrhosis, with slightly faster weight gain in animals that underwent cell therapy. Macroscopic analysis showed less friable and nodular livers of treated animals. Through staging and grading, we noted that treated animals were on a slightly lower score than non-treated animals after 90 days. Different cellular changes were observed, such as megalocitosis, steatosis, oval cells, hemossiderin, binucleated cells, apoptosis and duct proliferation, which were also more present on non-treated animals. However, vacuolizations and mitotic figures presence was higher on treated animals. Measurement of parenchymal collagen showed that interlobular collagen was decreased on treated animals. However, intralobular collagen levels were increased after treatment.
Serum biochemistry and immunohistochemistry are needed to conclude if treatment of cirrhosis was effective. However, current findings suggest that amniotic membrane stem cells reduce tissue damage and decrease collagen levels on fibrotic and cirrhotic rats.

Basic Research

90 - GENERATION OF INDUCIBLE PLURIPOTENT STEM CELLS (IPSCS) FROM DIFFERENT ANIMAL SPECIES

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Since the description of the induction of pluripotency in somatic cells, generated by forced expression of four transcription factors, these cells have been preferred over embryonic stem cells which inevitably depend on the destruction of developing embryos. These cells, called iPS (induced pluripotent stem) have characteristics similar to embryonic stem cells, such as morphology, potential for proliferation, expression of embryonic markers and differentiation in cells of all germ layers. Another important feature of iPS cells is the possibility of generating a stem cell with embryonic characteristics, self, with genetic material of each individual which, ultimately, would avoid problems of rejection when used in cell therapy protocols. However, many studies still need to be performed before the stage of clinical trials with these cells is achieved.
Our aim is to develop species-specific iPS cells for preclinical studies, which mimic the therapeutic effects of patient-specific iPS cells, targeting the treatment of humans.
Material and Methods: We used lentiviral particles (STEMCCA) carrying the four reprogramming genes (OCT4, SOX2, KLF4 and C-MYC) to reprogramm both human and rat adult cells.
We were able to produce iPS stem cells of both humans and rats aiming preclinical studies. Both of cells were derived with feeder layer, human iPS displayed a bFGF-dependence and rat iPS needed LIF to grow.
These cells, although utilize the same method of generation, have difficulties of reprogramming and dynamic of growth very different.
Supported by: FAPESP, CNPq, FINEP, BNDES, DECIT-MS

Basic Research

91 - STRATEGIES TO MODULATE AGGLOMERATION IN STIRRED CULTURES FOR EFFICIENT EXPANSION OF MESENCHYMAL STROMAL CELLS

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Use of mesenchymal stromal cells (MSCs) for applications in cell therapy and tissue engineering allowed a revolution in modern medicine, permitting pharmacological and clinical advances for treatment of degenerative and autoimmune diseases. Differentiation and regenerative potential of the MSCs becomes limited by the low levels of cells available in adult tissues (0.01 to 0.0005%) along with the high dose necessary in an infusion (approximately 106 cells/kg). Since traditional cell culture systems are shown to be inefficient to meet the demand, the use of culture spinner flasks and microcarriers (adhesive particles ~200µm size) as an alternative in large-scale cell expansion has been necessary. Although a well-established technique, it still requires adjustments, especially to the natural susceptibility of the MSCs to the formation of agglomerates caused by the accumulated extracellular matrix produced by the MSCs, which prevent access to nutrients and hampers cell recovery at the end of culture. It is known that additives such as dextran sulfate (DXS) have antiagglomerating properties, being an attractive option to modulate the formation of large agglomerates.
Thus, in this work it was measured the influence of dextran sulfate (DXS) and a commercial anticlumping (CAC) in MSCs cultures in spinner flasks and the addition of a physical barrier (calcium alginate microbeads) to prevent contact between microcarriers.
Therefore, were performed cultures of hMSC-TERT lineage in spinner flask containing Cultispher-S® microcarrier concentration of 3 g/L, 50 mL α−MEM supplemented culture medium (arginine, glucose, glutamine and 15% v/v fetal calf serum), maintained at 37ºC in a CO2 incubator with variable CO2 injection for pH control.
Results show that the addition of antiagglomeration additives inhibited cellular growth, such effect was more evident at 1 to 0,05% w/w DXS concentration, moderate at the addition of 0,01 % v/v CAC and low at 0,01% w/w DXS. The addition of physical barriers with sodium alginate microbeads at a particle number equivalent to the number of microcarriers (1:1) resulted in considerable cell death due to attrition, however, half of this value (1:2) permitted high cell growth and delayed the agglomerate formation, reaching a 13-fold expansion factor. Although the presence of agglomerates has been observed with the use of DXS, these did not reach the diameters observed in the control or alginate microspheres experiment, allowing a high cell growth by reaching a 8-fold expansion factor in the culture with 0,01% DXS. With recovered cells after expansion, it was proved the maintenance of the immunophenotypic and functional characteristics.
As a whole, results demonstrated that the use of additives and physical barriers in MSC culture are promising strategies to modulate the agglomerates formation.

Basic Research

92 - PLATELET-RICH PLASMA (PRP) AND CONDITIONED MEDIUM FROM HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS STIMULATE FIBROBLASTS AND KERATINOCYTES PROLIFERATION AND WOUND HEALING IN VITRO

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In the context of regenerative medicine for the treatment of chronic lesions, the clinical use of tissue bioengineering associated with cell therapy by using adult stem cells constitutes in a matter of great interest and therapeutic potential. The platelet-rich plasma (PRP) has the capacity to act as organizer of the extracellular matrix, stimulate angiogenesis and re-epithelialization. Mesenchymal stem cells (MSC) have shown potential to accelerate the resolution of ulcers, cell proliferation, as well as benefit the quality of skin repair in an additive or synergistic action with PRP.
Verify the effect of PRP and conditioned medium (CM) from MSC on the fibroblast and keratinocytes proliferation and migration in vitro.
MSC were obtained from adipose tissue of the abdominal region. CM was obtained from the culture of MSC in the absence of fetal bovine serum (FBS). A unit of whole blood was centrifuged to obtain PRP. Fibroblasts and keratinocytes were obtained from fragments of eyelid obtained by blepharoplasty. Proliferation assay (n=6) with CCK-8 kit was used to assess the growth of fibroblasts and keratinocytes in the presence of different concentrations of PRP, CM, CM+PRP or FBS. Fibroblasts were subjected to migration assay with PRP, CM, PRP + CM, FBS or medium without stimulation and the percentage of closure from scratch obtained by Image J. Statistical analysis was performed using the Kruskal-Wallis test, with significant result when $p<0.05$. The study is registered in the Platform Brazil (CAAE: 11214513.6.0000.5404).
After 48 h of culture, in accordance with the absorbance readings, there was a significant proliferative stimulus ($p <0.05$) in fibroblasts cultured with 10% of PRP (0.927 ± 0.087), 25% of PRP (0.628 ± 0.104), 100% of CM (0.530 ± 0.026) and PRP 25% + 25% of CM (0.550 ± 0.169) compared with no stimulus (0.368 ± 0.029). In keratinocytes, there was a significant proliferative stimulus in cultures with 25% of CM (0.492 ± 0.049), 50% of CM (0.629 ± 0.069), 100% of CM (0.544 ± 0.025) compared with no stimulus (0.225 ± 0.088). The migration test showed a statistically significant difference ($p <0.05$) on the percentage of closure from scratch by fibroblasts cultured with 10% PRP + 10% CM (17.78 ± 2.64) after 48 h, compared with the group without stimulus (8.25 ± 3.04).
The results of proliferation assays suggest that after 24 h of culture in vitro, fibroblasts and keratinocytes are stimulated to growth in response to stimulatory factors released by MSC from adipose tissue. After 48h, fibroblasts exhibit expansive and migratory behavior in response to exposure to PRP and CM. It is possible to postulate, based on the results obtained in vitro, the therapeutic potential of MSC from adipose tissue in association with PRP on the healing process and re-epithelialization of chronic wounds, so this addictive or synergic action could cooperate for the repair of skin lesions in vivo.

Basic Research

93 - EFFECT OF PHYSICAL TRAINING ON CELLULAR HOMING OF ADIPOSE DERIVED STEM CELLS USED IN CELL THERAPY AFTER ACUTE MYOCARDIAL INFARCTION

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Physical training can cause molecular adaptations that may influence the adipose derived stem cells (ADSC) homing after acute myocardial infarction (AMI).
To analyze the systemic expression of angiogenic and inflammatory factors, stromal derived factor 1 (SDF-1), and the myocardial expression of the receptors CXCR4 and CXCR7 in a murine model of AMI treated with ADSC, whose animals performed or not an exercise training.
40 female spontaneously hypertensive rats (SHR), 150 days old, allocated in 8 groups (n=5): sedentary (S), sedentary SHAM (S-SHAM), sedentary AMI (S-AMI), sedentary AMI stem cell therapy (S-ASC), trained (T), trained SHAM (T-SHAM), trained AMI (T-AMI) and trained AMI stem cell (T- ASC). Trained animals performed a running training during 10 weeks (5 days/week) on an ergometric treadmill (60 to 70% of maximal effort capacity). The groups S-ASC and T-ASC were injected with 2 x 105 ADSC at the moment of the AMI. The blood was collected before, 48 and 96 h after the surgery procedures to analyze endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), angiotensin II (Ang II), myeloperoxidase (MPO) and SDF-1 by ELISA assay. At 96 h the animals were sacrificed to obtain the heart tissue. Multifactorial ANOVA and Fisher's LSD was used for parametric data and Wilcoxon Mann-Whitney when non-parametric; The SDF-1 was analyzed by multiple comparisons adjusted by Bonferroni; $p<0.05$.
So far, the ejection fraction (EF) was reduced in the S-AIM, S-ASC, T-AIM, TAIM groups (38 ± 9; 42 ± 11; 37 ± 4; 47 ± 5%) when compared to S, S-SHAM ,T and T-SHAM groups (61 ± 7; 68 ± 2; 80 ± 2; 87 ± 3%; $P<0.05$). The EF in T-ASC was higher than in the S-AIM and T-AIM groups ($P<0.05$). The Ang II in all groups was reduced when compared to the S group (16 ± 3 pg/ml; $P<0.05$). The association of stem cell therapy with physical training significantly reduced ET-1 value in the T-ASC (23 ± 4 pg/ml) group to a similar value of that found in the T group (17.3 ± 8 pg/ml; $P>0.05$), and it was also lower than in the S-ASC group (37 ± 19 pg/ml; $P<0.05$). The VEGF was reduced in S-SHAM, S-AIM, S-ASC, T-AIM, TAIM and TASC (4 ± 1; 0.5 ± 0.1; 4 ± 1.5; 1.9 ± 1; 4.5 ± 1.2; 3 ± 0.6 pg/ml) when compared to the S and T groups (12 ± 3; 10 ± 3 pg/ml; $P<0.05$). SDF-1 was raised in the S-ASC 48 h (8.7 ± 1.5 pg/ml) after the AMI and it returned to the basal levels 96 h after de procedure (4.3 ± 1.3 pg/ml; $P<0.05$). In T-ASC the values didn't change at 48 and 96 h (8.8 ± 1.5; 8.2 ± 1.9 pg/ml; $P>0.05$). When the basal levels of SDF-1 the T group presented a higher values than the S group (5.1 ± 2.4; 12.8 ± 1.9 pg/ml; $p<0.05$). In 96 h, the SDF-1 was higher in the T-ASC (4.3 ± 1.3 pg/ml) group than in S-ASC (8.2 ± 1.9 pg/ml; $P<0.05$).
The trained animals who received the ADSC presented a reduced lost in the EF after AMI. This result is probably due to a lower inflammatory and vasoconstrictor response besides a raised SDF-1 level.
Apoio: Coordenação De Aperfeiçoamento De Pessoal De Nível Superior – Capes/PROSUP

Basic Research

94 - THE ADIPOSE TISSUE DERIVED STEM CELLS (ASC) CHANGED THE ACTIVATION PROFILE OF IMMUNE SYSTEM CELLS IN SCHISTOSOMA MANSONI ACUTE INFECTION

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Mesenchymal stem cells (MSC) have the ability of self-renewal and differentiation into various mesodermal cell lineages. Recently, it has been observed that MSC have potent anti-proliferative and anti-inflammatory effects in autoimmune and inflammatory diseases as a new strategy for immunosuppression. The control of inflammation in infectious and parasitic diseases by MSC was not evaluated so far.
The present study is aimed to evaluate if the ASC could regulate the inflammation in an experimental infection model of acute *S. mansoni* by immune system activation analysis.
The ASC were isolated from C57BL/6 mice, expanded in vitro and characterized phenotypic and functionally. These cells were injected by the tail vein into C57BL/6 mice (n=5) in two, four, six and eight weeks post-infection with *S. mansoni*. Fifteen and thirty days after the ASC injection, the splenocytes were obtained and lymphocytes activation evaluated by the expression of CD25, CD69, CD28 and CTLA-4 molecules. The IL-2, IFN-γ, TNF-α, IL-17, IL-4, IL-6 and IL-10 cytokine levels were measured in the serum blood by flow cytometry.
The results showed a decrease ($P<0.05$) in TCD4+ regulation as determined by CD25, CD69 and CTLA-4, mainly six weeks post-infection and after fifteen or thirty days post-injection of ASC. Interesting, the cytokine analysis reveled different profile in the IL-2 and IL-4 levels. Both cytokines showed significant levels after eight weeks post-infection and fifteen days post-injection of ASC.
In conclusion, our results shows that the ASC can modulate the immune response in *S. mansoni*, mainly after six weeks post-infection, and suggest that ASC can be evaluated for control the granulomatous reaction in this disease.
Financial support: CNPq and FAPEMIG

Basic Research

95 - ADIPOCITY MESENCHYMAL STEM CELLS (AT-MSCS) ADHERE AND PROLIFERATE ON POLI LATIC ACID SCAFFOLDS: AN OPTION TO ARTIFICIAL ORGAN?

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Regenerative medicine, a confluence of bioengineering and stem cell therapy, as an alternative for tissue and organ damages. Stem cells are indifferentiated cells with great capacity of proliferation, self-renew, differentiation into different lineages, being obtained from many sources. Mesenchymal stem cells (MSCs) have imunomodulatory capacity, and also differentiate into endo, ecto e mesodermal lineages. MSCs are easily obtained from adipose tissue (AT) by liposuction, which makes AT-MSCs an ideal source for cell therapy. Biopolymers, as poli latic acid, (PLA), are structures used as scaffold for tissue engineering, PLA could guide regenerative process without being removed due to their capacity of being absorbable, biodegradable and biocompatible.
This study aimed analyze adhesion and proliferation ability of AT-MSCs on PLLA, a PLA blend, scaffolds.
PLLA was manufactured from L-lactide, by ring opening polymerization. The tablets (0,5mm of diameter and 0,3mm of length) were molded by hot pressing (30', 90℃/130℃ of press temperature and 900PSI of pressure). PLLA was sterilized by ethylene oxid and porosity analyzed by scanning eletronic microscopy (SEM). AT was collected by liposuction. All donors signed free informed consent. AT was submitted to collagenase digestion, cultured (DMEM low glucose medium, SFB) for 3 days. Detached adherent cells, at passage four, were characterized as MSCs by flow cytometry, morphology changes (light and confocal microscopy) and mesodermal lineages differentiation. Genetic instability was analyzed by telomerase enzyme activity and karyotype analyses. AT-MSCs were placed on PLLA and cultured for 7 days. Adhesion and proliferation ability were analyzed by SEM.
PLLA presented about 100um of pore size. Adherent cells were confirmed as MSCs by morphology (fibroblasticd display), and immunophenotype results. Telomerase enzyme activity and karyotype analysis showed no AT-MSCs genetic instability. AT-MSCs were able to adhere and proliferate on the surface and inside of PLLA fully recovered the scaffold.
PLLA was biocompatible with AT-MSCs, being a promising alternative to artificial organs.

Basic Research

96 - EFFECT OF KAEMPFEROL AND PROLACTIN IN MESENCHYMAL-DERIVED STEM CELLS DIFFERENTIATION TO PANCREATIC BETA CELLS

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Type 1 Diabetes Mellitus is an autoimmune disease leading pancreatic beta cells (PBC) destruction and hyperglicemia Pancreas and islet transplantation would be effective, meanwhile immunossupression dependence, organ donation insufficiency is an impairment. Stem cells therapy could be an alternative.
This study aimed analyze whether kaempferol and prolactin when added to PBC differentiation medium could improve viability of PBC derived from mesenchymal stem cells obtained from adipocyte tissue (AT-MSCs).
AT-MSCs characterized by flow cytometry, light and confocal microscopy, differentiation to mesodermal lineage at passage four and submitted to genetic stability analyses were differentiated to PBC. Differentiation protocol accomplished 4 steps, eight different types were performed. Step I (low glucose DMEM, IBMX, FBS, fibronectin during 2days) and Step II (DMEM/F12 with 25 mM glucose, ITS-A, IBMX, fibronectin for1day) were the same in all protocols. Betacelulin, activin-A, kaempferol, prolactin were added alone or combining on step III (DMEM/F12, 5.56 mM glucose, nicotinamide, fibronectin, N2 and B27 supplements, BSA, for 3 days) and Step IV (step III medium and 25mM glucose, for 4 days). Differentiation analyses: morphology (light microscopy), gene expression (RT-PCR). Apoptosis performed by flow cytometry (annexinV/PI).
Morphology changes, gene expression results confirmed AT-MSCs differentiation to PBC. Cells apoptosis were the same at step I and II for all protocols (0.2% and 16.1%). Step III and IV showed different rates.
Kaempferol and Prolactin added to PBC differentiation medium increased cell viability (73.3%), diminishing apoptosis and oxidative stress secondary to high glucose concentrations necessary to PBC differentiation.

Basic Research

97 - STI1 SILENCING ALTERS MURINE EMBRYONIC STEM CELLS GROWTH

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Recent studies have shown that the deletion of Stress Inducible protein 1 (STI1) in mice results in the malformation of embryos after E6.5 and lethality after E10.5, indicating its essential role during the mammal embryonic development. Since pluripotent embryonic stem cells (ESCs) are able to mimic in vitro the early stages of the development, murine ESCs become an ideal model to study the physiological properties of STI1 in embryonic development.
Herein, the main goal is to evaluate the effect of STI1 in cell growth of murine embryonic stem cells, ES-E14TG2A, depleted to STI1. Methods: To evaluate the involvement of STI1 in murine ESC proliferation our group has generated ESCs populations with reduced STI1 expression by the use of shRNA-STI1 lentiviral transduction method. The confirmation of STI1 silencing in these different generated populations was obtained through western blotting analysis. To address the importance of STI1 in the ESCs biology, parental, shRNA-control and shRNA-STI1 populations were used in cell growth curve. The cell growth from four distinct populations was followed by 4 days and the number of cells was counted every day.
Our group has generated one control population (non-target shRNA) and four populations with different levels of STI1 expression – 2 populations with increased STI1 silencing rate (66%) and 2 with decreased silencing rates (38%). The cell growth from these four distinct populations was followed by 4 days and the number of cells was counted every day. Our results have shown that the STI1-silenced populations presented decreased cell growth when compared to parental and control populations.
These data suggest that STI1 could represent a key molecule in the regulation of ESCs cell growth.
This study is supported by FAPESP.

Basic Research

98 - MESENCHYMAL STROMAL CELLS DERIVED MICROVESICLES AS INDUCTORS OF IMMUNE TOLERANCE

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Transplant patients rely on life-long immunosuppressive drugs to prevent T cell activation and graft loss. However, this treatment risks infections and a range of side-effects, which, together with the inexorable chronic allograft injury, limit the graft and patient survival. Mesenchymal Stromal Cells (MSC) have recently emerged as one of the most promising candidates for cell-based immunotherapy due their inhibitory activity toward T cell subsets, dendritic cells (DCs), B cells, and natural killer (NK) cells. Microvesicles (MVs)/exosomes from MSC are one of the main mechanisms by which these cells exert their regenerative/anti-inflammatory actions. In addition, microvesicles trigger their in-vivo and in-vitro effects by RNA delivery to target cells.
In this study, we intend to verify whether MVs from MSC could be a strategy to modulate the immune response in vitro and evaluate their relevance in an experimental model of skin transplantation.
MSC were isolated from adipose tissue of C57BL/6 mice, expanded and characterized as routinely performed in our laboratory. MVs from MSC were obtained by ultra-centrifugation and their modulatory functions were examined in cultured DCs and T cell proliferation assay. In order to investigate the influence of MVs on DCs functions, DC-induced allogeneic T cell proliferation was evaluated in a mixed lymphocyte reaction (MLR). DCs were obtained from bone marrow of Balb/c mice and expanded in the presence of GM-CSF and IL-4. DCs expression of CD11c, CD80, CD86, CD40 and MHC II molecules was analysed via flow cytometry (FACS). Spleen cells from Balb/c mice were stimulated with syngeneic mature DCs in the presence of anti-CD3 antibody in T cell proliferation assay. T cell proliferation was determined by staining with CellTrace Violet and analysed via FACS.
To determine MVs effects on DCs maturation, MVs were added to differentiated immature DCs (at day 6) in the presence of maturation stimulus, LPS, for the last 24 hours. We observed that MVs treatment decreased CD80, CD86 and CD40 expression on DCs harvested on day 7. MHCII expression was comparable on MVs treated-DCs and untreated controls. Consistent with this date, in the MLR experiments, DCs previously treated with MVs showed less capacity of inducing allogeneic T cell proliferation and enhanced the percentage of Foxp3+ T cells (Treg). In order to evaluate the effect of MVs on T cell proliferation induced by anti-CD3 in the presence of syngeneic DCs, MVs were added since the beginning of T cell culture. MVs showed a trend for an inhibitory activity on T cell proliferation.
These results show that MSC derived-MVs can modify the surface phenotype of DCs. MVs treated-DCs have reduced capability to stimulate T cell proliferation and display increased ability to expand Treg. Finally, our data indicate a partial in vitro immunomodulatory effect of MVs on T cell proliferation.

Basic Research

99 - INDUCED PLURIPOTENT STEM CELLS (IPS) FOR CELLULARIZATION OF GELLAN GUM SPONGY-LIKE HYDROGELS AS REGENERATIVE STRATEGY IN CHRONIC KIDNEY DISEASE.

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The exponential increase of patients diagnosed with chronic kidney disease is a major health problem in Brazil and worldwide. Although dialysis and renal transplantation are used to treat this condition in the hope of delaying kidney failure or even stop the progression of the disease, the shortage of donors and the number of complications with immunosuppressive drugs, punctuate the need for alternatives to the patient chronic kidney. Knowing this, one of the major challenges in regenerative medicine is to improve current renal replacement therapies along with the discovery of new approaches for patients suffering from chronic kidney disease. The Tissue Engineering and Cell Therapy are the main fields of this research renal regeneration using scaffolds, in which stem cells are cultured, an ideal location for the shortage of organs for renal transplantation solution. Therefore, this study to meet the huge demand for new treatments for chronic renal lesions, developing innovative strategies for tissue engineering, to be seeded on Gellan Gum Spongy-like Hydrogels (National Invention Patent No 106890), wich also serves as a platform for growing the cells will guide tissue regeneration.
Process and characterize matrix seeded with iPS gellan gum, designed to deploy a larger number of cells with greater plasticity and better able to support or restore impaired kidney function in an experimental model.
Spongy-like hydrogels were obtained, following a patented methodology (Provisional patent 20131000027163), from GG hydrogels and upon re- hydration of a freeze dried polymeric network by dropwise addition of PBS or a cellular suspension prepared in adequate culture medium. Freezing thermodynamics under different freezing temperature and time, as well as after varying the solute amount and type, was analyzed. Differential Scanning Calorimetry (DSC) determined the onset of freezing temperature and the microarchitecture of the dried polymeric networks was characterized by micro-computed tomography (μ-CT). The precursor hydrogels and spongy-like hydrogels were characterized in terms of morphology by cryo-scanning electron microscopy (cryo-SEM), and regarding their water content and mechanical strength. Induced pluripotent cells were seeded in matrices of gellan gum. After 3, 7, 14 and 21 days of culture, cell adhesion was evaluated after phalloidin/DAPI staining, cell viability by calcein/PI and cell proliferation by kit-67staining.
The results of the MTT, calcein, serum alkaline phosphatase indicate that iPS cells were able not only to adhere to the gellan gum membrane, but did so while maintaining the viability and the state of pluripotency. Regarding the characterization of matrix tests were carried SEM as and micro CT.
This methodology is in contrast to traditional methods of cell administration until the time studied and used, giving all higher number of cells in the wound site, and may be an alternative pathway for cell therapy. The domain of this research represent an emerging area and the enormous potential of impact on quality of life of chronic kidney disease.

Basic Research

100 - ULTRASTRUCTURAL CHARACTERIZATION OF SUBPOPULATIONS OF MESENCHYMAL STEM CELL-LIKE FROM HUMAN UMBILICAL CORD

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Mesenchymal stem cells (MSCs) are multipotent cells obtained from a variety of adult and fetal tissues. These cells have been widely used in tissue regeneration. Studies have been suggested umbilical cord as promising for the MSCs isolation. A better understanding of these cell features are of great importance, since in vitro studies have been identifying mesenchymal stem cell-like subpopulations.
The aim of the present study was to identify and characterize subpopulations in cultures of MSCs-like cells obtained from human umbilical cord.
The umbilical cords were collected after delivery of the placenta, at Professor Bandeira Filho Maternity, Recife–PE (Human Ethical Committee -CPqAM nº. 353,807). The MSCs from cord vein were obtained by action of collagenase I, cultured in DMEM supplemented with 20% of fetal bovine serum and 1% penicillin-streptomicin. The cell suspension extracted from the culture in the third passage was fixed in 2% glutaraldehyde, 4% paraformaldehyde in 0.1M Sodium Cacodylate Buffer. After that, the sample was washed with sodium cacodylate buffer (0.1 M), post-fixed with 1% osmium tetroxide and dehydrated in increased concentrations of acetone. Later, it was blocked, ultra sectioned and contrasted. The image acquisition was carried out by transmission electron microscope 100CXII JEOL.
In the ultraestructural images was possible to identify two subpopulations of MSCs-like that was classified as MSCs-like types I and II. Both cells showed cytoplasmic projections and several empty vesicles or filled with material of different electron-density. The type I cells, which showed a small nucleus in relation to cytoplasm, are characterized by an enlarged endoplasmic reticulum, having many empty vesicles and few mitochondria. However, the type II cells had a large nucleus in relation to cytoplasm with evident presence of nucleolus and the cytoplasm was filled with rough endoplasmic reticulum, rich with ribosomes. In type II cells, was found an increased number of mitochondria with different shapes and sizes. Two subpopulations of MSCs-like isolated from human umbilical vein have been characterized.
The results suggest that both subpopulations cells-like may differ in their metabolic activities. The MSCs-like type II seem to have high metabolic activity and it may be related to cell proliferation. Studies to evaluate the therapeutic effect of these different cell populations are required.

Basic Research

101 - A COMPARATIVE STUDY OF BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS FROM DIABETIC AND A NON-DIABETIC RATS

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The worldwide increase in the prevalence of Diabetes mellitus (DM) has highlighted the need for increased research efforts into treatment options for both the disease itself and its associated complications. In this context, an alternative treatment using bone marrow derived Mesenchymal Stromal Cells (MSCs) appear as a new option. MSCs are multipotent cells that can be differentiated in adipocytes, osteocytes and chondrocytes. Additionally, the low immunogenicity and immunomodulatory potential are important properties of this cell type.
1- to compare the in vitro characteristics of MSCs obtained from (i) non-diabetic (MSCc) or (ii) diabetic rats (MSCd); 2- to evaluate the therapeutic potential of these cells to regulate the blood glucose levels in streptozotocin-induced diabetic rats.
In order to determine the MSCc and MSCd phenotype, surface markers were analyzed by flow cytometry. Furthermore, growth kinetic parameters and osteogenic and adipogenic potential were determined for both cell groups. To study the therapeutic potential, male Wistar rats were divided in four groups: Non- diabetic (n-DM), diabetic (DM), diabetic treated with MSCd (DM-MSCd) and diabetic treated with MSCc (DM-MSCc). Diabetic rats were induced by one injection of streptozotocin (60 mg/kg/i.p).
Both groups of cells showed a fibroblast-like shape in culture. But, MSCd showed a higher surface area when compared to MSCc. The typical MSCs surface markers were equally expressed in either MSCc and MSCd. Thus, both groups of cells were positive to CD29 and CD90 and negative for CD34 and CD45. The differentiation potential experiment showed that not only non-diabetic cells, but also diabetic cells were able to differentiate in adipocytes and osteocytes. However, the MSCd differentiation was slower than the non-diabetic ones. Furthermore, growth kinetics showed that MSCc proliferated at much hogher rates than MSCd. Both groups of cells were able to rescue the serum glucose level after one-week of transplantation and this effect was maintained for at least 4 weeks. Additionally, the cell treated diabetic groups showed similar gain of weight when compared to non-diabetic rats.
Our preliminary data shows that even though the MSCs showed slightly different properties in vitro, both were capable to rescue the blood glucose level in diabetic rats.

Basic Research

102 - SPHINGOSINE-1-PHOSPHATE ROLE IN CELL COMMUNICATION BETWEEN PLURIPOTENT STEM CELLS AND KIDNEY CELLS SUBJECTED TO HYPOXIA FOLLOWED BY REOXYGENATION

Juliane L. de Assis (UFRJ); Aline Marie Fernandes (UFRJ); Adalberto Ramón Vieyra (UFRJ); Marcelo E. Lamas (UFRJ)

Renal diseases are incapacitating epidemic disorders that represent a very high burden on the public health system, due to their poor prognosis and need of long and expensive treatments. RDs may be caused by nephrotoxic agents, ischemia/reperfusion and/or systemic inflammation. Human embryonic stem cells (hES) has appeared as an attractive alternative treatment especially due their ability to generate different cells types and also secrete bioactive molecules necessary to tissue repair. Today, a new way of cell-to-cell communication that occurs through microvesicles/exosomes secretion or soluble factors emerged: this mechanism starts differentiation process and induces paracrine responses. Sphingosine-1-phosphate (S1P) is a bioactive lipid involved in renal protection due to its role in tissue regeneration, survival and proliferation. Therefore, we hypothesized that S1P and its receptors might be involved in the crosstalk between stem cells and injured kidney cells which may be a crucial event for tissue regeneration.
Characterize the expression of S1P receptors in kidney cells and in human pluripotent stem cells either in basal conditions, or during respiratory blockade using Antimycin A (AA). We will also explore the possibility that the S1P receptors or their mRNA could be transferred in a crosstalk between pluripotent stem cells and kidney cells by microvesicles/exosomes.
Kidney proximal tubules cells (HK-2) grown in K-SFM medium without serum were subjected to treatment with either AA (0.1, 0.5, 1, 5 and 10 μM) for 1.5 h or incubated in a hypoxia chamber with 1% O2, both followed by 24 h reoxygenation. Protein expression and the presence of S1P receptors were analyzed by Western blotting, RT-PCR and immunocytochemistry. We have also used this methodology to investigate the presence of S1P receptors in hES grown in BRASTEM-2 medium and Matrigel.
We treated HK-2 cells with different concentrations of the drug and we observed less cell death using 1 μM for 1.5 h. Under the same conditions, H9 cell cultures presented a decrease in Ki67 positive cells. Interestingly, when H9 cells were incubated with AA, in the presence of S1P, we found proliferation rates similar to those found in control levels, possible due to S1P positive effect on cell survival. Moreover, we investigated the presence and possible alteration in the S1P receptors in the different culture conditions. We found transcripts encoding all five described isoforms of S1P receptors in H9 cells, confirmed by immunofluorescence and also found in HK-2 human proximal tubule cells.
Our data indicates that both cells have the S1P machinery and the role of S1P is important for cell survival. We propose that S1P can be responsible for tissue recovery, possible via microvesicles/exosomes secretion during the cross-talking between hES and HK-2.
FINANCIAL SUPPORT: CAPES/PROBITEC, CAPES/INL, FAPERJ, MS/DECIT and INCT-INBEB.

Basic Research

103 - MICROVESICULES FROM ADIPOSE-DERIVED MESENCHYMAL STEM CELLS ATTENUATES INJURY OF EXPERIMENTAL FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Rafael Luiz Pereira (Universidade de São Paulo); Raphael José F. Felizardo (Universidade Federal de São Paulo); Marcos Antonio Cenedeze (Universidade Federal de São Paulo); Clarisse Origassa (Universidade Federal de São Paulo); Alvaro Pacheco-Silva (Universidade Federal de São Paulo); Danilo Candido de Almeida (Universidade de São Paulo); Niels O. S. Câmara (Universidade de São Paulo)

Focal and segmental glomerulosclerosis (FSGS) is one of the most important causes of end-stage chronic renal disease. FSGS is a glomerular disease characterized by podocyte (visceral epithelial cells) foot processes effacement and reduced expression of the slit diaphragm-related proteins, an important and selective structure involved on filtration barrier. These alterations lead to development of physiological and histopathological alterations as proteinuria, vascular collapse, mesangial sclerosis and progressive segments deterioration of some glomeruli. In this context, the use of mesenquimal stem cells (MSCs) become a new and accessible approach to treatment of acute and chronic nephropathies. MSCs have important immunotherapeutic potentials, besides their role as effective agents on regulation of gene expression through microvesicles (Mvs). Mvs are loaded of miRNAs, which have abilities to transfer genetic information between MSCs and damaged cells into tissue, leading to a better functional outcome.
So, this study aim to evaluate the anti-inflammatory potential of microvesicles derived from adipose-derived mesenchymal stem cells (MV-AdMSCs) in an experimental model of FSGS in Balbc mice.
First, experimental FSGS was induced by a single intravenous injection of chemotherapic agent adriamycin (10mg/kg), which mimics the classical signs of corresponding human disease (n=5). After, the animals were treated with three consecutive doses of MV-AdMSCs with an objective to evaluate their anti-inflammatory and renoprotective properties.
Our preliminary results showed that the treatment with alogeneic MV-AdMSCs reduced dramatically total urine proteins, including albumin, a marker of glomerular lesion, in addition it also decreased the expression of classical inflammatory markers such as TGF-β e TNF-α. The renal parenchyma and basal lamina were improved after MV-AdMSCs injection. Expression of podocytes markers, such as podocin and nephrin, once reduced in disease, were re-established after MV-AdMSCs treatment. Finally, we observed an increase of expression of Heme oxigenase-1 and its transcription factor Nrf2 which we suggest be a possible way involved at regulation of Mvs.
We expect to demonstrate that one of paracrine mechanisms involved in FSGS tissue repair by MSCs, can involve miRNA expression, clarifying the repairing mechanisms and open new approaches to an efficient clinical practice.

Basic Research

104 - ULTRASTRUCTURAL COMPARATIVE ANALYSIS OF MESENCHYMAL STEM CELLS FROM UMBILICAL CORD AND ADIPOSE TISSUE OF HUMANS

Camila Carvalho (Centro de Pesquisas Aggeu Magalhães - FIOCRUZ.); Leyllane Moreira (Centro de Pesquisas Aggeu Magalhães - FIOCRUZ.); Fábio Santos (Centro de Pesquisas Aggeu Magalhães - FIOCRUZ.); Luiz Alves (Centro de Pesquisas Aggeu Magalhães – FIOCRUZ.); Rafael Padilha (Laboratório de Imunopatologia Keizo Asami – UFPE.); Pedro Pita (Serviço de Cirurgias Plásticas do Hospital das Clínicas - UFPE.); Silvia Montenegro (Centro de Pesquisas Aggeu Magalhães – FIOCRUZ.); Sheilla Oliveira (Centro de Pesquisas Aggeu Magalhães - FIOCRUZ.)

Mesenchymal stem cells (MSCs) are easily isolated, can be expanded in culture and have the property to differentiate in several cell types, making it a good tool for cell therapy. MSCs are mainly obtained from bone marrow, however new sources have been suggested, among them the umbilical cord and adipose tissue. The cells obtained from these tissues have shown therapeutic potential for tissue regeneration. Better morphological characterization of these cell populations is very important once the identification of phenotypic aspects can help to understand cell biological characteristics, making MSCs terapy safer.
Our aim was to analyze and compare the morphological aspects of the MSCs umbilical cord vein and adipose tissue of humans.
The umbilical cord vein was obtained from parturient (normal delivery or cesarean), at Professor Bandeira Filho Maternity, Recife–PE, and adipose tissue from abdominal region of patients after a plastic surgery liposuction in Esperança Hospital, Recife–PE (human ethical committee-CPqAM nº. 353,807). MSCs from cord vein and adipose tissue were acquired by action of collagenase I. In the third passage, the cell suspension was extracted and fixed in 2% glutaraldehyde, 4% paraformaldehyde and 0.1M sodium cacodylate buffer 0.1 M. The images acquisition was carried out by Scanning Electron Microscopy (SEM) JEOL JSM-5600 LV.
Both cell populations displayed cytoplasmic connections. However, morphological differences were observed. In MSCs obtained from umbilical cord, was identified the presence of many filopodia, rough surface showing pore-like structures. MSC adipose tissue, however, had long pseudopods with presence of the vesicles-likes structures.
MSCs obtained from umbilical cord vein showed distinct morphologies in comparison to adipose tissue. However, further characterization of these cells with respect to the application potential in regenerative medicine is required.

Basic Research

105 - DEXAMETHASONE INTERACTION WITH HUMAN MESENCHYMAL STEM CELLS STRUCTURE AND BEHAVIOR

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Glucocorticoids and immunosuppressive drugs are commonly used to treat inflammatory disorders, such as Inflammatory Bowel Disease (IBD), and despite few improvements, there is a need for novel therapeutic approaches. Mesenchymal Stem Cells (MSCs) have emerged as regulators of the immune response, secreting a range of cytokines and controlling inflammation. However, little is known about MSCs and drug interaction, because cell therapy could be affected.
The aim of this study was to evaluate the effect of Dexamethasone (DEX) in MSCs viability, nuclei morphology, cell polarity, F-actin distribution and cell migration.
After initial characterization, MSCs were treated with DEX (10 µM) for 24 hours, 48 hours or 7 days. MSCs viability was observed by MTT assay and nuclei morphology was analyzed by DAPI staining followed by NMA plugin. Cell polarity was assessed by Polarity Index and F-actin distribution with confocal images after Rhodamine-Phalloidin staining. Time-Lapse Microscopy was used to cell migration analysis.
DEX had no effects on cell viability or nuclei morphology. However, this glucocorticoid induced a more rounded cell shape with high presence of actin stress fibers and decreased lamellipodia formation. DEX also diminished cell directionality and migration speed in 24 hours and 7 days (-28.69% and -25.37%, respectively; $P < 0.05$, $n = 4$).
Our data shows that DEX can affect MSCs morphology and migration capacity, indicating that cell therapy in IBD may be altered by this immunosuppressive drug. More studies are necessary to determine if DEX can alter MSCs homing to the injury site.
Financial support: FIPE/HCPA – CAPES.

Basic Research

106 - THE EFFECT OF DELETION OF GD3 SYNTHASE ENZYME IN THE SUBVENTRICULAR ZONE OF ADULT MICE

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Gangliosides are glycosphingolipids that contain sialic acid and are present in the plasma membrane, with high concentrations in the central nervous system. The ganglioside 9-O-acetyl GD3 (9acGD3) expression correlates spatiotemporally with cell migration and axonal extension during development. In adult rodents, this ganglioside is no longer expressed in most regions of the central nervous system. Interestingly, 9acGD3 expression remains in the subventricular zone (SVZ), one of the few neurogenic regions in the adult.
The aim of this study is to evaluate the influence of the deletion of the enzyme GD3 synthase in SVZ neurogenic niche.
In this study we used wild type (WT) adult male mice 129 SvEv and knockouts for GD3 synthase enzyme (GD3S KO).
To analyze neurogenesis, we dissected the SVZ of WT and GD3S KO mice and compared the neurogenic potential of this region through neurosphere formation assay. The assays were carried out during seven days in culture in CO2 incubator in the presence of growth factors EGF (0.02 µg/ mL) and FGF-2 (0.01 µg/ mL).
To test the differentiation potential of the cells from both genotypes, the neurospheres derived from the SVZ cells were mechanically dissociated and plated in neurobasal medium without EGF and FGF-2. After five days in CO2 incubator the differentiated cells were fixed for analysis by immunocytochemistry and fluorescence microscopy.
After seven days in culture, we observed that the cells of both genotypes are capable of forming primary and secondary neurospheres, demonstrating the ability of self-renewing cells from the SVZ.
The GD3S KO primary neurospheres are significatively larger in diameter and are greater in number when compared to the neurospheres from WT mice (mean diameter: WT- 98.9 ± 3.2 mm; $p< 0.05$; n= 42/ GD3S KO- 110.6 ± 3.4 mm; $p< 0.05$; n= 51. Total number: WT- 10.7 ± 1.19 mm; $p< 0.05$; n= 24/ GD3S KO- 20.9 ± 1.6 mm; $p< 0.05$; n= 29). The withdrawal of growth factors promoted the differentiation of spheres into astrocytes and neurons, in both genotypes.
The neurospheres derived from the SVZ cells of GD3S KO mice have a higher percentage of differentiation into astrocytes and neurons when compared to neurospheres derived from the SVZ cells of WT mice (mean number of cells differentiated into astrocytes: WT- 21.1; n= 2/ GD3S KO- 23.7; n= 3. Mean number of cells differentiated into neurons: WT- 1.9; n= 2/ GD3S KO- 8.0; n= 3).
In this study, therefore, we conclude that adult mice lacking the enzyme GD3 synthase have greater number of progenitors / neural stem cells in the SVZ.

Basic Research

107 - ANALYSIS OF MOLECULES OF THE CELL HOMING IN STERNAL BONE MARROW OF PATIENTS WITH VALVULAR OR ISCHEMIC HEART DISEASE

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Cell homing is the mechanism by which a lesion releases signaling molecules (chemokines) that cause recruitment, proliferation and differentiation of progenitor cells, to regenerate damaged tissue. Stromal Cell-Derived Factor-1 (SDF-1) is the main molecule involved in this process. The receptors of this chemokine, CXCR4 and CXCR7, expressed on the plasma membrane as response to chemoattractant gradient, may contribute differently to SDF-1-mediated responses in different cardiopathies. Objective: To analyze the homing in bone marrow mononuclear cells through the expression of SDF-1 and its receptors, CXCR4 and CXCR7.

Bone marrow (BM) samples were collected by aspiration of the sternal bone in patients with ischemic (IHD, n = 41) and valve (CV, n = 30) heart disease, submitted to cardiac surgery. Control group (C, n = 9) was composed of healthy BM donors. The plasma was used for systemic SDF-1α analysis, by ELISA assay. The RNA was extracted and the cDNA obtained was used to analyze the CXCR4 and CXCR7 gene expression by quantitative PCR. Additionally, the presence of CXCR4 (CD184) positive cells was evaluated by flow cytometry.

SDF-1α level in the CI group was slightly reduced compared to the levels of the CV and C groups, but without significant difference (p = 0.483). A different pattern of receptor mRNA expression between the groups was observed, once the CV showed higher expression of CXCR4 (p = 0.071) and CXCR7 (p = 0.082). However, regarding the level of CXCR4+ cells present in BM, no significant difference was observed between groups (p = 0.36).

Although there is an unfavorable clinical condition of patients with ischemic heart disease in relation to cell homing, as soon as SDF-1α-CXCR4/CXCR7 signaling appears to be most affected in this study, valvular cardiac patients showed good profile among the molecules involved in the signaling of this mechanism. This fact can be considered in protocols that make use of mononuclear stem cells from bone marrow to cardiac diseases.

Basic Research

108 - ISOLATION AND CHARACTERIZATION OF UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS COMPARING ENZYMATIC AND EXPLANT PROCEDURES

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Mesenchymal stem cells (MSC) are multipotent cells with ability to proliferate, self-renew, and differentiate into many cell types. Minimal criteria determine that human MSC must be plastic-adherent, exhibit a specific cell-surface expression profile, and differentiate into osteocytes, adipocytes, and chondrocytes in vitro. MSC have a limited lifespan in vitro, with progressive reduction in capacity for self-renewal leading to irreversible arrest of cell division, which limits its use for therapeutic purposes. Bone marrow is deemed the “gold standard” for MSC derivation and use in clinical trials and graft versus host disease treatment. However, umbilical cord (UC) wall MSC are easy to collect and process, and proliferate rapidly in culture, but information on the variability of individual cell samples impacting upon in vitro expansion and aging processes is still needed.

We proposed to compare three different methods for MSC isolation from human UC and determine which protocol yields the highest number of viable cells with the best proliferation capacity.

Three different protocols were tested. Two were enzymatic procedures: protocol i (n=3) consisted in filling the umbilical vein with type 1 collagenase and protocol ii (n=5) included cutting the cord into 1cm pieces for digestion with collagenase. In the explant method, protocol iii (n=5) the cord was also cut, but into very small 1-2 mm pieces and transferred onto culture plates. Isolated cells were evaluated in terms of proliferation, differentiation capacity, and phenotype. Depending on the size and quality of the 8 UC studied, samples were processed using one or more protocols.

After passage 2 adherent cells were observed as a homogeneous population consisting of spindle-shape fibroblastoid cells identified as MSC. The cells obtained from all 3 protocols were able to differentiate into adipocytes and osteocytes and displayed the same standard phenotypic characteristics of MSC. However, our results show that isolating MSC from Wharton’s jelly (ii) is more advantageous than treating the UC vein (i). Furthermore, though neonatal, MSC exhibit different population doubling rates and reach senescence in different passages, irrespective of the protocol employed. Cells obtained from explants (iii) presented similar characteristics to cells isolated using enzymatic protocols, but always reached proliferation arrest earlier, irrespective of initial population doubling times. From the same UC, cells obtained with protocol ii reached later passages while exhibiting shorter doubling times in culture than cells from other protocols, that is, took longer to reach senescence.

We conclude that though population doubling time varies individually irrespective of the protocol used, mincing the cord and treatment with collagenase (protocol ii) yields the best MSC, which proliferate longer and reach later passages before becoming senescent.

Basic Research

109 - INFLAMMATION RESPONSE AFTER ARTERIAL INJURY IN ATHEROSCLEROTIC MICE TREATED WITH EPC IN THE PRESENCE OR NOT OF GLYCOSAMINOGLYCAN.

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Vascular interventions can damage endothelium affecting its regular functioning and regeneration. Cellular therapy using endothelial progenitor cells (EPC) originated from mononuclear (MNC) bone marrow cells can promote re-endothelization. Dermatan sulfate (DS) is an antithrombotic glycosaminoglycan that can have anti-inflammatory activity and inhibits thrombus formation.
The objective of this work is to verify if the treatment with EPC alone or with DS can inhibit the initial inflammatory response promoted by arterial lesion in apolipoprotein deficient mice (ApoE-/-).
We induced carotid artery lesion using a wire guided probe and intravenously administered EPCs obtained from C57BL6 mice bone marrow alone or with DS [20 mg/kg]. We analyzed molecules involved in adhesion (ICAM-1), leukocyte homing (P-selectin), vascular tone (eNOS) and stem cell migration (SDF-1) by western blotting and also analyzed apoptosis (TNF-alpha), extracellular matrix metabolism (TGF-beta) and anti-atherogenic cytokine (IL-10) by ELISA all experiments were done 1 and 3 days after lesion.
Treatment with DS alone was not efficient in decrease the expression of molecules such as ICAM-1 and P-selectin; it increased SDF-1 levels in plasma but this increase was not observed at the site of injury. Molecules like TGF-beta and TNF-alpha increased systematically. EPC alone was not able to avoid an increase in the expression of P-selectin, it increased the amount of IL-10 on plasma and it also decreased ICAM-1 expression. The treatment with EPC and DS presented the same results as EPC alone but failed to increase IL-10 levels. The arterial lesion caused the decrease of eNOS expression and no treatment was able to rescue it.
We can conclude that the treatment with EPC was capable to avoid a step in the leukocyte adhesion to the arterial wall and at the same time increased the amount of the anti-atherogenic molecule IL-10.
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Basic Research

110 - ENDOTHELIAL CELLS INCREASE THE OSTEOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED STEM CELLS COMBINED WITH CALCIUM PHOSPHATE SCAFFOLDS

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Adipose-derived stem cells (ASCs) have shown great potential for bone tissue engineering, particularly when combined with biomaterials such as calcium phosphate or hydroxyapatite. However, more studies are needed to develop the ideal combination of biomaterial/cellular components for bone repair.
The aim of this study was to evaluate the role of endothelial cells (ECs) co-culture with ASCs combined to calcium phosphate cement for osteogenesis in vitro.
ASCs were isolated from adipose tissue of Lewis rats by collagenase digestion and characterized for surface markers, cell proliferation and differentiation. Cells between passages 4 and 7 were associated with 3D scaffolds of calcium phosphate, with or without ECs of the EOMA line. Cell adherence was evaluated by counting non-adhered cells stained with Giemsa. Proliferation rates of ASCs in 2D and 3D conditions after 3-day incubation was determined using the MTT test. The osteogenic potential of ASCs combined with the calcium phosphate cement, with or without addition of EOMA cells, was determined by real-time RT-PCR analysis of the expression of bone marker genes.
Rat ASCs showed morphology, immunophenotype, and proliferation and differentiation potential characteristic of mesenchymal stem cells. Adherence to the calcium phosphate scaffolds was close to 100%, in the two cell concentrations analyzed. ASCs proliferated less when associated with the scaffolds than in conventional 2D conditions. However, expression of bone markers was enhanced by cultivation of ASCs in association with calcium phosphate cement, in normal or osteoinductive media. The addition of endothelial cells to the system increased significantly the expression of bone marker genes, particularly when osteoinductive media was used in the cultures.
These results show that the osteogenic potential of adipose-derived stem cells cultivated with calcium phosphate cement is increased by co-cultivation with endothelial cells. This co-culture system is relevant for the implementation of bone tissue engineering processes for clinical applications.
Acknowledgments: This work was supported by MCTI/CNPq/MS Brazil.

Basic Research

111 - THE RECK TUMOR SUPPRESSOR GENE SPLICING VARIANTS AND THEIR EXPRESSION PROFILE DURING HUMAN SKIN MESENQUIMAL STEM CELLS OSTEOGENIC AND ADIPOGENIC DIFFERENTIATION

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Cellular interaction with the extracellular matrix (ECM) is essential for different processes, such as: growth, migration, and differentiation. Matrix metalloproteinases (MMPs) are zinc-dependent enzymes which, in association with their inhibitors, are crucial for ECM remodeling. During osteogenesis and adipogenesis, MMP-2 and MMP-9 activities are known to be essential. Among the characterized MMP inhibitors, the REversion-inducing Cystein-rich protein with Kazal motifs, RECK, gene stands out. Only one RECK transcript has been characterized in the literature so far. The canonical RECK protein is anchored to the membrane by a glycosylphosphatidylinositol (GPI) anchor and has been shown to be involved with inhibition of MMP-2, MMP-9 and MT1-MMP activities.
Our aim is to identify and characterize RECK alternative splicing variants and analyze their expression profile during human skin mesenquimal stem cells osteogenic and adipogenic differentiation.
To this end, splice variants of the RECK tumor suppressor gene were identified by Expressed Sequence Tag (EST) analysis, isolated by RT-PCR, sequenced and cloned. Human skin mesenchymal stem-cells were submitted to osteogenic or adipogenic differentiation protocols. RNA was extracted from these cells at three different time periods of differentiation, namely: 7, 14 and 21 days. RECK transcripts expression was assessed by qRT-PCR.
Three novel alternatively spliced variants of the RECK tumor suppressor gene were identified and characterized. Our data show that the RECK canonical transcript has a higher expression compared to its alternative variants at early stages of the differentiation protocols. However, during late differentiation stages the balance between transcripts expression switches, with the alternatively spliced variants displaying higher expression levels, when compared to the canonical transcript.
Our results suggest that the balance of RECK transcripts expression may play an important role in osteogenesis and adipogenesis.
Financial support: BNDES, CAPES, CNPq, FAPESP, FINEP, MCTI and MS-DECIT.

Basic Research

112 - METABOLIC PROFILE ANALYSIS OF ADULT STEM CELLS DERIVED FROM ADIPOSE TISSUE

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In contrast to differentiated cells, many stem cells appear to rely to a greater extent on glycolysis than on oxidative phosphorylation to generate energy. Bioenergetics studies have revealed that human ESCs depend, in a large part on glycolysis for ATP production. Consistently, mitochondria are less complex and fewer in number in human ESCs than in their differentiated progeny. Could adult stem cells have the same characteristic? Or could a shorter differentiation period change this profile? Here we used adult stem cells and adipogenic differentiation model to address these questions.
The objective of this work is to find if human adipose tissue-derived stromal cells (hASC) can alter the metabolic profile after a brief period of differentiation induction.
hASCs from at least three patients were isolated, cultured, and characterized as previously described by our group. For adipogenic differentiation, hASCs were treated with induction medium containing the adipogenic inducers and maintained during 3 and 7 days. To verify if a minimum period of differentiation stimuli is already able to change oxidative and energetic metabolic profile, we first analyzed the mitochondrial number and membrane potential using the Mitotracker red and Rhodamine 123 green (Life Technologies®) respectively. Reactive oxygen species (ROS) generation was assessed using 2',7'-dichlorofluorescein diacetate (DCFH-DA) probe (Sigma-Aldrich®). Fluorescence was analyzed according to standard procedures using a FACSCanto flow cytometer (Becton-Dickinson - BD®). Thereafter we monitored oxygen consumption by high-resolution respirometry with an Oxygraph-2k (OROBOROS INSTRUMENTS, Innsbruck, Austria).
These assays showed us that 3 and 7 days of adipogenic induction may enhance mitochondrial membrane potential, indicating organelle maturation. Mitochondrial number and ROS production did not change after stimulus. Only cells that remained for 7 days under induction showed an increase in oxygen consumption.
The analysis of membrane potential showed us that three days of stimulus are sufficient to alter the metabolic profile of adult stem cells. This period is enough to stimulate mitochondrial activity. However, 7 days of induction are required to stimulate the cells to consume more oxygen, indicating a transition from glycolytic to oxidative metabolism. Thus we can conclude that a minimum period of differentiation can stimulate mitochondrial activity but this organelle needs more time to accomplish the oxidative phosphorylation more efficiently.
FUNDING SUPPORT: CNPq/CAPES/Araucaria Foundation/FIOCRUZ

Basic Research

113 - GENERATION OF INTERNEURONS FROM HUMAN PLURIPOTENT STEM CELLS

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The cerebral cortex comprises two major neuronal cell types: the excitatory glutamatergic neurons and the inhibitory GABAergic interneurons (~20% of the entire neocortical neuronal population). GABAergic interneurons are highly heterogeneous and supposed to be affected in schizophrenia, autism, epilepsy and Parkinson's disease. The generation of this specific neuron derived from human pluripotent stem cells is wishful for disease modeling and also as a tool for the discovery of new therapies for brain disorders. So far, no published protocol was efficient enough to generate a significant number of GABAergic interneurons from human pluripotent stem cells. Moreover, those protocols result on a low percentage of GABAergic interneurons even after 3 to 7 months.
Our goal is to develop a more efficient and less time-consuming protocol to generate GABAergic interneurons from human pluripotent stem cells.
Neural differentiation of human embryonic cell line (BR1) was performed in three stages. The first one is characterized by combined dual-SMAD inhibition and canonical Wnt pathway inhibition. The second stage is marked by induction of ventral fates through activation of Shh pathway. Finally, the third stage is related to neuronal maturation with trophic factors.
We obtained a cell culture presenting calbindin and parvalbumin positive GABAergic interneurons.
After ~ 3 months we were able to obtain a culture enriched in human GABAergic interneurons. These human cells will be used to model neurological and psychiatric disorders in vitro, including schizophrenia.
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Basic Research

114 - ALTERED EXPRESSION OF MIR-29A AND YOUR POSSIBLE TARGET, KCNIP2, IN DIABETIC PATIENTS

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MicroRNAs (miRs) are small RNAs that exert negative regulation of gene expression by inhibiting protein synthesis and are involved in various physiological and pathophysiological processes, including type 2 diabetes mellitus (DM). DM is the most prevalent metabolic syndrome worldwide. The characteristic profile of DM, comprising high levels of glucose, lipids and circulating inflammatory cytokines, leads to a number of vascular complications, such as coronary artery disease (CAD), which can culminate in acute myocardial infarction, and finally, heart failure (HF).
Since the molecular mechanisms involved in these events are still unclear, this study aimed to assess changes in miR expression in diabetic patients with or without HF and to look for possible targets of these miRs.
First, patients with CAD (CAD group), patients with CAD and DM (CAD+DM group) and patients with CAD, DM and HF (group CAD+DM+HF) had their clinical profiles assessed. Then, samples of heart tissue of these patients removed during coronary artery bypass grafting were analyzed by qPCR for relative quantification of 20 miRs (miR-1, miR-7, miR-9, miR-15a, miR-15b, miR-16 , miR-21, miR-29a, miR-34a, miR-126, miR-133a, miR-145, miR-185, miR-192, miR-200a, miR-208a, miR-208b, miR-210, miR -499 and let-7b), with CAD group being the control group. Finally, we used the database TargetScan to select possible target mRNAs of miRs found to be significantly altered ($p<0,05$). Target mRNA levels were also analyzed by qPCR. The statistical test used was One Way ANOVA with Bonferroni post-test for multiple comparisons.
The groups analyzed showed no differences in sex, age, body mass index, number of patients with hypertension or dyslipidemia. A significant difference was identified only in ejection fraction (CAD+DM+HF x CAD**** and CAD+DM+HF x CAD+DM***). Our findings for the expression of miRs was that miR-15a and -29a were downregulated in CAD+DM group when compared to the CAD group; miR-7 and -192 were upregulated in CAD+DM+HF group compared to the CAD group; and miR-16 and -126 were up regulated in group CAD+DM+HF relative to CAD+DM group. For miR-185, CAD+DM and CAD+DM+HF groups were upregulated when compared to CAD group, and miR-21 and let-7b were upregulated in CAD+DM+HF group in relation to the two other groups. The targets selected were transcripts of KCNIP2, KCNA5 and KCNJ2 genes, whose deregulation is associated with arrhythmias and atrial fibrillation. By qPCR, we found that KCNIP2, target of miR-29a, was altered in CAD+DM group, as expected, indicating a possible mechanism by which diabetes promotes electrical changes in the heart.
Our results indicate that the effects caused specifically by diabetes or by diabetes associated with HF are derived from different molecular pathways, since different miRs are involved in each case. Also conclude that KCNIP2 transcript is a potential target of miR-29a and both are changed analogously to the presence of diabetes.

Basic Research

115 - EMBRYONIC STEM CELL DIFFERENTIATION INTO INSULIN-PRODUCING CELLS (IPCS): FUNCTIONAL CHARACTERIZATION OF THE PURKINGE CELL PROTEIN 4 (PCP4) GENE IN THIS PROCESS

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Alternative cellular sources for type 1 Diabetes mellitus treatment have been previously investigated, the most promising of which seems to be the insulin producing cells (IPCs), obtained by stem cells differentiation. Some reports show that murine embryonic stem cells (mESCs) are able to form islet-like clusters, however, their insulin production is insufficient to render diabetic mice normoglycemic. This work aims at developing an adequate protocol for generation of IPCs by searching for new genes which may be involved in the pancreatic organogenesis process.
Early on during mESCs differentiation into IPCs, we observed the presence of progenitor cells, which were able to express pancreatic β-cell markers. At the end of the differentiation process, the islet-like clusters positively stained for the insulin-specific dithizone, were able to express proinsulin, PDX1, GLUT2, PAX4 and other β-cell markers. These clusters were microencapsulated in a novel biopolymer (Bioprotect®) generated and deposited as a patent by our group, and subjected to in vivo maturation by introducing them into normal animals. A dramatic increase in expression was observed for the above mentioned genes, indicating complete maturation of the differentiated cells. Moreover, when these encapsulated cell clusters were transplanted into diabetized mice, the animals achieved and maintained normoglycemia during 60 days. In addition, at day 42 after transplant, the glucose tolerance test (OGTT) response displayed by these animals was more similar to that of normal mice than to the control diabetized not treated mice ($p<0.05$).
In parallel, we compared undifferentiated mESCs to IPCs using a microarray platform and selected some of the upregulated genes for functional analysis. One of these genes is the Purkinje cell protein 4 (Pcp4), which is 1,000 times more expressed in IPCs than in undifferentiated mESCs. We adopted a functional genomics approach to investigate the role played by the Pcp4 gene in β-cells and in β-cell differentiation, by overexpressing and knocking down this gene in MIN-6 cells and mESCs. Overexpression of Pcp4 in MIN-6 did not interfere with the expression of the genes analyzed. On the other hand, Pcp4 knock-down caused increased cell growth rates, with increased doubling time and decreased cell viability. In addition, over-expression of Pcp4 in mESCs subjected to differentiation into IPCs apparently increase the expression of genes related to β-cell differentiation, such as the Isl1 gene (p
In conclusion, we developed a new protocol for ESCs differentiation into IPCs, which generates cell clusters that are able to revert diabetes in diabetized mice, and we also describe here, for the first time, that the Pcp4 gene is expressed in pancreatic β-cells, being possibly related to β-cell maturation and maintenance of their viability.
Support: BNDES, CNPq, FAPESP, MCTI, MS-DECIT.

Basic Research

116 - MORPHOLOGICAL COMPARISON OF MESENCHYMAL STEM CELLS OBTAINED FROM BONE MARROW AND HEPATIC STELLATE CELLS FROM MURINE MODEL

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The mesenchymal stem cells (MSCs) have emerged as a cell population with low immunogenicity and immunomodulatory capacity, being an alternative in the field of cell therapy. Direct relationship of MSCs with the pericytes has been suggested. The pericytes are present in blood vessel walls and as the MSCs, have potential to differentiate into multiple lineages. Hepatic pericytes are recognized as hepatic stellate cells (HSCs), which play a key role in fibrogenesis. Thus, a better knowledge of MSCs and HSCs is very important for studies that aim to use cell therapy in liver diseases.
So it is our aim to compare the morphological features between MSCs and HSCs obtained from uninfected and Schistosoma mansoni infected mice, respectively.
For this purpose, the study was performed with C57BL/6 mice (animal ethics committee license number 032/2011- CPqAM), which were divided into two groups: uninfected and S. mansoni infected mice. The MSCs were acquired from uninfected mice bone marrow and the HSCs were obtained from infected mice. The cells were cultured in DMEM supplemented with 20% of fetal bovine serum and 1% penicillin-streptomicin. The morphological characteristics of the cells were evaluated by Scanning Electron Microscopy (SEM) 100CXII JEOL.
In culture, it was possible to observe fibroblastoid morphology in both cell populations. However, MSCs had longer and thinner cytoplasmic extensions. Besides presenting short extensions cytoplasmic the HSCs had star shape. Both cell populations possessed plastic-adherent capacity. The findings observed in cell culture were confirmed by SEM.
MSCs and HSCs showed differences regarding to morphology and plastic-adherent capacity. These results suggest a possible distinction between these cell populations, generating a perspective of new studies.

Basic Research

117 - TISSUE-ENGINEERED IN VITRO: RECELLULARIZATION OF WELL-PRESERVED ACELLULAR KIDNEY SCAFFOLD USING PORCINE PROXIMAL TUBULE CELL LINE

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The extracellular matrix (ECM) is produced by the resident cells of each tissue and organ and is in a state of dynamic equilibrium with its surrounding microenvironment. It has been postulated that only organ-derived intact ECMs can be carefully decellularized to retain the innate matrix constituents needed for promoting cell adhesion, migration, proliferation, differentiation, and regulation of intercellular signaling.
The goal of this study was the production of a decellularized kidney scaffold with structural and mechanical properties necessary for engineering basic renal structures in vitro.
All animal procedures were approved by the Institutional Animal Care and Use Committee (CCS UFRJ - Macaé). The rats were sedated and anesthetized with 10 mg/kg xylasine and 100 mg/kg ketamine solution. Following sedation and anesthesia, 200 UI of heparin solution was intraperitoneally administrated to avoid blood clotting. After 5 minutes a laparotomy was performed for kidney excision. The kidneys were washed twice with PBS followed by decellularization with 1% SDS for 15 hrs followed by perfusion for 15 minutes with phosphate buffered saline solution. The kidney extracellular matrix (ECM) was fixed in 4% paraformaldehyde (PF) at room temperature for 3 days and embedded in paraffin or stored in antibiotic-antimicotic solution in PBS to use as scaffolds for cell culture. The porcine proximal tubule cell line (LLC-PK1) was expanded in DMEM medium supplemented with 5% FBS, 100 U/ml penicillin, 100 U/ml streptomycin at 37℃ in 5% CO2. The LLC-PK1 cells were plated in ECM recovered from decellularized kidney, after antibiotic-antimicotic treatment, using the medium described above. 3 and 14 days after culture initiation, partially recellularized ECM were fixed in 4% PF and embedded in paraffin for histochemical analyses with H&E, periodic acid-Schiff (PAS) and picrosirius.
After 15hrs of decellularization process, the kidneys became translucid. Qualitative analysis of decellularized tissues with 1% SDS at room temperature revealed well-preserved architecture and demonstrated the absence of cell nuclei, confirming removal of intact cells. The detergent-based perfusion protocol successfully produced acellular kidneys that retained the web-like basement membrane. HE and PAS staining confirmed the removal of cellular material. After LLC-PK1 recellularization procedure cell adhesion on renal ECM was observed. H&E and PAS staining showed cell adhesion to the decellularized kidney ECM.
These findings demonstrate that decellularized kidney sections retain critical structural properties necessary to use as a three-dimensional scaffold and promote cellular recellularization. This study provides the initial steps in developing strategies for renal tissue engineering and repair.

Basic Research

118 - EFFECT OF LASER THERAPY ON ADIPOSE-DERIVED STEM CELLS SUBMITTED TO CRYOPRESERVATION

Fernanda Ginani (UFRN); Diego M. Soares (UFRN); **Mardem P. e V. Barreto (UFRN);** Carlos Augusto G. Barboza (UFRN)

The aim of this study was to evaluate the effect of low-level laser irradiation (LLLI) on the proliferation and viability of murine adipose-derived stem cells previously submitted to cryopreservation.
Fragments of adipose tissue were extracted from the inguinal region of a male Swiss mouse, two-months old, and kept in a phosphate buffer. The specimens were submitted to three washes with alpha-MEM medium supplemented with antibiotics and then an enzymatic digestion was performed with collagenase I for 1 h at 37℃. After processing, the cells were cultured in α-MEM supplemented with antibiotics and 10% fetal bovine serum (FBS) in a humid atmosphere with 5% CO2 at 37℃. In order to confirm the multi-lineage differentiation potential of adipose-derived cells, aliquots of cells were cultured in osteogenic, chondrogenic, or adipogenic differentiation media for up to 21 days. In the first passage (P1), the cells were submitted to cryopreservation in FBS with 10% dimethylsulfoxide (DMSO), with gradual decrease of temperature (2 h at 4℃, 18 h at -20℃ and then kept at -80℃) and stored for 30 days. Next, the cells were thawed and maintained in culture. After the third passage (P3) cells were submitted to irradiation with an InGaAIP diode laser (wavelength 660nm, power 30 mW), using two energy densities (0.5 J/cm2 and 1.0 J/cm2). A new irradiation was performed 48 h after the first one, using the same parameters. A control (non-irradiated) group was kept under the same experimental conditions of culture. The evaluation of cell proliferation and viability was performed by Trypan blue exclusion method and MTT assay at intervals of 0, 24, 48 and 72 h after the first irradiation.
The irradiated groups showed an increased cell proliferation when compared to the control group at intervals of 24, 48 and 72 hours ($p<0.05$). The mitochondrial activity, measured by MTT assay, showed similar results to cell counting by Trypan blue assay, with the irradiated groups (0.5 and 1.0 J/cm^2) showing a significantly higher activity of MTT at intervals of 48 and 72 h when compared to the control group ($p<0.05$).
It can be concluded that LLLI has stimulatory effects on the proliferation of adipose-derived stem cells submitted to cryopreservation.

Basic Research

119 - LOW LEVEL LASER IRRADIATION INDUCES PROLIFERATION OF DENTAL PULP STEM CELLS

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The aim of this study was to evaluate the effect of low level laser irradiation (LLLI) on the proliferation of dental pulp stem cells (DPSCs).
Human DPSCs were isolated from two healthy third molars extracted due to surgical indication. The multipotential nature of the cells was confirmed by expression of stem cell surface markers CD105, CD73, and CD90 by flow cytometry and conversion into osteogenic and adipogenic phenotypes after culture in differentiation medium. In the third subculture, the cells were irradiated or not (control), with a laser diode InGaAlP, power of 30 mW, wavelength of 660 nm, continuous action mode, and using two different energy densities (0.5 and 1.0 J/cm2). The cells were irradiated at 0 and 48 h, with the laser probe fixed perpendicular to each plate at a distance of 0.5 cm from the cells. Cell proliferation and viability was evaluated by Trypan blue exclusion method and measuring mitochondrial activity using the MTT-based cytotoxicity assay at intervals of 24, 48, 72 and 96 h after the first laser application. Events related to cell death were evaluated by expression of Annexin V and PI and the cell cycle was also analyzed by flow cytometry.
The analysis of the number of cells in the different groups by the Trypan blue exclusion method revealed an increase of cell proliferation over time in all groups. A higher proliferation rate was seen in the irradiated groups when compared to the control group, with statistically significant difference ($p<0.05$) at the interval of 72 h. At the 96 h interval, a significant difference was only observed with an energy density of 1.0 J/cm2. There was no difference in cell viability analyzed by the Trypan blue exclusion method among the groups at all time points studied. Mitochondrial activity in the irradiated groups followed the pattern observed by the Trypan blue exclusion method. Irradiation with energy densities of 0.5 and 1.0 J/cm2 promoted significantly higher number of cells when compared to the control group after the second irradiation at interval of 72 h ($p<0.05$). It was observed that the cells had low positive staining for Annexin V and PI, markers of cell death. No significant changes were observed in cell viability throughout the experiment, although a slight increase in the percentage of viability was observed in the two irradiated groups after the second application of LLLI. The analysis of the distribution of the cells in the cell cycle phases showed a higher percentage of cells in G0/G1 phase (over 50%) 24 h after plating, but no statistically significant difference was found among the groups. In the last two intervals examined (72 and 96 h), approximately 85% of the cells were distributed in phases S and G2/M.
It can be confirmed that the patterns of LLLI used in the present study (power 30 mW, wavelength of 660 nm and energy density of 1,0 J/cm2) promotes proliferation of DPSCs and maintaining its viability.

Basic Research

120 - INFLUENCE OF LOW-LEVEL LASER IRRADIATION ON THE PROLIFERATION AND VIABILITY OF STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

Fernanda Ginani (UFRN); Mardem P. e V. Barreto (UFRN); Lélia B.de Souza (UFRN); Carlos Augusto G. Barboza (UFRN)

The aim of this study was to evaluate the potential effect of low-level laser irradiation (LLLI) on the proliferation and viability of stem cells from human exfoliated deciduous teeth (SHED).
Cells obtained from the pulp of three deciduous teeth at end-stage exfoliation and with indicated extraction were expanded in α-MEM culture medium supplemented with antibiotics and 15% fetal bovine serum. The cells were characterized as stem cells by expression of cell surface markers and by differentiation into osteoblasts and adipocytes under induced conditions. In the third subculture, the cells were irradiated or not (control), with a laser diode InGaAlP, power of 30 mW, wavelength of 660 nm, continuous action mode, with a tip diameter of 0,01 cm2, and using two different energy densities (0.5 J/cm2 for 16 seconds and and 1.0 J/cm2 for 33 seconds). The cells were irradiated at 0 and 48 h, with the laser probe fixed perpendicular to each plate at a distance of 0.5 cm from the cells. Cell proliferation and viability were analyzed at 0, 24, 48 and 72 h after the first laser application in the control (not irradiated) and irradiated groups using Trypan blue exclusion method and MTT-based cytotoxicity assay. Events related to cell death were evaluated by expression of Annexin V and PI and the cell cycle was also analyzed by flow cytometry.
The analysis of the number of cells by the Trypan blue exclusion method revealed that an energy density of 1.0 J/cm2 promoted an increase of cell proliferation at 48 and 72 h when compared to the control and 0.5 J/cm2 groups ($p<0.05$). A higher proliferation rate was seen in the irradiated groups when compared to the control group, with statistically significant difference ($p<0.05$) at the interval of 72 h. Analysis of cell viability by the Trypan blue exclusion method showed no variation in the time points studied. MTT assay comproved the results of Trypan blue exclusion method – the group irradiated with energy density of 1.0 J/cm2 showed a significantly higher number of cells when compared to the group irradiated with 0.5 J/cm2 at intervals of 48 and 72 h ($p<0.05$). In relation to cell viability, all groups showed low positive staining for Annexin V and PI throughout the experiment which indicated that the viability was not affected by the energy densities studied. The distribution of cells in cell cycle phases revealed that the irradiated groups exhibited the majority of the cells in S and G2/M phases.
It can be concluded that the patterns of LLLI used in the present study promote proliferation of SHED and maintaining its viability.

Basic Research

121 - IMMUNOMODULATORY PROPERTIES OF MESENCHYMAL STEM CELLS-DERIVED MICROVESICLES IN CLASSICALLY ACTIVATED MACROPHAGES

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Macrophages (Mϕ) have been associated to participate in acute kidney injury (AKI), and may play a pivotal role in both kidney development and recovery. According to microenvironment stimuli these cells may be characterized into classically activated Mϕ (M1), which secrete large amounts of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and reactive oxygen species or alternatively activated Mϕ (M2), which in contrast, may solve the inflammation releasing anti-inflammatory molecules such as IL-10, TGF-β and arginase-1. Additionally, M2 Mϕ have a reparative function which promote cell proliferation, reduce apoptosis and stimulate angiogenesis. In this sense, the obtaining of the distinct population of regulatory Mϕ could be extremely important for the establishment of alternative therapies in the AKI context. The mesenchymal stem cells (MSCs) have an important immunomodulatory potential evidenced in the regulation of Mϕ, DC cells, lymphocytes and NK cells. MSCs also promote the secretion of microvesicles (MVs) which participate actively in the reprogramming of target cells. However, the real participation of MSC and their MVs on Mϕ modulation yet remain to be elucidated. According to this question, the aim of this study was evaluate the immunomodulatory role of mesenchymal stem cells-derived microvesicles (MSC-MVs) in classically activated Mϕ.
Murine bone marrow-derived Mϕ were co-cultured with both MSC or MSC-MVs for 48 hours, after polarization to M1 (24 hours with LPS / IFN-γ). To MSC treatment were used a cell proportion 1:1 (MSC:Mϕ), and to MSC-MVs were utilized doses with 50 µg every 8 hours. MSC differentiation assays and MVs characterization also were performed.
Firstly, we extensively characterized and confirmed the obtaining of classically M1 and M2 Mϕ populations. After, the treatment with MSCs, it was shown an expressive reduction of classical M1 markers such as CD86, CCR7 and Class II MHC. In addition, we found that MSC also provided an increase of M2 markers (CD206 and CD36) in classically activated Mϕ. Complementarily, treatment with MSC-MVs shown to be effective in attenuating the expression of pro-inflammatory molecules in M1 Mϕ such as CCR7, IL-1-β and IL-6. Moreover, we identified that oxidative stress index (iNOS and NO levels) was also modified in treated M1 Mϕ. Furthermore, we observed in classically activated Mϕ exposed to MVs, the acquisition of alternative phenotype, evidenced by increasing of IL-10, Arginase-1 and CD206. Finally, observed that SOCS3, a molecule with strong immunomodulatory effect, was increased in Mϕ treated with MSC-MVs.
Our preliminary findings suggest that MSC and MSC-MVs have similar properties to modify the classically activated M1 Mϕ into a regulatory profile (M2-like). We believe that this modulation may be related to signaling pathways involved with SOCS3 activation. However, further experiments should be performed to confirm this hypothesis.

Basic Research

122 - QUALITY CONTROL OF A BRAZILIAN DISEASE-SPECIFIC iPSCs BIOBANK

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Human induced pluripotent stem cells (iPSCs) have been proving to be a valuable tool for biomedical research and regenerative medicine. Their capacity to self-renew, differentiate into any tissue of the human body, preserve genetic background of the donor and propagate into culture dishes make them well suited for modeling diseases and tests of toxicity. Because of these remarkable iPSCs characteristics, twelve Brazilian laboratories were recruited to establish a biological iPSCs bank of several disorders. To assess the quality of the cells that will be stored at the biobank, our laboratory will provide quality assurance service to all generated cells prior to storage. We will process samples to rule out mycoplasma contamination, which can modify gene expression and cell functioning, as well as, perform karyotype analysis to ensure genetic integrity in order to monitor the stability of the cells.
To establish and apply until the end of 2016, a quality control of all iPSCs to be stored in the Brazilian disease-specific iPSCs biobank.
The Mycoplasma test will be performed using Mycoalert Plus luminescence kit. Pluripotency will be assessed by immunocytochemistry for Nanog, Oct-4, Sox-2, SSEA-4, TRA-1-60 and TRA-1-81 in iPSCs, as well as for AFP, Brachyury, GFAP, Nestin, SMA and Sox-17 in iPSCs-derived embrioid bodies. Cytogenetics analysis will be performed by using G-band and/or DAPI staining. Our method of screening consists in analyzing 20 G-banded metaphases for each cell line provided, and carefully evaluate all numerical or structural chromosomal aberrations in order to identify possible clonal aberration.
Tests are being carried out in order to establish the best protocols for quality assurance of samples shipped from different regions of Brazil. Distinct methodologies related to freezing, transport, cell plating and fixation have been included in the workflow of these tests. Mycoplasma could be successfully detected in all cells tested independently of supernatant freezing, confirming that supernatant shipped in dry ice are suitable for testing by luminescence. Testing of plating ranging from 1/35 to 1/100 (per well) of an 80% confluent 100mm human pluripotent stem cell dish resulted in an appropriate confluence in 96 well plates. Results indicate metanol fixation allows dry material kept at 4 Celsius for 24 hours to be correctly stained for the pluripotency markers Nanog, TRA-1-60, TRA-1-81 and SSEA-4, although Oct-4 staining displays a different pattern than freshly 4% PFA fixed cells.
Warranting the quality of all iPSCs to be included in the Brazilian Biobank will ensure the achievement of the full potential of such cells (capacity to generate tissue of interest, reproduce molecular phenotypes) that will then be available for the scientific community.
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Basic Research

123 - CHARACTERIZATION OF MESENCHYMAL STEM CELLS FROM GREATER HUMAN OMENTUM

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Adult stem cells can be isolated from different sources such as placenta, cord blood and various tissues of adult individuals. The most well studied and characterized cells isolated from adult individuals tissues are the hematopoietic stem cells and mesenchymal stem cells. Adult mesenchymal stem cells can be found in adipose tissue. The greatest human omentum protects visceral organs against mechanical shocks, but can also be a reservoir of mesenchymal stem cells.
It is important to discover and characterize new stem cells sources for cell therapy use.
Initially, isolation and cultivation of stem cells from human greater omentum were performed. Cells extraction was done by treatment of the human greater omentum piece (about 30 g) with a solution of 0.1% collagenase for 45 minutes at 37 ° C. Then, the material underwent centrifugation at 1400 rpm for 7 minutes. The pellet was resuspended in DMEM supplemented with 10% human serum and cells were cultured at 37° C with 5% CO_2. After reaching the fourth passage, cells immunophenotype was characterized by flow cytometry assay and their transformation into different cell lines – chondrogenic, adipogenic and osteogenic – was performed by exposure to inducing culture mediums.
Evidence of differentiation processes was found by staining techniques Oil Red, Von Kossa and Ocean Blue. Phenotypic characterization by flow cytometry assay confirmed the presence of characteristic mesenchymal stem cells membrane proteins (90-95% of cells stained) and the absence of markers of mature cells (less than 5% markup) as calculated by flow cytometric analysis Flow Jo software.
Mesenchymal stem cells from human omentum also differentiate into chondrocytes, osteoblasts and adipocytes after stimulated, as evidenced by staining of specific structures already differentiated cells followed by optical microscopy. Mesenchymal stem cells isolated from human greater omentum are a new alternative for research in regenerative medicine.

Basic Research

124 - CHONDROGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELL IN SUGARCANE BIOPOLYMER MEMBRANES

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The human mesenchymal stem cells (hMSCs) are a rare population of progenitor cells with multi-potential differentiation capacity and seem to be an attractive tool in the context of tissue engineering and cell therapy. The development of new materials may be cited as a leading sector for tissue engineering and biopolymers are considered innovative biomaterials with widespread application in cell biology by outstanding biocompatibility.
In this study was evaluated the morphological aspects of human umbilical cord mesenchymal stem cells (hUCMSCs) and its condrogenic differentiation when cultured in membranes of biopolymer sugarcane.
Umbilical cords (n=20) were obtained from healthy donor in accordance with the Ethical Committee of Health Sciences Center of UFPE (n.482/10). Veins of umbilical cords were filled with collagenase IV and maintained at 37℃. Cells were c entrifuged for 10 min and resuspended in DMEM supplemented with 20% fetal bovine serum, 100U/mL penicillin, 100 µg/mL streptomycin and plated in flasks (105 cells/mL). Adherent cells were cultured until reached 80-90% confluence. Mesenchymal lineage was confirmed by flow cytometry. Membranes of a biopolymer extracted from sugarcane molasses produced by synthesis of the microorganism Zoogloea sp. were used as new support. Morphological aspects were analyzed by light phase-contrast microscopy and the cells were processed for Safranin-O staining for glycosaminoglycans.
hUCMSCs cultured in commercial support (porcine gelatin) exhibited fibroblast-like morphology (72h) and the monolayer occurred at five days. When cultured in membranes of sugarcane biopolymers these cells showed similar to fibroblasts, but were also observed cells with rounded shapes and some irregular. During the chondrogenic differentiation in membranes for three weeks, it was noted cells that became progressively bigger and more rounded attached to sugarcane biopolymer.
Our preliminary results proved that the sugarcane biopolymer membrane promotes chondrocytes adhesion and it could be represent a possible biomimetic microenvironment for these cells seeding.

Basic Research

125 - HYPOXIA AND SERUM DEPRIVATION DO NOT IMPAIR PROLIFERATION AND ANTIOXIDANT ENZYMES EXPRESSION IN MESENCHYMAL STEM CELLS DERIVED FROM MENSTRUAL BLOOD

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It is well known that most of stem cell injected in ischemic tissue die in a few days. This may reflect the harsh, proapoptotic microenvironment, which may not be favorable for mesenchymal stem cells (MSC) survival. Thus, it is essential to identify a MSC capable to survive in a poor blood supply and low oxygen environment for successful cellular therapy. Among the different sources of MSC, the ones derived from menstrual blood (mbMSC) might survive since their niche undergoes with intense necrosis and increased oxidative stress monthly.
The aim of this work was investigate the impact of reduced oxygen concentration and the absence of fetal bovine serum (FBS) in mbMSC adhesion, proliferation and resistance to oxidative stress.
Menstrual blood was obtained from healthy women. All the experiments were approved by our local institutional review board. The experimental conditions used in vitro were: normoxia (21% O2) and hypoxia (5% and 1% O2) in the presence and absence of FBS. Cells cultured in normoxia with serum were used as controls. For cell adhesion assay, the mbMSC were plated and after two hours, the adherent cells were stained with crystal violet and analyzed at 570 nm. Cellular proliferation was analyzed after two pulses of 5-bromo-2-deoxyuridine (BrdU – 10 µM) at 12 hours interval by immunofluorescence. The expression of genes encoding antioxidant enzymes and growth factors were analyzed by real-time PCR after 48 hours of cultivation.
No significant difference was found in the percentage of adhered cells cultured in different conditions of hypoxia (1% and 5%) and also in the absence of serum when compared to control. In regard to cellular proliferation, 5% hypoxia with or without FBS induced a significant increase in BrdU+ cells (79±11% and 73±2%) compared to normoxia, with or without FBS (53±11% and 10±5%), and 1% hypoxia with or without FBS (75±4% and 39±17%). It is clear that serum deprivation leaded to a significant decrease in BrdU+ cells in normoxia and 1% hypoxia, but not in 5% hypoxia. Moreover, serum deprivation in 5% hypoxia increases about 4-fold higher for catalase, SOD1, VEGF, BMP4. In serum presence, 5% hypoxia increases twice RNA levels for SOD2 and 3. On the other hand, 1% hypoxia with and without FBS decreases about twice RNA levels for catalase, SOD1 and BMP4 in qPCR assay.
The mbMSC maintains the ability of adhesion when exposed to hypoxic environment and serum deprivation. Furthermore, cells continue to proliferate in different O2 concentrations and when deprived of serum showing that even in extreme conditions they might maintain this vital characteristic. Moreover, 5% O2 increases expression of antioxidant enzymes and VEGF, indicating that in these environment mbMSC have higher chances of survival. Additionally, it implicates that mbMSC injected in ischemic tissues can also induce neovascularization, which may prevent tissue damage.

Basic Research

126 - ROLE OF ENDOTHELIAL PROGENITOR CELLS IN ARTERIAL THROMBOSIS AND VASCULAR REMODELING IN ATHEROSCLEROTIC MICE

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Arterial thrombi may trigger the pathological processes that lead to ischemic stroke and myocardial infarction. Recanalization of the thrombus is part of the vascular bead recovery after lesion and is directly linked to the cells and molecules associated to the thrombus. Endothelial progenitor cells (EPCs) were first described by Asahara et al, 1997, and were isolated from human peripheral blood. These cells are characterized by the expression of CD34, CD133, VEGFR2 e CD31, CD144, Tie-2, LDL internalization and formation of tube like structures in vitro. They can be mobilized from bone marrow to peripheral blood going to the lesion site, differentiating into endothelial cells (EC) and promoting re-endothelization.
Our study intends to verify the role the EPCs in the formation, resolution and re-endothelialization of arterial injury in atherosclerotic mice.
In this work, we obtained the differentiated endothelial progenitor cells from mice bone marrow mononuclear cells expanded in culture for 30 days in a differentiation medium and verified their EPC characteristics using flow citometry. These cells were tested in a carotid artery injury model using ferric chloride. We used LDL receptor deficient male mice fed a high fat diet for 4 weeks later. The lesion was induced as follows: an incision was made in the middle neck to isolate the carotid artery and it was injured with a small piece of paper embebed with 15% ferric chloride and put over the vessel for 30 seconds. In treatments with injection of EPC, were injected via tail vein, 2 x 106 cells, 15 min before the induction of injury. We measured the occlusion time that was considered to be the time between the initial flow and the time the flow stopped and occurred the vessel occlusion by thrombus formation in the artery. We also analyzed histologically the vessels at time zero and 7 or 14 days after injury.
We observed a statistically significant difference in the vessel occlusion time between the control (6 min) and the injected mice (14 min). We observed an increase in the number of cells in the lumen of the vessel of the injected animals. We also observed the presence of atherosclerotic plaque in all animals and a decrease in thrombus size in the injected animals after 7 days of injury.
Conclusions: We conclude that the injection of EPC affects thrombus formation time and its resolution in atherosclerotic mice and that probably the presence of these cells in the thrombotic matrix alters its composition affecting the behavior of the thrombus in the vessel wall.
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Basic Research

127 - SUGARCANE-DERIVED BIOMATERIALS AS SUPPORT FOR CULTURE OF HUMAM MESENCHYMAL STEM CELLS AND ITS DIFFERENTIATION IN KERATINOCYTES

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The tissue bioengineering is a multidisciplinary field that seeks to develop or improve technologies that may assist the recovery of injured tissues. The biomaterials are a sector vanguard for tissue engineering and biopolymers such innovative tools arise due to its biocompatibility. Human (hMSCs) are a population of cells capable of multipotential differentiation, being fundamental to the development of cell therapy.
Evaluate the biocompatibility of human umbilical cord mesenchymal stem cells (hUCMSCs) cultured on sugarcane biopolymer (gel and membranes) and its ability to differentiate in keratinocytes.
Umbilical cords (n = 20) were donated by the puerperal of Clinical Hospital of Pernambuco/UFPE according to protocol approved by the Ethics in Research (n. 482/2010). Each cord had its vein filled with collagenase type IV for the extraction of hUCMSCs. After this procedure, the cells were cultured in Dulbecco's modified Eagle medium/Ham’s F-12 supplemented by 20% fetal bovine serum (FBS), antibiotics mix and maintained at 37oC with a water-saturated atmosphere and 5% CO2. The confirmation of the mesenchymal lineage was by flow cytometry. The cell morphology on gel and biomembranes was observed by light phase-contrast microscope and scanning electron microscopy (SEM). The differentiation in keratinocytes was performed on both materials (gel and biomembranes) about 14 days and simultaneous photographic were recorded by light phase-contrast microscope.
Colonies of hUCMSCs with fibroblastoid aspects were observed within 72 hours. Phenotypic analysis revealed positive expression of molecular markers such as surface CD 29, CD 44 and CD 90. Negative expression was also confirmed for typical markers of endothelial and hematopoietic lineages (CD31, CD 34 and CD 45). Both supports (gel and biomembranes) favored adhesion, growth and differentiation of MSCs, exhibiting relevant biocompatibility. In sugarcane gel, the cells grew with elongated morphology and, under the action of keratinocyte inductors presented themselves concentrated in the periphery of the support. On the membrane, it was possible observe cells with rounded morphology and some with irregular aspects. Under the effect of differentiator, grouped and rounded-cuboidal conformation cells were observed, typical of keratinocyte like cells.
The cultivation of hUCMSCs on the biopolymer sugarcane showed significant biocompatibility, by being able to maintain the development and differentiation of cells with high plasticity, as MSCs. More studies should be developed to better characterize the process of keratinocyte differentiation until the establishment of its therapeutic application.

Basic Research

128 - BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AND THEIR CONDITIONED MEDIUM ATTENUATE FIBROSIS IN AN IRREVERSIBLE MODEL OF UNILATERAL URETERAL OBSTRUCTION

Andrei Silva (UNIFESP); Kleiton Silva (UNIFESP); Vicente Teixeira (UNIFESP); Nestor Schor (UNIFESP)

The therapeutic potential of mesenchymal stem cells (MSC) and their conditioned medium (MSC-CM) has been extensively studied. MSC can repair tissue, reduce local inflammation and modulate the immune response. Persistent renal tubular interstitial inflammation results in fibrosis and leads to Chronic Kidney Disease (CKD). Unilateral ureteral obstruction (UUO) is a very well-accepted renal fibrosis model.
In this study, we evaluated factors influenced by the administration of MSC or MSC-CM in the UUO model.
MSCs extracted from rat bone marrow were cultivated in vitro and characterized by flow cytometry and cellular differentiation. Eight groups of female rats were used in experiments (n=7, each), including SHAM, UUO (Ureteral Unilateral Obstruction), UUO+MSC (Obstruction + MSC) and UUO+CM (Obstruction + MSC-CM) for 7 days of obstruction and SHAM, UUO, UUO+MSC and UUO+CM for 14 days of obstruction. The MSC or MSC-CM was administered via the abdominal vena cava after total ligation of the left ureter. After 7 or 14 days, rats were euthanized and serum and obstructed kidney samples were collected.
MSC or MSC-CM decreased the expression of molecules, such as COL1A1, α-SMA and TNF-α. We also observed reductions in the levels of caspase-3, α-SMA and PCNA in treated animals by immunohistochemistry.
Our results suggest that the intravenous administration of MSC or MSC-CM improves fibrosis progression and factors involved in apoptosis, inflammation, cell proliferation and epithelial-mesenchymal transition in Wistar rats subjected to unilateral ureteral obstruction, indicating a potential tool for preventing CKD.

Basic Research

129 - RELEVANCE OF AKT SIGNALING PATHWAY ON HUMAN PLURIPOTENT STEM CELLS SURVIVAL

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Human embryonic and induced pluripotent stem cells (hESCs and hiPs, respectively) have great capacity to differentiate in vitro and huge potential to be used as cellular sources for regenerative medicine. The basic fibroblast growth factor (bFGF) is essential for survival, maintenance of pluripotency and self-renewal of hESCs and hiPs. PI3K and its most prominent effector, Akt, regulate cell viability in many cell types. bFGF activates PI3K signaling pathway in these pluripotent cells.
The aim of this work is to study the contribution of Akt in hESCs and hiPs viability.
hESCs lines WA01 (H1) - WA09 (H9) and hiPs line FN2.1 (generated in our laboratory from human foreskin fibroblasts) were cultured until confluence in the presence of bFGF. Then, cell survival was analyzed in the presence of three structurally unrelated Akt inhibitors. Inhibitors used were: Akt inhibitor IV (specific of a kinase which activates Akt), Akt inhibitor VIII (prevents binding of Akt to cell membrane) and GSK690693 (blocks ATP-binding site of Akt). In particular, cell viability was measured by the XTT colorimetric assay and Trypan blue exclusion test. Annexin V exposure to outer cell membrane and DNA fragmentation are two of the most important criteria used to indentify apoptotic cells, therefore changes in apoptotic rate were analyzed assessing DNA fragmentation with a Cell Death Detection Elisa Kit and determining Annexin V/propidium iodide double staining with a flow cytometer. Activation of initiator and effector caspases (like caspase-9 and caspase-3, respectively) is another hallmark of apoptosis induction. In that sense, caspase-9, caspase-3 and PARP (caspase-3 substrate) cleavage were measured by Western blot. Besides, changes in Bcl-2 family members abundance were also quantified by Western blot.
The pluripotent nature of confluent hESCs and hiPs was confirmed by the presence of the pluripotent markers: TRA1-60, TRA1-81 SSEA-4, Oct-4 and Nanog in immunofluorescence and quantitative RT-PCR assays. Cell viability significantly decreased in a concentration dependent manner in the presence of each inhibitor. Moreover, in all cases, the percentage of viable cells also diminished upon Akt inhibition. Furthermore, an increase in the number of Annexin V+/propidium iodide- cells and in the extent of DNA fragmentation were observed after 8 hours of Akt inhibitors addition in H9, H1 and FN2.1 cells. Moreover, Western blot analysis revealed the activation of caspase-9, caspase-3 and PARP cleavage at different time points upon Akt inhibitors treatment. Finally, no changes in Bcl-2 family members expression levels were detected by Western Blot under the same experimental conditions.
Taken together, we can conclude from the above results, that AKT activity is anti-apoptotic and thus relevant to hESCs and hiPs survival.

Basic Research

130 - MONONUCLEAR BONE MARROW CELLS TRANSPLANTATION PREVENTS HIPPOCAMPAL DAMAGE IN RATS WITH STATUS EPILEPTICUS

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Injection of pilocarpine into rats is a well-known animal model of status epilepticus (SE), resembling human temporal lobe epilepsy. In this model, hippocampal damage leads to a reduced long-term potentiation (LTP), decreasing synaptic plasticity and compromising memory processing. We have tested the effects of mononuclear bone marrow cells (BMC) transplantation on the hippocampal function in pilocarpine-injected rats, and studied the mechanisms underlying these effects.
Male Wistar rats (45-50 days-old) were initially treated with methylscopalamine (1 mg/kg; i.p.) and thirty minutes later with a single injection of pilocarpine (260 mg/kg; i.p.). SE was determined by the presence of generalized tonic-clonic seizures in the animals. Diazepam (10 mg/kg, i.p.) was administered 1h after the onset of SE, and the rats were randomly assigned to receive saline (SE) or BMC (SE-BMC; 1x107 cells in 0.2 mL PBS intravenously). The animals in the control group were not submitted to SE and only received saline solution (control). At one, three and ten days after SE animals were anesthetized with thiopental (40 mg/kg i.p) and decapitated; one hippocampus was separated for in vitro electrophysiological study (LTP) and calcium influx measurements. To further examine the impact of inflammatory changes on synaptic plasticity, we also investigated the potential effect of indomethacin (IND-100 µM) and celecoxib (CEL-10 µM) on the LTP. The other hippocampus was homogenized to quantify the levels of IL-1β, IL-1R, NMDAƐ1, NMDAƐ2, and of synaptic proteins by western blotting.
LTP was obtained in the animals from the SE group only at the day 1 after pilocarpine treatment; however it could not be induced at the days 3 and 10 after this treatment. Conversely, in the SE-BMC group, LTP could be induced at the day 10 after treatment. We found that IND and CEL did not significantly change the induction and maintenance of LTP in the control group, but they were able to keep the LTP induction in the SE group at the day 1 post-SE. Calcium influx was diminished at the days 1 and 10 in the SE group, however, this effect was fully prevented by BMC transplantation. Western blot results showed that pilocarpine robustly activated the expression of the pro-inflammatory protein IL-1β and its receptor IL-1R. Besides, there was a rapid increase in the levels of NMDAƐ1 and NMDAƐ2 receptors and of the protein synapsin at the day 1 post-SE. BMC transplantation followed SE prevented the increase of all these hippocampal proteins.
BMC treatment could decrease the inflammation 1 day after SE, and maintained the calcium influx, the levels of NMDAƐ1 and NMDAƐ2 receptors and of the protein synapsin similar to the controls. This treatment could also prevent damage to the hippocampal circuitry, allowing LTP induction.

Basic Research

131 - RECELLULARIZED EXTRACELLULAR MATRIX: AN ALTERNATIVE TO LIVER TRANSPLANTATION

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Severe hepatic failure is the result of long-term liver injury. Liver transplantation is the only efficient treatment, but is currently limited by organ shortage. The demand for new livers continues overcoming the availability. In this context, the creation of a bio-artificial liver might solve this clinical problem.
This work aims to produce a 3D liver recellularized scaffold with intact components of extracellular matrix (ECM) and vascular system.
Wistar rats were used to liver excision surgery. Twenty minutes before this procedure, heparine was administrated. Livers were perfused through portal vein using an infusion pump at 4 ml/min with water for 1 hour livers followed by Triton X-100 for 30 min and with SDS 1% for 12h. After total decellularization, livers were washed with distilled H2O for 30 min to remove residual SDS and then were preserved at 4 ºC for 7 days. To analyze the ECM integrity post decellularization protocol, DAPI, H&E, sirius red, DNA quantification, electronic scanning microscopy and immunohistochemistry assays against collagen type I, III, IV, laminin and fibronectin, were performed. Toluidine blue was used to examine the vasculature. For recellularization, approximately 109 HEPG2 (Human hepatocyte carcinoma) was injected through portal vein and allowed to attach for 2 hours at 37oC. Cells were continuous perfused with medium and FBS 10% using an infusion pump at 4ml/min for 3 and 7 days. DAPI, H&E, sirius red, DNA quantification, electronic scanning microscopy and immunohistochemistry assays against albumin, CK-18 were performed.
Toluidine blue showed that the vascular system was totally preserved. Macroscopy, microscopy and histological staining showed that the decellularization process preserves the structure and components of the ECM. After 7 days, cells were detected in the decellularized tissue. Conclusions: Our decellularization method was efficient removing resident cells and preserving the liver’s ECM and vascular system. After 7 days HEPG2 cells were able to adhere on liver 3D scaffold generating new perspectives on building bioartificial liver.

Basic Research

132 - PROCESSING BODIES AND STRESS GRANULES COMPONENTS ARE NECESSARY TO ACHIEVE FULL hASC DIFFERENTIATION

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Messenger ribonucleoprotein particles (mRNPs) are complexes formed by messenger RNAs (mRNAs) and a pool of different proteins bound directly or indirectly to the mRNA. Different proteins might form different mRNPs with different functions. Aggregations of mRNPs form granules that are visible to the microscope. Two types of granules commonly found in eukaryotic cells are the Processing Bodies (PB) and the Stress Granules (SG). They have essential roles on posttranscriptional regulation, more specifically on degradation (PB) and storage (PB and SG) of the mRNA. While PB are constitutively observed, SG are seeing only during stress conditions. TTP and RCK are a component present in both granulesTIA1 and TIAR are exclusively SG. Since cell differentiation induction is stressful for the cell and little is known about the role of those granules during stem cells proliferation and differentiation, one objective of our laboratory is to elucidate the dynamic and function of mRNAPs containing RCK, TTP, DCP2 and TIAR in stem cells.
To understand the importance of PB and SG components during stem cells differentiation.
Human Adipocyte Stromal Cells (hASCs) were used. RCK, TTP, TIAR granules were analyzed by immunofluorescence after 24 hours of adipogenic differentiation. Specific siRNA oligos for RCK, TTP, TIAR were transfected and immediately induced to adipogenic differentiation and kept in CO2 incubator for 24 hours, 4, 7 and 14 days of differentiation with specific adipogenic media. Differentiation was measured by fluorescence intensity after staining with AdipoRed.
SGs were absent in undifferentiated or differentiated hADSCs as determined by TIAR immunolabeling which localized mainly in the nucleus. PB varied in number and size on differentiated cells. Interestingly, in hADSC there is a very low number of PB and is even absent in some cells although, PB number significantly increases after 24 hour of differentiation. Under oxidative stress hADSC have an increase of PB and the formation of SGs. These SG may vary in composition since eIF4E is absent and eIF4B immunolabeled SGs are more perinuclear than TIAR immunolabeled SGs. siRNA of PB and SG essential proteins in undifferentiated hADSCs (time 0) showed that there is a significant increase of adipocyte differentiation after 4, 7 and 14 days of adipogenesis induction. Interestingly, this increase was due to adipocyte maturation, i.e. size of lipid vesicles, and not to the number of differentiated cells.
mRNPs are in low number in undifferentiated hADSCs but there is an increase of PB during differentiation and those granules seems to be important for late adipocyte maturation once siRNA of PB and SG components drives to an early differentiation. Also, SG are formed after oxidative stress induction and those granules are almost deprived of eIF4E showing that the composition of those granules may vary when comparing to differentiated cells.

Basic Research

133 - HUMAN ADIPOSE TISSUE-DERIVED STROMAL CELLS (hASC) FOR CYTOTOXICITY ASSAYS: ALTERNATIVE METHOD FOR ANIMAL TESTS FOR TOXICITY PREDICTION

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The objective of this study is to develop alternatives to animal testing for drug screening and correlate its use with toxic effects in humans using human adipose tissue-derived stromal cells (hASC). Our hypothesis is that hASC are excellent substrates for toxicity prediction since they are normal human cells. Moreover, toxicity can be tested along cellular differentiation processes with a better output than those obtained from available methods.
We have chosen a well known and validated methodology for initial estimation of doses for acute oral toxicity prediction: the inhibition of neutral red uptake (NRU) using hASC from 2 donors in comparison with BALBc 3T3 clone A31 cell line as reference. We tested 12 reference chemicals, 2 drugs for each class of toxicity. We determined IC50 (Inhibitory Concentration 50%) and these values were used for prediction of LD50 (Lethal Dose for 50% of animals) either in mg/kg or mmol/kg. Both LD50 predictions were used to predict toxicity and, evaluate which one would suit our study better.
We also evaluated non-toxic concentrations of 1 drug for each class of toxicity using hASC from 1 donor (estimated by NRU test) in order to assess its effect on adipogenic differentiation of hASC for 14 days. Nile Red/DAPI staining was used to evaluate cell number and adipogenic differentiation. Statistical significance was determined with ANOVA and Dunnet´s post-test.
For both cells, the LD50 prediction when it was used regression in mg/mL was 50% accurate, while when it was used regression in mmol/kg the prediction was 50% accurate for AD-MSC and 46% for 3T3. Thus, hASC has the same ability for toxicity prediction as well as 3T3 cell line for the drugs evaluated. This prediction is even better when the regression was done in mmol/kg.
To verify if adipogenic differentiation inhibition would be used as a toxicity indicator, we tested non-citotoxic concentrations for 1 drug of each class of toxicity during cell differentiation into adipocytes for 14 days. We were able to determine inhibition of lipid drops area and/or inhibition of % of differentiated cells for class 2,3, 4 and 6.
hASC is an excellent model for predicting toxicity, similar to the well established 3T3 cell line. Moreover, inhibition of adipogenic differentiation can be an indicator of citotoxic effects that can be correlated to toxicity predictions in humans. To our knowledge, it was the first time that hASCs have been used with this aim. The methods developed in this study have great potential for application in industry and for regulatory purposes. They can be used to evaluate the cytotoxicity of chemical compounds, products already established or in development by reducing or replacing the use of laboratory animals.
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The authors indicate no conflicts of interest.

Basic Research

134 - IMPACT OF THE DEPLETION OF CO-CHAPERONE STI1 IN THE BIOLOGY OF MURINE EMBRYONIC STEM CELLS

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Stress-inducible protein 1 (STI1) is known as an adaptor protein that coordinates the activity of heat shock proteins (70 and 90kDa) during protein synthesis, but when engaged to prion protein on cell surface modulates important neurotrophic functions. The role of STI1 in developmental biology has been characterized and recent data have shown that the loss of STI1 expression in mice causes embryonic death in embryos at E10.5, which can be rescued by transgenic expression of STI1, demonstrating an indispensable role during embryonic development.
Herein, the main goal of this study is to investigate the main functions of STI1 protein in early mammalian development, evaluating its involvement with stemness, survival and differentiation properties. To address these issues murine embryonic stem cells (mESCs) populations with different levels of STI1 expression will be used as model of study.
Firstly, derivation of mESCs populations from inner cell mass (ICM) from STI1 transgenic mice embryos with distinct genotypes (STI1+/+, STI1+/- and STI1-/-) has been performed. Alternatively, STI1 has been silenced by shRNA in mESC cell line (ES-E14TG2a). Levels of STI1 expression were checked by Western Blotting assays and BrdU incorporation was used to evaluate proliferation assays. Teratoma and embryoid bodies (EBs) formation were performed to evaluate differentiation properties of mESCs.
Blastocyst flushing, zona pellucida removal and immunosurgery of trophoectoderm were successfully performed, moreover, the expansion of mESCs in culture was impaired and an alternative protocol has been employed. On the other hand, ES-E14Tg2a cells were successfully characterized to their pluripotency before the functional studies be started. STI1-silenced mESCs, cultured in the presence of LIF, presented reduced cell number and colony formation besides several membrane protrusions when compared with control and parental cell populations. In BrdU incorporation assays, the downregulation of STI1 impairs ESCs proliferation. In addition, cell differentiation assay assessed by EBs formation using STI1-silenced mESCs showed that STI1 protein is required for proper EB size and morphology of cell aggregates.
These results suggest an interesting role of STI1 in stemness maintenance and proliferation regulation of mESCs. This study will contribute to establish the main functions of STI1 in early mammalian development. This study is supported by FAPESP.

Basic Research

135 - ISOLATION, CHARACTERIZATION AND DIFFERENTIATION OF PROGENITOR CELLS FROM RABBIT (*Oryctolagus cuniculus*) AMNIOTIC MEMBRANE

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Amniotic membrane originates from the epiblast, thus having high potential pluripotency, which is confirmed by the expression of markers such as Sox-2, Nanog, Oct-4 and Rex-1. However, the potential application of amniotic membrane cells is not only the feature of pluripotency, but also its immunogenicity feature. Because of these characteristics, the amniotic membrane has emerged as an important new source of stem cells in different species including the man, however nothing is known in rabbit.
The aims of this study were to isolate, characterize and differentiate progenitor cells from rabbit amniotic membrane.
For this, explants of amniotic membranes were obtained from 8 rabbit fetuses with 16 days-old. Cells were cultured in DMEM-HIGH glucose with 15% of fetal bovine serum and incubated at 37℃ in a humidified atmosphere of 5% CO2. Every 3 days, 70% of the medium was replaced and at 80% of confluence, the cells were harvested with 0.25% trypsin solution. The cells were characterized by standard methods including morphology, immunophenotyping by fluorescence, growth and differentiation potential (adipogenic and osteogenic, using oil red and Von Kossa staining, respectively).
Predominantly, fibroblast-like cells with elongated cytoplasm and centrally located nuclei were observed in the cultures. After freezing assay, the cells remained the same morphology showing satisfactory growth potential and uniformity. The cells were immunopositive for mesenchymal stem cells markers (CD105, CD73, and Stro-1), pluripotency (Nanog and Oct ¾), and cytoskeleton (vimentin and cytokeratin). In contrast, cells were negative for CD117, a precursor of hematopoietic stem cells. In the adipogenic differentiation, cells had a rounded shape with a nuclei displaced to the periphery of the cells. Several lipid vesicles were observed in the cytoplasm. Osteogenic differentiation was characterized by a bone matrix with calcium deposits formation.
In conclusion, the amniotic progenitor cells had satisfactory abilities of expansion, expressed typical mesenchymal stem cells markers, and showed multipotent potential of differentiation in vitro.

Basic Research

136 - EFFICIENCY IN PRODUCTION EQUINE INDUCED PLURIPOTENCY CELLS (IPS) USING FIBROBLASTS AND UMBILICAL CORD MESENCHYMAL STEM CELLS

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An alternative to creating banks of pluripotent stem cells involves reprogramming the somatic cell nucleus. This induced reprogramming results from the action of different molecules, including transcription factors and other proteins. Recently, several groups have reported the induction of pluripotency in human fibroblasts through the transduction with viral vectors expressing OCT4, C-MYC, KLF4 and SOX2 genes. The cells called induced pluripotent stem cells (iPS) have a morphology characteristic of embryonic stem cells, express pluripotent markers and are capable to differentiate in tissues from the three germ layers in vitro and in vivo. Thus, the generation of iPS cells from the reprograming of different cell types by specific factors, could represent an alternative to obtaining a bank of stem cells with pluripotent characteristics.
Equine fibroblasts and umbilical cord cells (EUCC) were genetically modified using lentiviral vectors containing STEMCCA excitable polycistronic human cDNA of OCT4, SOX2, c-MYC and KLF4 (EF1a-hSTEMCCA) in order to transform unipotent and multipotent cells in pluripotent cells. EUCC and fibroblasts were plated at a concentration of 5x103 cells per well of 9.6 cm2 and cultured in DMEM high glucose with 20% FBS, 1% penicillin/streptomycin and 1.2% amphotericin. When cultures reached approximately 60% confluence they were transducted in a medium containing 50mL of viral concentrate plus 8 ηg/ml polybrene (hexadimethrine bromide, Sigma). The medium was renewed after 14 hours of incubation. Five days post-transduction, the cells were transferred into MEFs. During reprogramming period cell were cultured in a specific medium for iPS. Between days 6 and 15 post-transduction we evaluate the time to the beginning of the appearance of colonies, colony morphology and number of colonies (greater than 1 mm diameter) formed per well.
In fibroblasts´ cultures the colonies began to form on day 7, and in EUCC´ cultures on day 6. Both cultures developed thin colonies, with well-defined edges, formed by small cells of uniform size and hexagonal appearance, with evident nucleus containing one or two nucleoli. In each well 18 colonies were formed, on average, independently of the cell type. The cultures remained viable and colonies multiply in the course of 15 days.
Lentiviral vectors were efficient to deliver the transcription factors to cells, formed colonies showed morphological characteristics similar to embryonic stem cells between 6 and 7 days post-transduction. Future experiments will be conducted for imuno genotypic characterization of these cells.

Basic Research

137 - ISOLATION AND DIFFERENTIATION OF MESENCHYMAL STEM CELLS DERIVED FROM OLFACTORY EPITHELIUM OF DOG FETUS

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The olfactory epithelium is a sensory organ located in the dorsal-caudal region of the nasal cavity, formed by a neuroepithelium, olfactory cells and bipolar neurons responsible for olfactory sensitivity.The olfactory epithelium is a promising source of mesenchymal stem cells to be used in cell therapy.
This study is aimed to isolate and establish the primary culture of mesenchymal-like cells from olfactory epithelium of dog fetus, in order to perform the in vitro differentiation trials.
Six dog fetuses of about 60 days were collected after a routine ovarian hysterectomy in dogs. The olfactory epithelium cells were collected and subjected to enzymatic digestion with 0.25% trypsin for 30 min. The isolated cells were seeded in cell culture dishes with DMEM F12 supplemented with Fetal Bovine Serum, non-essential amino acids, L-glutamine and penicillin and streptomycin. For cell viability assays, the cells were frozen for 1 week, then were harvested and counted in each passage with Neubauer´s chamber by dilution (1:1) in trypan blue. For colony-forming units assay, $1x10^3$ cells were seeded in 100mm culture dishes. The canine olfactory epithelium cells were subjected to osteogenic, adipogenic and chondrogenic differentiation assays using StemX VivoTM kits and neurogenic differentiation using 3 – mercapto-1, 2-propanediol.
The olfactory epithelium cells demonstrated plate adherence and the cell morphology was similar to fibroblastic cells. Cell viability analysis revealed high percentage of living cells in culture (67%) and low percentage of dead cells (33%). When plated at low density, they formed an average of 118 colonies of more than 200 cell aggregates. Under specific conditions in vitro, the olfactory epithelium cells differentiated into osteogenic, chondrogenic, adipogenic and neurogenic cell lineages.
In culture medium, the olfactory epithelial cells showed morphology similar to fibroblastic cells and extensive self-renewal capability. They can be cryopreserved without depreciation in the cell viability. Under specific conditions they undergo into osteogenic, chondrogenic, adipogenic and neurogenic cell lineages. These findings suggest that the olfactory epithelium of dog fetus may be a potential source of mesenchymal stem cells to be used in regenerative medicine and cell-based therapy.

Basic Research

138 - STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH WITH IMMUNOSUPPRESSIVE ACTIVITY CAN BE CULTURED IN THE PRESENCE OF HUMAN SERA

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The Stem Cells from Human Exfoliated Deciduous teeth (SHED) have been currently isolated from deciduous teeth. They present mesenchymal and embryonary markers, rendering these cells as interesting in the therapeutic point of view. Recent publications show the SHED to have immunosuppressant activity.
Evaluate the immunosuppressant activity of the SHED in vitro.
The immunosuppressant activity of the SHED isolated by our protocols was evaluated by MTT assay. When the SHED were co-cultivated with T lymphocytes CD2+, the T-cells proliferation was inhibited in a DSCPs ratio-dependent manner. In addition a comparison of the cell in vitro proliferation using human sera versus FBS was performed by MTT assay for two SHED lineages on days 4 and 8 of culture.
The inhibition rates were of 2.56%, 27.82%, 46.39% and 51.62% when the respective T CD2+ cells to SHED proportions were 103:1, 102:1, 10:1 and 2:1. We observed that the SHED have a strong inhibitory effect on the activation of the T cells, considering that their proliferation was inhibited when in a mixed culture. When co-culturing the T lymphocytes CD+2 with SHED in the presence of a mitogenic agent (phytohaemagglutinin), the lymphoid cells proliferation was markedly inhibited. Furthermore, statistical analysis showed that there exists no significant difference when compared the proliferation on human serum or FBS (Tukey test, $p<0.01$).
Our data suggest that the SHED supress the allergenic proliferation of T cells in a non-dependent of MHC fashion. Aiming at future applications of these cells for cellular therapy, the use of foetal bovine serum (FBS) should be avoided according to the national sanitary surveillance agency (ANVISA), in order to prevent transmission of bovine pathogens to the patients from happening. The results of the MTT assay comparing the proliferation among distinct sera support that SHED are suitable for cellular therapy, mainly due to their immunosuppressant activity. ANVISA prohibiting the use of products with animal origin for cellular therapy should be stressed as an importance of searching for alternative methods of cultivating cells with that purpose. Our work suggests that SHED can be cultured using human serum as a substitute for the traditional use of FBS.

Basic Research

139 - PRODUCTION OF BIOLOGICAL SCAFFOLDS FROM DECELLULARIZED BOVINE MUSCLE TISSUE

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Sprains, contusions and lacerations are examples of well-known lesions of skeletal muscle that can be completely regenerated over time. The most frequently used alternative for the treatment of such lesions consists in transplantation of muscle mass coming from nearby or distant location to the injured regions. Bioengineered tissue emerges as a promising alternative for the regeneration of muscle tissue because it is associated with low morbidity. A commonly used strategy consists in growing cells in a scaffold that provides the proper three-dimensional structure for adhesion, proliferation, differentiation and secretion of a new Extracellular Matrix (EM). Regarding the use of biomaterials, the commonly used approach consists in the use of synthetic biodegradable polymers, but such polymers lack of signals enabling cells recognition thus hindering the formation of functional tissue. Another problem is the structural uniformity of scaffolds composed of only one type of synthetic polymer that can not reproduce the complex hierarchy of tissues. Alternatively, decellularized tissue has been widely used as scaffold in surgical reconstruction for replacement of organs and tissues. The use of EM as a scaffold can be explained by its role in the maintenance of tissue architecture, besides participating in the regulation of processes such as proliferation, migration and differentiation. The reconstitution of skeletal muscle tissue through decellularization and repopulation of tissues is also the target of numerous studies, but all of them have used muscles of small animals like dogs, mice, rabbits, rats.
Thus, this work aims to produce a scaffold constituted by EM by the decellularization of bovine skeletal muscle.
The study material is composed of the gastrocnemius muscle of beef cattle originating from slaughterhouses. The muscles were processed for the removal of fat and connective tissue and sliced into pieces. For decellularization a set of different detergent baths was applied. For the determination of decellularization the following criteria were used: (1) DNA double strand content less than 50 ng/mg; (2) DNA fragments smaller than 200 bp; and (3) absence of nuclear structures by histological analysis. After that, the scaffold will undergo other analyses in order to evaluate its biocompatibility with the aim to use it in cell therapy.
After the decellularization process the material presented translucent appearance consistent with the decellularization process and maintained initial 3D structure. The DNA content was below 1ng/mg and it was not observed presence of DNA bands in agarose gel.
Thus, we successfully obtained a scaffold of bovine muscle that showed much higher degree of decellularization than recommended in the literature. It represents an excellent candidate for future works on stem cell repopulation.

Basic Research

140 - DEMINERALIZATION AND DECELLULARIZATION OF BOVINE BONE TO PRODUCE HYDROGELS AS BIOLOGICAL SCAFFOLDS AND REPOPULATION STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH AND FROM ADIPOSE TISSUE

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According to the World Health Organization, there are around 150 illnesses, conditions and syndromes related to bone abnormalities. Skeletal insufficiencies linked to orofacial conditions are a public health issue. During the quadrennium 2008-2011, 20985 ambulatory care procedures have been performed with total cost being R$ 283333.48. The most common treatment option for severe bone loss are the grafting techniques, which may be classified autogenous, xenogenous or alogenous according to its origin. Xenogenous grafts are those, in which the donor belongs to a different species of the receptor. This sort of procedure may cause great nuisance to the patient due to the non-self tissue rejection. Over the last decade, there has been growing interest in biological scaffolds composed by extracellular matrix derived from tissue decellularization, considering that the two main immune response generating antigens are the DNA and the membrane oligosaccharide, α-gal. Thus, the production of biological material from decellularized animal tissue would lower the costs, enabling patients in treatment by the Brazilian Unified Health System (SUS) to profit from a safer grafting procedure, which prevents immune response.
Obtain demineralized and decellularized bovine bone tissue to produce hydrogels to serve as biological scaffolds and repopulate it with stem cells isolated from human exfoliated deciduous teeth and adipose tissue, aiming its future application in grafting procedures.
The trabecular part of the bovine femur obtained from a abattoir was ground and demineralized with chloric acid, followed by the removal of lipids by using methanol and chloroform. Subsequently, the material was decellularized by enzymatic digestion. In order to confirm decellularization, DNA was extracted by the CTAB method, its concentration was determined by Nanodrop® and in order to determine integrity, a 1% agarose gel was employed. The hydrogel will be obtained by the extracellular matrix solubilisation in pepsin and jellification by temperature alterations.
After the demineralization and decellularization process, we obtained a fine powder. DNA concentration obtained from the extraction performed on this powder was below 50 ng per mg of dry weight of the matrix, which is recommended by the literature. The agarose gel showed smear, indicating that the remaining DNA in the extracellular matrix was degraded.
Demineralization and decellularization were successfully performed and the amount of DNA remaining in the matrix was inferior to what the literature recommends. After the obtainment of hydrogels and its recellularization with human originated stem cells, the material has great potential for grafting.

Basic Research

141 - THE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS (BMSCS) OR CONDITIONED MEDIUM (BMSCS-CM) REPAIR THE ACUTE KIDNEY INJURY (AKI) INDUCED BY ACYCLOVIR (AC)

Joelma Santina Christo (UNIFESP); Clevia Passos (UNIFESP); Nestor Schor (UNIFESP)

The AC is an antiviral drug used to treat herpes simplex type 1 and 2 and varicella zoster and may induce nephrotoxicity with AKI. Several groups have reported the contribution MSC in repair AKI processes.
The aim of this study was to investigate the role of BMSCs or its conditioned medium BMSCs-CM in this model of AKI.
They were cultured and used at 4th passages for all experiments. The female Wistar rats received AC (80mg/Kg/BW) or water (CTL) daily, for 5 days i.p. (N=05) and 72 hours after the 5th day of treatment, they received i.v. BMSCs ($1X10^6$cells) or BMSCs-CM (500µl) injection (1 doses). Then blood were collected for urea (U) and creatinine (Cr) as well as histological evaluation.
The AKI model was confirmed by increase in creatinine and urea after AC, compared CTL group. The AC+BMSCS groups, after 72 h, presented lower creatinine and urea compared with ACY alone. Also the AC+ BMSCs-CM group presented lower values for creatinine and urea compared with ACY alone group. Were observed in the groups treated with BMSCs or BMSCs-CM. AC induced increases in pro-inflammatory cytokines 1L1α, 1L-β, IL-3, IL-6, TNFα, IFNγ and decreases in anti-inflammatory cytokine IL-10.
These effects were much lower after MSC or MSC-CM administration.

Basic Research

142 - ENDOCANNABINOID SYSTEM CHARACTERIZATION IN RENAL INJURY BY ISCHEMIA AND REPERFUSION

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The endocannabinoid system (ECS) is formed by the cannabinoid receptors (CB1 and CB2), endogenous lipids (endocannabinoids) and different enzymes responsible for the synthesis and degradation of these lipids that are involved on modulation of physiological responses. Several studies suggest its involvement in pathophysiological conditions, such that caused by ischemia and reperfusion (IR) in different tissues. Our group is studying the role of different bioactive lipids in the paracrine interaction between stem cells and kidney cells, and although there are evidences for the involvement of the ECS in renal injury, little is knower about the ECS during cell-based therapy for ischemic kidney injury.
Evaluate the involvement of the ECS in renal IR injury and its role in protecting the lesion generated by treatment with stem cells derived from bone marrow (BMSCs) in an in vivo model for IR.
Male Wistar rats had the renal arteries clamped, followed by release of circulation after 30 min, and kept for 24 hours prior to the experiment. In the treated group BMSCs (107 cells in saline) were administered subcapsularly in both kidneys, 1 hour before ischemia (Beiral et al., 2012). To evaluate the presence of CB1 and CB2 immunofluorescence staining was performed.
Using immunofluorescence we show the presence of cannabinoid receptors renal proximal tubule (PT), and noted a significant increase expression of CB1 after the IR. Whereas, pretreatment with BMSCs, prevents these increase. The CB2 expression in PT increased only in animals that were pretreated with BMSCs tissue.
ECS receptors are expressed in the renal proximal tubule of rats and its expression is modulated in case of injury by IR. Several studies in the literature indicate that increased expression of CB1 condition after IR to be involved in the role of ECS compound the establishment of injury, while the role of CB2 would provide protection to the tissues. Our data suggest that ECS could play a crucial role in the protection profile that treatment with BMSCs offers the kidney tissue in cases of IR.
Financial Support: Capes-PROBITEC, Capes-INL, CNPq e FAPERJ-CONICET.

Basic Research

143 - ISOLATION AND CULTIVATION OF MESENCHYMAL STEM CELLS FROM CANINE AND FELINE AMNIOTIC MEMBRANE FOR EVALUATION OF MESENCHYMAL MARKERS AND TERATOGENIC POTENTIAL

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In current days the amnion has been used as a promising source of mesenchymal stem cells in several animal models, for their application in cell therapy. This material is easily obtained because the amnion is discarded after birth, therefore, no ethical conflicts for its use.When cultured under specific conditions the amniotic mesenchymal stem cells reveal high plasticity and can differentiate into various cell lineages.Several tests have been performed to clarify some questions about possible complications their application: biosecure, tissue function deterioration and carcinogenic potential.Thus, the purpose of this study is to isolate and culture mesenchymal cells from canine and feline amniotic membrane, to perform a comparative analysis of the molecular profile and teratogenic potential between canine and feline species. Previous studies reported no teratoma formation in feline amniotic cells but the effects of canine amniotic cells are not well understood.

The cells were isolated from fetal membranes collected after a routine ovarian hysterectomy in dogs (35-45days). The amnion was mechanically separated, washed repeatedly with sterile PBS and the pieces were minced to reach homogenous solution. The isolated cells were seeded in cell culture dishes with DMEMF12, 38,5^{o}C and 5% of CO_2. After 24 hours in culture medium, amniotic mesenchymal-like cells demonstrated plate adherence and the cell morphology was similar to fibroblastic cells. When the plated cells reached 80% confluence, the culture was recovered using trypsin 0,25%. The cell solution was centrifuged, the supernatant were discarded, and the cell pellet was resuspended with 1ml of the culture medium for subsequent passage. The dog and cat amniotic mesenchymal stem cells ($10x10^6$) were transplanted into immunodeficient mice Balb c/nude for teratogenicity assay. The expression of specific mesenchymal surface markers (CD73, CD90, CD105) and teratogenicity markers (CD30) were accessed by immunocytochemistry.

In this study we successfully established the primary culture of dog and cat amniotic stem cells. Regarding to teratogenicity assays, none teratome were observed 50 days after cell transplantation into immunodeficient mice, and the animals survived without complications. These animals will be euthanized for histopathology assays. The mesenchymal stem cell specific markers (CD73, CD90) were expressed in both species, but the CD105 were positive only in cats. The teratogenicity marker (CD30) was positive in canine amniotic cells and negative in feline amniotic cells.

We successfully established the mesenchymal stem cell lineage from canine and feline amnion, and these cells expressed the specific MSCs surface markers. After transplantation into immunodeficient mice, non teratome were observed although the teratogenicity markers were positive for canine amniotic MSCs. The level of expression of these surface markers in these cells will be accessed by quantitative methods for better understanding (flow cytometry).

Basic Research

144 - BEHAVIOR OF THE MESENCHYMAL STEM CELLS DERIVED FROM DIFFERENT DENTAL AND PERIODONTAL REGIONS AFTER CHEMICAL STRESS BY SODIUM ARSENATE

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The study of the reactions of mesenchymal stem cells (MSCs) to stressors is relevant aiming to recognize behaviors that such cells may exhibit when used in permeated environments by aggressors which ones contribute to the generation of reactive oxygen specimens. It must be considered that in situations of cell transplants, the environment that will be the cell receptor site may present unfavorable conditions to homeostasis. Thus it is necessary the understanding of the relationship between stem cells and the environment inhabited by them.
The study aimed to observe the cell behavior of dental and periodontal origin and lineage of fibroblasts when submitted to stress.
The sample consisted of human teeth-derived MSCs from the following sources: pulp of permanent teeth, periodontal ligament, apical papilla, dental follicle and commercial line of fibroblasts (control). The isolation of cells from periodontal and dental tissue used enzymatic dissociation with collagenase and dispase II. MSCs were grown until they reached approximately 80% confluence, when they were dissociated using 0.25% trypsin-EDTA (Sigma-Aldrich) and continually expanded for several passages. Characterization of MSC considered adherence to plastic, immunophenotyping by flow cytometry and cell differentiation. The stress test used cells grown in the passage 3 (P3) and stressful medium as sodium arsenate. The viability evaluation used the Annexin V and 7-AAD. The analysis was carried out on days 2, 12 and 24 hours (T1, T2 and T3) and compared to control. To detect differences between means observed between trials (control, T1, T2 and T3) for the markers 7-AAD and Annexin V, analysis of variance for repeated measures (ANOVA) with Post Hoc Bonferroni test were used. In all comparisons the index of significance was 5%.
The result indicated that there were no significant differences between trials for the marker 7-AAD ($P = 0.451$) and there is significant differences between trials for the marker Annexin V ($P = 0.001$) in comparison of T3 with the control and T1.
According to the model studied, it was observed that in the first 12 hours of cellular injury by sodium arsenate, the events of apoptosis were not as evident as those observed at 24 hours.

Basic Research

145 - ISOLATION AND CHARACTERIZATION OF UNDIFFERENTIATED CELLS OF EMBRYONATED EGGS FROM Aedes aegypti

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Aedes aegypti is an insect from the Family Culicidae, being the vector responsible for the dengue virus transmission. It is a dark mosquito easily identified by white lyre shaped markings on its body. A major problem of public health in the world is directly linked to dengue, where approximately 550 million people are infected annually and about 20,000 have died. In Brazil, about 82,039 severe cases of the disease were registered in the past ten years, with 2,931 deaths. This disease is caused by a virus of the family Flaviviridae, transmitted by the female mosquito to the host during its blood feeding. The study of insect cells has been widely used in the production of insecticides, pests and vectors control and new biotechnologies. Insect cells are usually grown in suspension, extending freely into the culture medium.
This study aimed to establish a cell culture protocol for undifferentiated cells of Aedes aegypti, using the isolation and characterization of these cells as a source of research in cell and structural biology of these vectors.
Aedes aegypti eggs were obtained in a breeding site at Universidade Estadual do Maranhão – UEMA (Caxias, Maranhão, Brazil). After sampling, they were kept at room temperature until further analysis. Histological analysis with Hematoxylin-eosin (HE), for morphological study of embryos contained in the eggs, was performed. Immunohistochemistry tests were conducted to identify pluripotent cells using Stro-1, Nanog and SSEA-4 antibodies. Then, a cell culture protocol was established for these cells, through primary culture and first passage (with 24 and 48 h of culture, respectively), after which their morphology and cell cycle were evaluated.
Histological analysis at the end of the embryonic period (48 to 62 h) showed embryos divided into head, thorax and abdomen, composed of 8 abdominal segments, as found in the first larval instar. Aedes aegypti cells had globoid format, small size and heterogenic population. In the immunohistochemical assay, pluripotent cells positive for SSEA-4, Nanog, and Stro-1 markers were observed on embryos at the end of the embryonic period.
Through these markers it was possible to observe that these cells had a high proliferative index. The analysis of cell cycle phases Sub-G1, G0-G1, S and G2-M in P0 were 27.5%, 68%, 30.2% and 1.9%, respectively; and in P1 were 10%, 92.4%, 6.2% and 0.6%, respectively. By analysis of cell cycle phases, it was observed that the protocol was favorable for cell culture due to the high number of viable cells found performing its metabolic activities normally.
Eggs of Aedes aegypti at the end of the embryonic period have pluripotent cells, which may represent a promising tool for further research in biotechnology and biological control of this vector.

Basic Research

146 - GENERATION AND CARDIAC DIFFERENTIATION OF FEEDER FREE INDUCED PLURIPOTENT STEM CELLS

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The aim of this work was to generate induced pluripotent stem cells (iPS) from human dermal fibroblasts (HDF) using the STEMCCA system and to differentiate these cells into cardiomyocytes. Lentiviral particles were generated by transfecting the HEK293FT cells with policistronic plasmid STEMCCA (OCT4, SOX2, KLF4 and c-MYC), VSV-G, Tat, Rev and Gag/Pol. HDF in the 4th passage were transduced (MOI1) and the cells were transferred to plates treated with Matrigel™ and cultivated in mTeSR1. G-band karyotyping was performed and iPS were characterized by RT-PCR, flow cytometry, immunofluorescence and spontaneous differentiation. iPS were differentiated into cardiac lineage following 12 days of induction. In day 0-1 the cells were aggregated and cultivated with STEMPRO34, transferrin, ascorbic acid, monothioglycerol (basal medium) and BMP4. In days 1-4, mesoderm differentiation was induced with basal medium with the addition of BMP4/ActivinA in the following concentrations: 3/2, 10/6 and 30/10 ng/mL. The evolution of CD56 and PDGFRα expression was analyzed by flow cytometry. For the differentiation in cardiac mesoderm (days 3-8), cells were cultivated with basal medium with the addition of VEGF 10 ng/mL and XAV 939 (10μM). In days 8-12 the cells were cultivated with a basal medium. In day 15, cells were evaluated for the troponin T expression.
Our results demonstrated that virus production was efficient, with 11.6% of the cells transduced being positive for OCT4 after 72 hours. iPS emerged during days 12-18 and were selected manually for expansion. iPS had a normal karyotype (46, XX) and expressed OCT4, SOX2, NANOG, REX1, KLF4, DNMT3B, TDGF, TERT, GDF3, LIN28 and NODAL by RT-PCR. By flow cytometry and immunofluorescence, we observed expression and characteristic staining of OCT4 (90.7%), SOX2 (84.7%), NANOG (97.7%), SSEA4 (78.5%), TRA1-60 (98.2%), TRA1-81 (79.2%). Cells also had a positive staining for c-MYC and LIN28. iPS showed spontaneous differentiation and expressed by RT-PCR Nestin, TUBB3 (ectoderm), MSX-1, Brachyury, BMP4 (mesoderm), Gata6 and Sox17 (endoderm) and had positive staining for Nestin and Brachyury. On day 2 of the cardiac differentiation, the cells did not express CD56 and PDGFRα in any conditions. On days 3 and 4, the 10/6 condition expressed CD56 (44.7% and 52.6%) and had a discrete expression of PDGFRα, inducing the cardiac mesoderm. On day 8 beating cells in all conditions were observed and on day 15 flow cytometry for Troponin T demonstrated that the best condition was 10/6 with induction of cardiac mesoderm on day 3: 3/2 (32.1%), 10/6 (41.7%), 30/10 (36.3%).
The viral production was efficient and the generated feeder free iPS had normal karyotype with pluripotent characteristics. These iPS cells were able to differentiate into cardiomyocytes.

Basic Research

147 - QUANTITATIVE ANALYSIS OF THE ENZYMATIC VERSUS MECHANICAL ADIPOSE TISSUE DISSOCIATION TO OBTAIN MESENCHYMAL STEM CELLS

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Regenerative medicine (RM) has emerged of different sciences boost, including biology of the stem cell. The mesenchymal stem cells (MSCs) isolated from adipose tissue (AT) have occupied more space in RM. One of the major concerns of modern science is danger of amplification and purification of MSC by the cell culture method. In previous work we identified the DNA damage is more related to the dissociation method than the culture itself.
Given these findings and knowing that mechanical dissociation (MD) determines a lower rate of DNA damage measured by Komet test, this study aims to assess the quantitative performance of mechanical and enzymatic techniques dissociation.
The statistical analysis will be the multivariate variance for repeated measures. Inguinal adipose tissue fragments of rats with a mean age of 2 months and average weight of 350 g were obtained. In MD, samples were processed using a cell scrapper and in ED, using colagenase I. This work is the first step of a larger project to assess the contribution of biophotonics in cell growth and modulation of paracrine effects of adipose tissue-derived MSC through biostimulation of MSC cultured with continuous and pulsed LED of 630 nm.
The partial results of 18 samples obtained and processed recorded the average weight of adipose tissue collected for the MD technique 2.95 g, with maximum and minimum values of 5.67 and 1.17 g, respectively. The median time to the second passage was analyzed in 2 ways: culture free from contamination culture or change of fetal serum batch, and this time was 20.7 days versus 41.7 days for culture with complications. And the corresponding number of cells obtained was 2.98 x 10^6. The recorded mean time to the third passage was 57.84 days and cell count was 1.86 x 106 cells. For the ED technical, the average weight of collected adipose tissue was 2.01 g with maximum and minimum values of 3.73 and 1.32 g, respectively. The time for the second passage was 22 days in stable culture and 4.78 days in culture with complications. The number of cells obtained at this stage was 3.6 x 10^6 cells. As for the third passage time was 64.21 days and cell count was 2.53 x 10^6 cells. These results show that complications such as contamination of the culture or change fetal serum batch can increase up to twice the time of passage of adipose tissue-derived MSC. On average, the method of MD obtained, in 21 days of culture, 0.98 x 10^6 MSCs per gram of AT collected while ED method obtained 1.79 X 10^6 cell per gram in 22 days of culture.
It is evident that the ED method allows obtaining a larger number of CTM with similar intervals time. Therefore, the original project aims to decrease these differences, evaluating the expansion time in culture and paracrine factors related to the secretion of cytokines, without determining DNA damage with the use of irradiation type LED.

Basic Research

148 - EFFECTS OF VESICULES (Vs) DERIVED FROM RENAL PLURIPOTENT STEM CELLS (rPSCs) ON THE LPS TOXICITY IN IMMORTALITY HUMAN MESANGIAL CELLS (iHMCs) AND RENAL EPITHELIAL LLCP-K1 CELLS IN CULTURE

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The iHMCs and LLCP-K_1 lesions induced by LPS are characterized by reductions in proliferation and increases in pro-inflammatory cytokines productions and as well enhanced apoptosis. The rPSC therapy becomes promising tool in the acute kidney injury (AKI) treatment.
In this study we investigate the effect of Vs derived from rPSCs on the iHMCs or LLCP-K_1 treated with LPS.
Mice (C57BL6-J) were used to obtain rPSCs using standard techniques following renal cortex isolation by differential sieving. The rPSCs were characterized by immunofluorescence and FACS utilizing positive antibodies (Wnt1, CD24, PAX2 and ZO1) and negative antibodies (CD45, Thy-1 and pan cytokeratin). In order to enhance the production of Vs, the rPSCs were previously treated with LPS (100µg/ml), gentamicin (G; 2mM) or cisplatin (Cis; 6µM) (preconditioned - PC) or not (nPC) for 24 hours. Then, the Vs were obtained by ultracentrifugation technique and characterized by electronic microscopy transmission (TEM) and Western blot utilizing CD81, CD9 and CD63. The iHMCs or LLCP-K_1were treated with LPS (100µg/ml) or PBS (vehicle) during 72 hours. Additional iHMCs and LLCP-K_1 groups were treated with LPS+Vs nPC (35µg/ml) or PC (35µg/ml) for 24h with LPS, G or Cis. At the end of these treatments, cells were utilized by cellular viability for Tripan blue, acridine orange and caspase 3 cleavages. Also, IL2, IL6 and IL10 were evaluated by Western blot and ELISA assays and NO production by Griess method.
The rPSCs positive were marked by Wnt1, CD24, PAX2 and ZO1 for immunofluorescence and PAX2, ZO1 for FACS assays, and they are not marked for CD45, Thy-1 and pancytokeratin. Vs were positively marked to CD9, CD81 or CD63 by Western blot and TEM. The treatment with LPS decreased cell viability when compared to PBS 24h (150±7.9 *vs.*408±18.8; 82.3±7.6 *vs.* 97.8±9.3 %; $p<0.05$) or 72h (67.5±7.4 *vs.* 385±12.6; 74.3±6.7 *vs.* 98.1±13.4%; $p<0.05$) by Tripan or acridine orange techniques, respectively. When the cells were treated with nPC Vs, it is observed an increase in cell viability at all treatment times 24h (312±7.4 *vs.* 150±7.9; 89.4 *vs.* 74.3±6.7%; $p<0.05$) or 72h (266±14.6 *vs.* 76.6±5.7; 88.4±4.9 *vs.* 76.6±5.7%; $p<0.05$) respectively, for both methods. The treatment with LPS 72h increased expression of cleavages caspase 3, IL2 and IL6 and decreased IL10 *vs.* CTL groups. The pro-inflammatory cytokines and caspase 3 expression decreases when iHMCs received nPC-Vs and increased IL10. NO production (nmoles/mg) increase in LPS *vs.* CTL groups 72h in iHMCs (82.8±9.5 *vs.* 171.2±27.8; $p<0.05$) and decrease in nPC-Vs (145.4±4.6 *vs.* 171.2±27.8; $p<0.05$) and PC-Vs with LPS, G or Cis for 24h, respectively (109.5±11.2; 115.8±5.8; 103.2±9.3; $p<0.05$). Similar results of NO were obtained with the LLCP-K1, NO increased in LPS vs. CTL groups (24.5±1.8 *vs.* 129.8±16.4; $p<0.05$) and decrease in nPC-Vs (129.8±16.4 *vs.*72.6±6.6; $p<0.05$) and PC-Vs with LPS, G or Cis for 24h, respectively (76.3±5.9; 41.7±5.1; 33.8±1.3; $p<0.05$).
These results suggested that the Vs derived from rPSC have the ability to protect the toxicity in iHMCs and LLCP-K_1 induced by LPS observed by apoptosis, cytokines pro-inflammatory and NO modulation. The protective effects were mediated by Vs-derived from rPSCs and intensified when the Vs-rPSC were previously stimulated by toxins, especially with different insult. In the present case the stimulation by Cis presented higher protective effects.
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Basic Research

149 - IN VITRO AND IN VIVO BIOCOMPATIBILITY OF NANOTEXTURIZED POLY (lactic-co-glycolic acid) WITH TACROLIMUS (FK506) FOR NERVE REPAIR

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The recovery of peripheral nerve injury that leads to variable degrees of functional losses is uncommon without surgical intervention. Different surgical techniques are applied for nerve repair. Among these, the tubulization allows the regenerative factors to be used into the scaffold to make a closer contact with the nerve ends. Biomaterials and neurotrophic factors are indicated as a therapeutic alternative for peripheral nerves reconstruction. Poly (lactic-co-glycolic acid) (PLGA) nanotexturized with sodium hydroxide (NaOH) and tacrolimus have positive effects on peripheral nerve repair.
Develop a nanotexturized absorbable tube made of poly (lactic-co-glycolic acid), and to evaluate its in vitro and in vivo biocompatibility with and without tacrolimus incorporation.
Nanofunctionalization of absorbable PLGA films were made with the addition of NaCl (sodium chloride) and NaOH (sodium hydroxide). Tacrolimus (3% w/v) was associated during polymer preparation. The in vitro cell viability was obtained by NIH 3T3 cell assays: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to evaluate the biomaterial cytotoxicity (1, 2, 7, 14 and 30 days). After 24 hours, DAPI staining (4, 6-diamidino-2-phrnykinodole) was performed to measure cell adhesion on the films by fluorescence imaging. To evaluate the in vivo biocompatibility the biomaterial was implanted in the paralombar region of rats randomized in two groups: T1=PLGA (30 rats), and T2=PLGA+tacrolimus (30 rats). Three rats were used as a control (without implants). After 7, 14, 21, 30, and 90 days, the surrounding tissue was removed and stained with hematoxylin/eosin and picrosirius for histological evaluation.
The in vitro cell viability of PLGA films, associated or not with tacrolimus, was different when compared with the controls ($p=0.000$). Regardless the culture period, biomaterials containing tacrolimus proved to better sustain cell viability ($p=0.026$) when compared with the biomaterial alone. Acute inflammation was detected by in vivo biocompatibility evaluation in the implants surrounding tissue although with lower intensity over time. Collagen deposition was detected since the seventieth day after surgical procedure and increased up to the ninety day ($p\leq0.020$).
Nanotexturized PLGA alone or associated with tacrolimus is biocompatible, sustain cell adhesion and growth. FK506 seems to decrease the inflammatory response to implant and surgical procedure. Therefore is possible to apply this strategy to improve nerve regeneration.

Basic Research

150 - THE EFFECT OF MESENCHYMAL STROMAL CELLS ON CARDIAC MITOCHONDRIAL RESPIRATION IN A MURINE MODEL OF ACUTE MYOCARDIAL INFARCTION

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Acute myocardial infarction (AMI) remains an important cause of death and heart failure worldwide. Myocardial ischemia is characterized by severe hypoxia, alterations in ion homeostasis and mitochondrial dysfunction. Inhibition of mitochondrial respiration results in changes in contractility stimulates the production of reactive oxygen species and causes cell death. Cell therapy with mesenchymal stromal cells (MSCs) has therapeutic potential in AMI due to their paracrine effects, secretion of vesicles and mitochondrial transference.
This study aimed to evaluate the effect of MSCs from bone marrow on the mitochondrial respiration of infarcted cardiac tissue in a co-culture model.
Female Wistar rats between 8 to 10 weeks of age underwent to permanent left anterior descending artery ligation (n=4). Age matched sham procedures (n=5) were used as a control. Confirmation of AMI was performed by electrocardiogram and were only included in the study rats that showed pathologic Q wave and ST-elevation. In mitochondrial respiration assays, rats were euthanized 24h after AMI; slices of heart tissue (about 6 mg in triplicate) from infarcted zone (IZ) and border zone (BZ) were obtained with a Tissue Chopper and maintained in co-culture with MSCs (3×10^5) for 24h using a Transwell system. Slices (previously co-cultured or not with MSCs) or MSC alone (1×10^5) were placed into the chambers of an Oroboros Oxygraph-O_2k to quantify their respiration.
After 24h of culture BZ and IZ slices (2.2 ± 0.6 and 0.9 ± 0.5 pmol O_2 s- mg-1 respectively) from infarcted rats presented a decrease in mitochondrial respiration when compared to sham group (3.5 ± 0.4 pmol O_2 s- mg-1). Treated MSCs-IZ slices from infarcted rats showed no difference in mitochondrial respiration when compared to non-treated slices (1.3 ± 0.3 vs. 0.9 ± 0.5 pmol O_2 s- mg-1; $p>0.05$). However, treated BZ slices presented an improved mitochondrial respiration when compared to non-treated BZ slices (3.3 ± 0.4 vs.2.2 ± 0.6 pmol O_2 s- mg-1; $p<0.05$). MSCs alone, co-cultured with sham or with BZ slices showed an O_2 consumption of 129.6 ± 6.4, 91.9 ± 12.4 and 83.4 ± 16.6 pmol O_2 s- mg1 respectively with significant difference between MSCs alone and MSCs in co-culture with BZ slices and with sham group ($p<0.05$). Interestingly, MSCs showed barely detectable mitochondrial respiration when co-cultured with IZ slices.
Co-culture with MSCs improved basal mitochondrial respiration in BZ but not in IZ infarcted heart, suggesting that the MSCs mechanism of action involves a paracrine effect, which is able to preserve only the less affected region. The conditioned medium recovered after co-culture of MSCs with IZ affected MSCs respiration, suggesting the existence of a cross-talk between the infarcted tissue and MSCs.

Basic Research

151 - MESENCHYMAL STROMAL CELLS FROM DIFFERENT NON INVASIVE SOURCES TO BE USED IN BONE TISSUE ENGINEERING FOR CLEFT LIP AND PALATE PATIENTS

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Cleft lip and palate (CLP), one of the most frequent congenital malformations, which affect about 2-3% of newborns, and the reconstruction of this defect still represents a challenge in the rehabilitation of these patients. The gold standard in alveolar bone reconstruction is autogenous bone grafts. However, these surgical procedures may be subjected to complications such as donor area morbidity, post-surgical reabsorption and infections. To circumvent these problems, researchers have focused on bone tissue engineering as alternative methods.
The purpose of this study is identify a non-invasive alternative source of stem cells with osteogenic potential without conferring morbidity to the bone donor area.
To isolate the mesenchymal stem cell from non-invasive sources we have used dental pulp (DP) obtained from deciduous teeth and orbicular oris muscle (OOM) fragments of CLP patients, which are regularly discarded during surgery repair (cheiloplasty) to make bone tissue engineering. We obtained cells from DP and OOM fragments, of CLP patients using previously described (Bueno et al, 2009) pre-plating technique. The mesenchymal stem cells were characterized through flow cytometry analysis. They were also induced, under appropriate cell culture conditions, to chondrogenic, adipogenic, osteogenic, and skeletal muscle cell differentiation, as evidenced by immunohistochemistry. To evaluate “in vivo”, osteogenic potential these cells associated with biomaterial were transplanted to craniofacial bone defect in animal model.
The mesenchymal stem cells obtained from non-invasive sources (DO and OOM), through flow cytometry analysis, were positively marked for mesenchymal stem cell antigens (CD29, CD90, CD105, CD73, CD166), and negative for hematopoietic and endothelial cell markers (CD45, CD34, CD31). After induction under appropriate cell culture conditions, these mesenchymal cells obtained from OOM and DP were capable to undergo chondrogenic, adipogenic, osteogenic, and skeletal muscle cell differentiation, as evidenced by immunohistochemistry. We also demonstrated that these cells together with a biomaterial lead to bone tissue reconstitution in animal model
In conclusion, we showed that cells from OOM, LPM and DP have phenotypic and behavior characteristics similar to other adult stem cells, both in vitro and in vivo. Our findings suggest that these tissues (OOM, LPM, and DP) represent promising sources of stem cells to be used in alveolar bone grafting treatment (bone tissue engineering), particularly in young CLP patients.

Basic Research

152 - EFFECT OF TRANSPLANTATION OF BONE MARROW MESENCHYMAL STEM CELLS IN MURINE MODEL OF DIABETIC PERIPHERAL NEUROPATHY

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Diabetes is a highly prevalent disease that often compromises the peripheral nervous system. Clinical characteristics of diabetic peripheral neuropathy include spontaneous symptoms, changes in sensitivity, hyperalgesia and allodynia, with patients often suffering from severe pain that can last for many years. Currently there is no gold standard for the treatment of neuropathic pain. Based on the potential of stem cells for functional reestablishment of the damaged nervous system, the cell therapy represents a promissory alternative to the neuropathic pain control.
Evaluate the potential of mesenchymal stem cells derived from bone marrow in a mice model of diabetic peripheral neuropathy.
Male C57BL/6 mice were injected intraperitoneally (i.p.) with streptozotocin (STZ) 80 mg/kg (Sigma) diluted in a citrate buffer. Negative control group received vehicle only. Mice were considered diabetic if glycemia were above 250 mg/dl. After 28 days, hyperglycemic mice were transplanted by orbital plexus injection with 1 x 10^6 cells/mouse in a final volume of 200 µl or saline (200 µl). Paw mechanical and thermal nociceptive thresholds were evaluated by using von Frey filaments and Hargreaves test, respectively. Nociceptive threshold, body weight, blood glucose level and motor function (rotarod test) were evaluated during the experimental period of 90 days. Animal care and handling procedures were in accordance with International Association for the Study of Pain guidelines for the use of animals in pain research and the Institutional Animal Care and Use Committee FIOCRUZ (L-IGM-025/09).
Blood glucose levels and body weight loss were reduced in diabetic cell-treated mice when compared to diabetic saline-treated controls during the follow up period. STZ-induced mechanical allodynia and thermal hypoalgesia was also reduced after the cell therapy. Sixteen days after transplantation, cell-treated diabetic mice exhibited nociceptive thresholds similar to that of non-diabetic mice, an effect maintained throughout the 90-day evaluation period. In addition, the cell therapy improved the motor function in diabetic mice.
The present study demonstrates that mesenchymal stem cells produces a powerful and long-lasting antinociceptive effect on diabetic neuropathy. Our results suggest stem cell therapy as an option for the control of diabetes complications such as intractable diabetic neuropathic pain.

Basic Research

153 - CHARACTERIZATION OF UNDIFFERENTIATED STEM CELL FROM EMBRYONATED EGGS OF Caligo illioneus illioneus

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Caligo illioneus illioneus, popularly known as eye owl is an important representative of Lepidoptera in Brazil, with Neotropical distribution. Few studies have been conducted with Caligo subspecies and the first descriptions of biology and immature stages were performed in British Guiana, from oviposition on leaves of sugarcane and banana, its targets of predation when in the caterpillar stage. Insect cell lines have been valuable tools since the discovery of the first stabilization by Grace (1962). More than 500 lines have been established until this year, and those that had higher success in stabilization and development were obtained from ovaries, hemocytes, midgut and adipose tissue. However, few studies showed positive results using lines of embryonic stem cells. These studies contributed to the advancement of knowledge about the physiology of the species described, using the in vitro propagation of pathogens, research on pest control and production of recombinant proteins.
This study aimed to obtain and characterize undifferentiated stem cells from embryonated eggs of Caligo illioneus illioneus obtained from primary culture using a specific cultivation, storage in liquid nitrogen and evaluated in vitro maintenance and expansion of a new protocol for culture stem cell of Lepidoptera.
Eggs were collected in Diadema Butterfly Vivarium, at Diadema, São Paulo and they were stored at room temperature. Eggs and embryos removed were submitted to ultrastructural morphological analysis by scanning electron microscopy. Subsequently, it was established a protocol for culture of embryonic stem cells. 2nd, 4th and 6th passages (P) of cells (at 7, 14 and 21 days of culture, respectively) were analyzed by flow cytometry in relation to their morphology and cell cycle phases.
The egg has rounded format, somewhat flattened at upper pole, with 2.0 mm diameter keeping the standard of Caligo genus for staining, milky white. Cell cultures from Caligo illioneus illioneus showed cells with globoid format, small and variable size, composing a heterogeneous population and unattached, distributed in formation of multicellular aggregates. The analysis of cell cycle phases G0-G1, S and G2-M at P2nd was 50.2%; 44.9% and 4.9%, at P4th: 19.6%; 65.8%, 14.7% and at P6th: 32.1%; 63.7% and 4.18%, respectively. The data obtained by analysis of cell populations distributed at different phases of cell cycle showed that the protocol established for cell culture was favorable in relation to significant yield at cell number, expansion and cryopreservation, confirmed by an increase in the proportion of cells in S and G2/M phases.
It was concluded that this new protocol is viable to obtain and expansion of stem cells and precursors to embryonated eggs from Caligo illioneus illioneus, representing a promising tool for further research in biotechnology, physiology and conservation of subspecies of Caligo.

Basic Research

154 - ARE CARDIAC NICHE-DERIVED SIGNALS SUFFICIENT TO INDUCE MESENCHYMAL STEM CELL DIFFERENTIATION?

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Stem cells are undifferentiated cells that are able to self-renew and give rise to multiple tissues; therefore, they are widely studied as a source for cell therapies in order to provide tissue regeneration and may represent a major technological breakthrough towards the treatment of several pathologies, including cardiovascular diseases. It is known that stem cells depend on a microenvironment whose function consists in controlling the cell behavior, providing several signals that may lead to differentiation in a defined cell type and the maintenance of cell function within the tissue. Among the several signals, there are those which are derived from extracellular matrix (ECM) proteins, and soluble factors such as growth factors and cytokines. Several studies indicate that these proteins are extremely important when it comes to promoting cardiomyogenic differentiation of stem cells.

In this study, we used ECM and soluble factors secreted by human cardiac cells; these cells, which actively migrate from explants of human heart tissue in culture, were previously characterized as a heterogeneous population of multipotent stromal cells and cardiac fibroblasts.

Our main objective is to evaluate the potential of these cells to secret trophic factors that may induce the cardiomyogenic differentiation of mesenchymal stem cells (MSCs) derived from human adipose tissue.

In order to respond these questions, culture of cardiac cells were decellularized to obtain ECM proteins and also conditioned medium (CM) of cardiac cells was collected for three consecutive days. Subsequently, MSCs derived from adipose tissue were culture on ECM with/without CM. In order to evaluate the influence of ECM and/or CM on stem cells behavior, cell viability (neutral red), cell death (Anexin-V/7-AAD) and cell differentiation assays were performed.

Cell viability of stem cells was not altered after 3 days of culture with any of the treatments and after 7 days, no death by apoptosis or necrosis was observed, therefore, ECM and CM were not toxic to the cultures. Similarly, after 7 days of culture, MSCs were evaluated for the presence of key markers of cardiac differentiation, suggesting that primarily in cells cultured on ECM with CM, the expression of cardiac proteins such as Gata-4, Troponin I and Alpha-actinin became more pronounced.

We believe that ECM and CM derived from cardiac cells may mimic the tissue niche by secreting factors that are able to induce cardiomyogenic pre-differentiation of MSCs derived from adipose tissue.

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The authors indicate no conflicts of interest.

Basic Research

155 - LABELING MESENCHYMAL STEM CELLS WITH METALLIC NANOPARTICLES FUNCTIONALIZED WITH DIMERCAPTOSUCCINIC ACID (DMSA).

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Assess if iron oxide and gold nanoparticles, both functionalized with 2,3-dimercaptosuccinic (DMSA) acid, can act as tracers for dental pulp mesenchymal stem cells (dpMSCs), allowing the visualization and tracking of these in a computed microtomography apparatus, based on an experimental model of pulmonary fibrosis.
a) The toxicity of nanoparticles on dpMSCs was assessed by MTT and Trypan Blue viability assays; by light microscopy morphological analysis; and by transmission electron microscopy (TEM) analysis. b) The visualization and measurement of nanoparticles Fe-DMSA uptake were performed by staining technique "Prussian Blue" and by colorimetric dosage of this dye, respectively. Visualization and uptake quantification of Au-DMSA nanoparticles were performed by confocal microscopy and analysis by optical emission spectrometry with inductively coupled plasma (ICP-OES), respectively. c) Changes in physiological parameters, such as osteogenic and adipogenic differentiation; proliferation rates; and lymphocyte suppression, were checked in labeled dpMSCs. d) A murine model of pulmonary fibrosis, induced by bleomycin, was employed to give theoretical support about the labeled dpMSCs' migration when inoculated by two routes of administration: intravenous and intranasal. e) After inoculation of $5x10^{5}$ labeled cells in mice, these were analyzed daily in the microtomograph in order to detect uptaken Fe-DMSA and Au-DMSA, checking their efficiency as tracers of dpMSCs.
There was no statistically significant reduction in the dpMSCs viability and characteristic signs of apoptosis were not detected after Fe-DMSA and Au-DMSA uptake, at all concentrations tested. Photos obtained by TEM confirmed the presence of these nanoparticles in the cytoplasm: while the Au-DMSA were close to the cell membrane, Fe-DMSA were observed in some organelles, especially mitochondria, causing structural changes there. Thus, there was mitochondrial toxicity and formation of apoptotic bodies, especially in dpMSCs exposed to Fe-DMSA. The uptake measurement tests indicated an average amount of 17 picograms (pg) of Fe-DMSA per cell and 4 pg of Au-DMSA per cell. There were also no significant changes in adipogenesis and osteogenesis; growth curves and inhibition of lymphocyte proliferation between labeled and unlabeled dpMSCs. Although the biocompatibility between both nanoparticles and cells was proved, Fe-DMSA as well as Au-DMSA could not be detected in microtomograph after being incorporated by dpMSCs.
In terms of biocompatibility, the use of Fe-DMSA and Au-DMSA as tracers for dpMSCs was assured. However, these metal nanoparticles shown to be not suitable for visualization and tracking of these cells in vivo by computed microtomography, considering that they were not detected the equipment, at the concentrations tested.

Basic Research

156 - INTRANASAL INSTILLATION OF MESENCHYMAL STEM CELLS FOR IDIOPATHIC PULMONARY FIBROSIS TREATMENT: AN EFFECTIVE ALTERNATIVE

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It is known that pulmonary alveoli – the structures responsible for gas exchange – are very delicate and frequently exposed to certain substances (e.g. chemicals, medicines, and smoking) that may injure them. As a result of successive lesions in lung alveoli, many patients develop Idiopathic Pulmonary Fibrosis (IPF). Recently, some studies have suggested the therapeutic potential of Mesenchymal Stem Cells (MSC), demonstrating that these are able to migrate toward the injured sites and attenuate disease evolution. However, there is no agreement about the amount of arriving and remaining MSC in lungs after their administration.
The present study aimed to evaluate the efficacy of intranasal administration of MSC in a murine model of IPF, comparing it with intravenous route.
Initially, saline solution (control group) or bleomycin sulphate solution (6mg/kg) were administered intratracheally into black mice (C57BL6) in order to induce of pulmonary inflammation. Then, 24 hours later, MSC labelled with iron oxide nanoparticles coated with DMSA (Fe–DMSA) were injected into the tail vein or administrated by intranasal instillation ($5x10^5$ MSC per animal). During seven days, mice's weights were measured. On the eighth day, the animals were euthanized and their lungs were collected for histological analysis; moreover, the staining techniques employed were hematoxylin-eosin (H&E) (to verify alveolar structure changes), Gömöri trichrome (collagen deposition) and Prussian blue staining (to detect MSC labelled with Fe-DMSA).
Our results firstly showed a significant weight loss of the animals that received MSC intravenously in comparison with the control group; on the other hand, animals treated intranasally had their weight constant. Since this reduction suggests IPF progression in animals, histological analysis was then performed in order to confirm it: the alveolar structure in intravenously treated animals was thickened; furthermore, there were several lymphocytes infiltrates. Secondly, as expected, were found more MSC in intranasally treated mice's lungs, usually next to the bronchioles. The alveolar integrity verified in these animals suggests the MSC effectiveness in diminishing the inflammatory effects. Interestingly, intravenously treated animals had marked MSC in abundance in lymphnodes, suggesting that this route of administration fails by retaining large quantities of cells in these organs, thus preventing their arrival in the injured lung tissue and permitting the IPF advancement. A fact that does not happen in intranasally treated animals, since this route is probably the most direct.
In summary, administration of MSC by intranasal instillation is a promising alternative for the treatment of IPF considering that it allows the cells to migrate more easily and directly towards lesioned lung alveoli.

Basic Research

157 - DENTAL PULP STEM CELLS AS POTENTIAL MODEL FOR NEUROFIBROMATOSIS TYPE 1 INVESTIGATION

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Neurofibromatosis type 1 (NF1) is the most prevalent among the genetic disorders, among the neurofibromatoses, which are characterized by high probability of developing nervous system cell neoplasia. The core findings in NF1 are the neurofibromas, tumours developed on the peripheral nerve sheathes, in addition, the usual signs are: presence of hyperpigmentation areas; iris Lisch nodules; optic nerve glioma; and severe bone deformities (50% of the cases). This phenotype is described to be expressed either in a inherited dominant way due to a mutation in the neurofibronin (NF) gene located on chromosome 17q11.2, or as a de novo mutation in the same gene, leading to lack of cell cycle control. However, clinical findings in NF1 are highly variable in presence and intensity, hindering the prediction of future symptoms, prognosis, as well as in proposing a palliative treatment. Thus, arises the necessity to create suitable models, which can be used to predict the patterns of pathogenesis in NF1. Adult stem cells (ASCs) are reported to have been used as biological models for a range of diseases. The dental pulp stem cells (DPSCs) are ASCs, which are particularly useful, since they are isolated from an easily extracted tissue, which is available during the early life of the patient.
Evaluate the use of DPSCs as a model for NF1 studies: investigation of cell proliferation rate and bone deformities.
We successfully isolated and characterized DPSC from the pulp of deciduous teeth of a 6-year old NF1 patient and two other healthy children of similar age. Cell proliferation was assayed by counting in haemocytometer after successively cell re-plating. In order to compare osteogenic differentiation, we used osteoblast-differentiating medium. and quantified by alizarin stain which relates to degree of calcification; and evaluated the expression of osteoblastic markers by RT-PCR.
The DPSCs isolated from the NF1 patient displayed a greater rate of proliferation when compared to the control cells. Osteogenic differentiation occurred as expected for both NF1 and control, in what concerned cell morphology and osteoblast marker genes expression, ALP; BMP2; BMP4; OCN; and SPP1. However, alizarin staining denoted markedly lower calcification level in the cells from the NF1-diagnosed child, considering that less calcium deposits were visualised under light microscopy and a smaller amount of alizarin could be quantified by spectrophotometry after extraction from the stained cells.
DPSCs seem to be useful as a model for studying NF1 and predicting prognosis of patients, since their in vitro behaviour seems to mimic at least two features of this disorder – higher tendency to develop bone abnormalities and neoplastic cell proliferation. More tests are still to be done in order to evaluate the actual applicability of DPSCs in this sense.

Basic Research

158 - SYSTEMIC TRANSPLANTATION OF MESENCHYMAL STEM CELLS PROMOTE LOCOMOTOR RECOVERY AND TISSUE PRESERVATION AFTER COMPRESSIVE SPINAL CORD INJURY IN MICE

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Spinal cord injury (SCI) causes motor and sensory deficits that impair functional performance, and significantly impacts expectancy and quality of life. These functional deficits occur because of axonal degeneration, neuron and glial cells death and demyelination.
The aim of this study was to investigate the effect of the systemic transplantation of mesenchymal stem cell (MSC) as a treatment in a compressive spinal cord injury model.
For this purpose, we used adult female C57BL/6 mice that underwent laminectomy at T9 level, followed by spinal cord compression for 1 minute with a 30g vascular clip. One week after SCI, characterizing a subacute lesion, the animals received an intraperitoneal (i.p.) or an intravenous (i.v.) MSC injection (8x105 in a volume of 500 µL) or vehicle (DMEM – 500 µL) as treatment. After transplantation, up to 8 weeks, we performed behavior testing using global mobility test and Basso Mouse Scale (BMS). After that, the animals were sacrificed and the samples were processed for light microscopy and immunohistochemistry.
The results of cell transplanted groups revealed an improvement on locomotor performance, including a better global mobility (MSC i.p.=5,53±1,06 cm/s, DMEM i.p.=2,96±1,33 cm/s, MSC i.v.=5,93±1,10 cm/s, DMEM i.v.=3,75±1,22 cm/s) and higher scores in BMS test (MSC i.p.=3,042±2,436, DMEM i.p.=1,083±2,662, MSC i.v.=3,458±2,511, DMEM i.v.=1,208±2,596) in both, i.p. and i.v. groups. These animals also presented better white matter preservation in comparison to DMEM group (MSC i.p.=51,84±1,30%, DMEM i.p.=42,53±0,99%, MSC i.v.=51,60±1,85%, DMEM i.v.=43,67±0,48%) and the semithin analysis revealed several preserved nerve fibers (MSC i.p.=969,3±140,5, DMEM i.p.=450,3±82,2, MSC i.v.=991±141,1, DMEM i.v.=441±57,6), and these fibers presented higher caliber (MSC i.p.=184,3±24,31 µm2, DMEM i.p.=73,65±6,38 µm2, MSC i.v.=237,3±32,07 µm2, DMEM i.v.=55,84±3,79 µm2) and higher axonal (MSC i.p.=69,42±9,73 µm2, DMEM i.p.=37,78±5,47 µm2, MSC i.v.=97,53±8,28 µm2, DMEM i.v.=29,99±1,21 µm2) and myelin (MSC i.p.=114,8±14,57 µm2, DMEM i.p.=35,87±3,91 µm2, MSC i.v.=139,8±25,82 µm2, DMEM i.v.=25,85±4,01 µm2) areas. In addition, in the treated groups it was found higher levels of trophic factors (MSC i.p.=2,24±0,42%, 1,2 ±0,35%, 1,99±0,46%, 0,58±0,08%, DMEM i.p.=0,26±0,04%, 0,28±0,07%, 0,41±0,10%, 0,15±0,03%, MSC i.v.=1,50±0,25%, 1,85±0,30%, 1,47±0,43%, 0,54±0,09%, DMEM i.v.=0,31±0,06%, 0,61±0,14%, 0,19±0,03%, 0,24±0,04% for NT3, NT4, BDNF and NGF, respectively) and reduction in astrocytosis (MSC i.p.=3,86±0,44%, DMEM i.p.=7,35±1,52%, MSC i.v.=2,66±0,25%, DMEM i.v.=5,84±0,82%).
So, our results suggest that the therapies used in this work showed beneficial effects, indicating that this treatment increased white matter sparing, nervous fibers preservation and contributed to functional recovery. In addition, we can also conclude that systemic transplantation of mesenchymal stem cell is a feasible choice for SCI treatment.

Basic Research

159 - CO-CULTURE WITH MESENCHYMAL STEM CELLS COULD PROMOTE HUMAN ISLET SURVIVAL IN VITRO

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Islet transplantation is a promising treatment for patients with type 1 diabetes mellitus (T1DM) with an instable metabolic control. However, the yield of viable pancreatic islets isolated from a single donor is negatively affected by the inflammatory environment related to the donor's brain death (BD) and also due to the stress caused by the isolation procedure per se. In this context, in vitro co-culture of islets with mesenchymal stem cells (MSCs) might improve the quality of the isolated islets since MSCs have cytoprotective action, attenuating inflammation and decreasing apoptosis of the isolated islet from a BD-donor.
To investigate whether co-culture of human pancreatic islets with adipose derived MSCs, in an indirect contact environment, can improve islet viability in vitro.
Human islets were isolated according to the method described by Ricordi (1989). MSCs were isolated using the protocol established by Zuk (2001), from lipoaspirates of patients who were not undergoing plastic surgery. All patients (for adipose tissue samples) or donor's relatives (for pancreas) signed an informed consent form. Islets were cultivated alone or in indirect contact with MSCs (passages P5-P6), using an insert in 6-well plates. Islet viability was determined after 24h, 48h and 72h of culture by the evaluation of the cell membrane integrity after FDA and PI dye incubations and counting of at least 50 islets by condition using a fluorescence microscope.
Islet viability after 24 hours of culture was 98.4 ± 3.0 (for the group of co-culture with MSCs) and 92.5 ± 20.7 (islets alone-group); after 48 hours was 93.6 ± 16.6 and 88.9 ± 22.0, and after 72 hours was 91.3 ± 16.7 and 87.6 ± 19.8, respectively.
Our preliminary results indicate that the co-culture of pancreatic islets with adipose derived-MSCs promotes an improvement in islet viability, which might reduce the loss of these cells during isolation and transplantation procedures, and further improve islet transplantation outcomes.

Basic Research

160 - DISTRIBUTION OF CANNABINOID RECEPTORS IN HUMAN CEREBRAL ORGANOIDS

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The endocannabinoid system is involved in several neurodevelopmental processes such as differentiation and migration. An imbalance in this process may contribute to the development of neuropsychiatric disorders.
The aim of this study is to characterize the distribution of cannabinoid receptors within 3D neuronal cultures compared with adherent cells.
Cerebral organoids recapitulate formation of the human cortex and can, therefore, contribute to developmental biology as well as disease modeling studies. Organoids were prepared as described by Lancaster (2013) and the entire process was carried out in spinners. Shortly, MatrigelTM embedded embryoid bodies (EB) were grown in a neuronal differentiation medium containing retinoic acid. Cannabinoid receptor distribution was analyzed by immunocytochemistry to verify their co-expression with mature neurons.
Our preliminary unpublished results show that cerebral organoids express CB1 cannabinoid receptors in cortical plate-like zones, similarly to what was previously observed in rat pups (Vitalis et al, 2008) and cerebral tissue of human fetuses (Zurolo et al, 2010). The advantage of this in vitro method is that it resembles the human brain development. We are confident that this approach will be game changing for the field, specifically to study disease associated with neurodevelopment and abnormal cannabinoid receptor express in cerebral tissues.
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Basic Research

161 - OXYGEN MATTERS: CULTURING HUMAN NEURAL PROGENITOR CELLS IN PHYSIOLOGICAL CONCENTRATIONS OF OXYGEN

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In order to properly model diseases using human embryonic stem cells and their derivatives, they should be grown, in vitro, in conditions as similar as possible to its original niche, in vivo. In this sense, research groups are now analyzing the consequences of physiological oxygen concentrations to pluripontent stem cells and derived neural cells. However, so far, they have barely described the metabolic alterations suffered by these cells.
In this work, we aim to investigate cellular and metabolic changes of human pluripotent stem cell (hPSC)-derived neural progenitors grown under physiological oxygen concentrations.
For this purpose, neural progenitor cells (NPCs) were generated from hPSCs and then introduced into a 3% oxygen (hypoxia) controlled environment for 18 days. The NPCs were then characterized and assessed for growth, as well as glicolytic and oxidative metabolism.
Although the cells grown under hypoxia maintained their neural progenitor markers and growth rates similar to NPCs under normoxia (21% oxygen), they had a 42% decrease in mitochondrial content, but presented 2 times increased oxygen consumption. Furthermore, their mitochondria were 2, 7 times more efficient in producing ATP, which resulted in a 30% decrease in rates of oxygen reactive species production.
Our results indicate that the environment in which human NPCs are cultured influence their oxidative metabolism and oxidative stress. We propose that oxygen concentrations should be reduced in vitro so that NPCs and hPSCs are exposed to a similar niche they experience in vivo, making them more suitable for disease modeling.
Financial support:CAPES, CNPq, BNDES, FAPERJ, Ministério da Saúde, FINEP

Basic Research

162 - EXTRACELLULAR VESICLES DERIVED FROM HUMAN CARDIAC TISSUE MODULATES IMPORTANT CELLULAR PROCESSES INVOLVED IN CARDIAC REGENERATION

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Cardiovascular diseases are the leading causes of mortality and morbidity throughout the world. Alternative approaches are under study to recover cardiac tissue after injury as for example cell therapy. More recently, a sub-product of stem cells – extracellular vesicles (EVs) – is under investigation. However, EVs obtained from whole tissue as human heart has not been characterized yet.

In this work, we standardize, isolate and characterize extracellular vesicles derived from conditioned medium of human cardiac explants (hC-EVs).

The hC-EVs were evaluated functionally in three cell types of interest for cardiac regeneration: mesenchymal stem cells (MSC), endothelial progenitor cells (EPC) and a rat cardiac myoblastic cell line, H9C2. In addition, conditioned medium of human cardiac explants depleted of hC-EVs was used as control for functional tests (WEV, without EVs). The size of hC-EVs was distributed between 50-350 nm. By using transmission electron microscopy it has been shown, either by morphology or by the presence of CD63 and CD9 in the membrane of many vesicles, that our samples of hC-EVs contain exosomes. In addition, we detected by immunostaining subpopulations of microvesicles derived from plasma membrane of MSC/fibroblasts and from plasma membrane of cardiac fibroblast.

Gene ontology analyzes using a set of proteins of hC-EVs identified by mass spectrometry showed a single term GO with statistical reliability related to oxidative stress. In functional assays, the treatment of MSC with hC-EVs or WEV in the concentration of 10µg/mL increased cell proliferation, did not affect cell death and, both treatments provide a quite moderate migration of MSC. In addition, MSC have positive labeling for cardiac protein GATA-4 when induced to cardiomyogenic differentiation mainly with hC-EVs. Angiogenesis assays using CPE showed that the hC-EVs have the potential to positively modulate the formation of vessels, which remain assembled for 24 hours. In H9C2 cells, hC-EVs do not increase the proliferation; however, a significant percentage of cells are rescued from cell death induced by oxidative stress. Finally, H9C2 cells induced to myogenic differentiation exhibited positive labeling for both GATA-4 and Troponin I and, while hC-EVs did not affect their differentiation potential, WEV negatively affected cardiac differentiation as determined by quantitative analysis of Troponin I fluorescence.

Thus, our results show for the first time the presence of a rich variety of EVs in conditioned medium of human heart explants and their therapeutic potential for cardiac regeneration.

163 - MRI-BASED APPROACHES TO QUANTITATIVELY STUDY STEM CELL TRAFFICKING AND FUNCTION IN VIVO

Eric T. Ahrens (University of California at San Diego)

Magnetic resonance imaging (MRI) is experiencing a rapid expansion in technologies to enable the visualization of stem cell populations in vivo. These capabilities are facilitated by the development of new imaging probes that tag stem cells prior to transfer or alter a cell's proteome to facilitate MRI detection.

This talk will first cover a new approach for stem cell tracking developed in our lab called 'in vivo cytometry,' where cell populations of interest, such as stem cells, can be tracked and quantified in vivo.

We formulate novel perfluorocarbon (PFC) emulsions to label cells ex vivo. The labeled cells are then introduced into the subject and their migration can be monitored using fluorine-19 (19F) MRI. The 19F images are extremely selective for the labeled cells, with no background signal from the host's tissues. Moreover, the absolute number of labeled cells in regions of interest can be estimated directly from the in vivo 19F images. Additionally, the PFC emulsion reagents have bio-sensing properties that report on the absolute level of intracellular oxygen and can potentially be used to monitor cell differentiation or apoptosis in vivo.

Our view is that in vivo cytometry will have a major impact in the clinical development of new generations of cellular therapeutics. We have been actively pursuing translation of in vivo cytometry to monitor the delivery and modes of action of cell therapies, and we will report on recent clinical trial results.

Looking ahead, MRI will be able to harvest the power of modern molecular biological tools to impart exogenous image contrast to living tissue in a cell-specific or event-related manner. This will be accomplished using transgenic and vector technologies to express reporter genes coding for paramagnetic metalloproteins. Towards this goal, I will describe efforts to develop and characterize new generations of nucleic-acid based MRI reporters that render cells paramagnetic and detectable in vivo. For example, MRI reporters can be used for labeling stem cells for long-term tracking in vivo. This talk will also address imaging hardware considerations for MRI cell tracking.

Pre-clinical Research

164 - HUMAN DENTAL PULP STEM CELLS ASSOCIATED WITH A BIOMATERIAL REGENERATES KNEE LESION IN SHEEP

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Osteoarthritis (OA) affects people worldwide decreasing their quality of life. The disease is caused by degradation of the articular cartilage, being often the knee's joint the most affected. In addition, the articular cartilage is avascular, thus presenting limited capability of regeneration. Cell therapy using stem cells (SC) has emerged as an alternative for the treatment of diseases that have no effective treatment. In this work we used stem cells from human dental pulp from exfoliated deciduous teeth (SHED) in association with biomaterials in order to treat osteoarticular injury in ovine model. The biomaterial was made mainly with chitosan and hydroxyapatite and was specially manufactured for this study at Friedrich-Baur-Forschungs-Institute Für Biomaterilien, in Germany.
Use SHED cell in association with a biomaterial to treat OA in knee of sheep model.
The sheep underwent medial parapatellar arthrotomy where were created four circular osteochondral defects in the femoral trochlea (6x6 mm). The defects created were immediately submitted to the following treatments: infusion of SHED associated with the biomaterial, just biomaterial, just SHED or no treatment (experimental control). After the surgery, the animals were monitored clinically and under image analysis (X-ray), ultrasonography (US) and arthroscopy. The surgical procedure was adequate, and the animals recovered one week after surgery.
The x-ray analyses revealed that the biomaterial was properly inserted into the cleft created in the animals. The US and arthroscopy analyses revealed that SHED associated with the biomaterial produced a reconstitution of damaged tissue, confirmed by histology. This study provided a protocol to cure OA. Moreover, the association of SHED with the biomaterial decreased significantly the time required for osteochondral regeneration in the animal, fact to be highlighted when considering the time for recovery in the patient.
The ovine model created to this study could be useful to study OA and SHED associated with biomaterial seems to be effective to treat OA. More studies should be carried out to elucidate the mechanisms involved in this model.

Pre-clinical Research

165 - THERAPEUTIC POTENTIAL OF G-CSF AND MESENCHYMAL STEM CELL OF BONE MARROW IN PULMONARY ARTERIAL HYPERTENSION INDUCED BY MONOCROTALINE IN RATS

Emanuelle F. Baptista (Universidade Federal do Rio de Janeiro (UFRJ)); Ana Paula F. de Oliveira (Universidade Federal do Rio de Janeiro (UFRJ)); Luciana M. Camilo (Universidade Federal do Rio de Janeiro (UFRJ)); Alysson R. S. Carvalho (Universidade Federal do Rio de Janeiro (UFRJ)); Antonio Carlos C. de Carvalho (Universidade Federal do Rio de Janeiro (UFRJ)); José Hamilton Matheus Nascimento (Universidade Federal do Rio de Janeiro (UFRJ))

Pulmonary arterial hypertension (PAH) is a disease characterized by increased pulmonary vascular resistance. PAH can be experimentally induced by monocrotaline (MCT) administration. G-CSF (granulocyte colony-stimulating factor) and mesenchymal stem cells (MSCs) appear as new alternatives for the treatment of PAH, by mobilizing hematopoietic progenitor cells and endothelial cells from bone marrow to the peripheral circulation, as well as MSCs by presented angiogenic and immunomodulatory effect.
This study aimed to evaluate the therapeutic potential of G-CSF and MSCs in experimental PAH induced by monocrotaline in rats.
Adult male Wistar rats were distribute in 5 groups (n=51): CTL (control), MCT (PAH), MCT+MSCs (PAH treated with MSCs), MCT+G-CSF (PAH treated with G-CSF) and MCT+MSCs+G-CSF (PAH treated with G-CSF and MSCs). Monocrotaline (60 mg/kg, i.p.) was injected 14 days before starting the treatments with G-CSF (50 µg/kg/day i.p.,) for 14 consecutive days and/or MSCs (5x106 cells) injected at day 14 post-MCT (via jugular vein). We analyzed the survival rate, body weight, electrocardiogram (ECG), echocardiogram (ECO), right ventricular systolic pressure (RVSP), systemic blood pressure (SBP) and organ weights (heart, lungs and liver).
The hypertensive rats (MCT) showed increased RVSP and right ventricular hypertrophy, increased QT interval, decreased SBP and weight of the lungs and heart corrected by body weight increased. Treatments with MSCs and G-CSF showed a tendency to higher survival rate compared with the MCT group. The MCT+MSC group showed body weight gain similar to CTL group and greater than the others. In ECG, all hypertensive groups showed higher QT interval not reversed by treatments. In ECO, the increase in RV area was not reversed by treatments. G-CSF and G-CSF plus MSCs reduced pulmonary vascular resistance indicated by increased TAP (acceleration time of pulmonary flow), TAP/ET (TAP/ejection time of pulmonary flow) and VTI (time velocity integral of pulmonary flow), and pulmonary arterial pressure (indicated by reduced RVSP) compared to MCT group. The treatments did not reverse the reduction in SBP and RV hypertrophy in hypertensive groups, although the ratio heart weight: body weight was decreased.
The treatment with G-CSF and MSCs alone or in association, decreased pulmonary artery pressure and showed a tendency for higher survival rate, but did not reverse the remodeling of the right ventricle.

166 - ANALISYS OF HUMAN ADIPOSE DERIVED STEM CELL AND DERMAL FIBROBLASTS IN BLOOD AND CHITOSAN SCAFFOLDS: A FOCUS ON CYTOKINES DISPOSAL

Helga C. Nunes (Universidade Estadual Paulista - Júlio de Mesquita Filho – UNESP); Ana Carolina P. Pasian (Universidade Estadual Paulista - Júlio de Mesquita Filho – UNESP); Henrique de S. Vieira (Universidade Estadual Paulista - Júlio de Mesquita Filho – UNESP); Michele J. A. Valerio (Universidade Paulista – UNIP); Flávia Cilene Alves (Universidade de Araras – UNIARARAS); Andrei Moroz (Universidade Estadual Paulista - Júlio de Mesquita Filho – UNESP); Elenice Deffune (Universidade Estadual Paulista - Júlio de Mesquita Filho – UNESP); Rosana R. Ferreira (Universidade Estadual Paulista - Júlio de Mesquita Filho – UNESP)

Cytokines are small glycoproteins,weighting from 8 to 30 kDa, important in cell signaling.They are produced by many types of cells,including cells located next to injury and immune cells through mitogen-activated protein kinases.Not as classical hormones,they are not stored as preformed molecules,acting especially by paracrine and autocrine mechanisms.They influence the activity,differentiation, proliferation,and survival of immune cells,as well as regulate the production and activity of other cytokines that can increase (proinflammatory) or decrease (anti-inflammatory)inflammatory response.Some cytokines can have a pro(Th1) or anti-inflammatory(Th2)actions,according to the microenvironment in which they are located.Among proinflammatory ones, we can mention interleukins (IL)1,2,6,7and TNF (tumor necrosis factor).Anti-inflammatory cytokines include IL-4,IL-10,IL-13,and TGFβ(transforming growth factor).Scaffolds (SC) are tridimentional structures developed to promote mechanical support and favorable microenvironment to cells,this technology opens new gates to regenerative medicine.Adipose tissue represents an accessible and promising source of adult mesenchymal stem cells (ADSCs) providing good number and concentration.This tissue is composed by a variety of cells like pre-adipocytes, adult adipocytes,fibroblasts,vascular fraction muscle cells,endothelial cells, monocytes, macrophages and limphocytes.
The aim of this study was to analyze the interaction between ADSCs, fibroblasts and three types of SCs developed in Botucatu´s School of Medicine Blood Center composed by fibrin glue, platelet gel and chitosan incorporated with platelet derived hormones.The study was designed to access and measure some cytokines (interleukine released on the medium supernatant of cells in contact with SCs.
Two samples of ADSCs and fibroblasts were cultivated into this SCs and the IL-8, IL-1β, IL-6, IL-10, tumorigenesis/necrosis(TNF), and IL-12p70 were accessed by Human Inflammatory Cytokines(CBA) BD®kit analysis using FCAP Array Administrator software BD®by flow citometry method.
During the medium change the supernatant of the cells seeding after 24, 48 and 72h was collected and frozen in -80ºC freezer. The results showed that all samples seeded in all SCs presented significant levels of IL-6 and IL-8. The expression of other cytokines didn´t changed significantly.Chitosan incorporated with platelet derived hormones SCs and platelet gel SCs on time 24h, presented the most significant IL-6 and IL-8 release. IL-6 and IL-8 were the cytokines mostly released by the two types of cells.These glycoproteins are known to have ambiguous and proinflammatory properties, respectively. It is important to point out that control scaffold (pure chitosan) showed inibitory effect on cell secretion.
In general it’s possible to conclude that all SCs were livable to cells allowing carrying them out its natural functions.

Pre-clinical Research

167 - EFFECTS OF RADIOTHERAPY AND THE USE OF MESENCHYMAL STEM CELLS ASSOCIATED WITH PLATELET RICH PLASMA ON DENTAL IMPLANT OSSEOINTEGRATION: EXPERIMENTAL STUDY IN BRAZILIAN MINIPIGS

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This study evaluated the effect of radiotherapy (RT) and the use of mesenchymal stem cells associated with platelet rich plasma (MSC+PRP) on dental implant osseointegration.
MSC from 12 male and adult Brazilian minipigs were isolated from the iliac crest and expanded in culture. The animals were divided into 3 groups: Group A (no irradiated), Group B (implants placement 15 days before RT) and Group C (implants placement three months after RT). The total radiation dose for each side of the mandible was 24 Gy. 48 implants were placed on the control side and 48 on the experimental side.
The implant loss rate in control and experimental sides were respectively 25.0% and 18.7% in group A (p=0.686), 31.2% and 25.0% in group B (p=0.686) and 68.7% and 68.7% in group C (p=1.000), with a statistically difference between the three groups at the control side (p=0.041) and at the experimental side (p=0.047). The percentage of bone-implant-contact in control and experimental sides were respectively 39.0 and 27.7 in group A (p=0.110), 20.9 and 16.7 in group B (p=0.347) and 16.0 and 7.1 in group C (p=0.310), with statistical significance between the groups at the control side (p=0.033) and at the experimental side (p=0.046). The bone density inside the threads in experimental and control sides were respectively 46.8 and 36.5 in group A (p=0.247), 29.3 and 24.1 in group B (p=0.379) and 21.0 and 11.6 in group C (p=0.421), with statistical significance between the three groups only at the control side (p=0.025).
The results showed a negative effect of RT on dental implant osseointegration. The use of MSC+PRP before the implant placement did not show any significant positive effect on peri-implant bone regeneration, although the implant loss rate was lower when the combination was used.

168 - RABBIT NEOTRACHEA TRANSPLANTATION PRODUCED BY TISSUE ENGINEERING

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Tracheal lesions whose length is more than 50% in adults and a third of the trachea in small children require curative treatment. The biological scaffolds from decellularized organs have been widely used in pre-clinical studies with animals and in clinical applications in humans.
The objective of this study is to compare the transplantation of the neotrachea produced by tissue engineering in rabbits using a decellularization protocol with 5 and 10 cycles.
The tracheae (1 cm) of the donor rabbits were decellularized with this protocol: freezing/defreezing + blue light-emitting diode (LED) irradiation with a dose of 90 J/cm2 at a total exposure of 60 minutes + sonication (Ultrasonic cleaner- Unique model USC 1400®) with 40 Khz during 10 minutes + sodium deoxicholate detergent 4% during 48 hours under 180 rpm agitation with 5 cycles and 10 cycles. After these treatments, it was realized the application of the mesenchymal stem cells, chondrocytes and smooth muscle cells from receptors rabbits on the outer face of the produced scaffolds. The surgeries were performed in 3 groups (n=4): Group 1 – transplantation of the decellularized tracheae with 5 cycles, Group 2 – transplantation of the decellularized tracheae with 10 cycles, Group 3 – Sham.
After 5 cycles of treatment, it was observed the presence of some cellular elements, mainly in the cartilaginous area. After 10 cycles of decellularization, it was observed the presence of some chondrocytes. All Group 1 animals died within 10-12 days after surgery showing difficulty breathing due to a fibrous proliferation with luminal obliteration. It was observed inflammatory reaction with lymphocytic infiltration in the transplanted area and also in distant organs such as liver and lung. These findings suggest graft-versus-host disease, due to the presence of cells from donors after 5 cycles of decellularization protocol. All the animals in group 2 and 3 are alive so far, 22 days after the surgery without difficulty breathing or signs of rejection. These animals did not lose weight, and will be sacrificed after 90 days.
This is the first report about the potential of LED decellularization in trachea scaffolds, a new LED application in tissue engineering. However, 5 cycles of decellularization protocol were not sufficient to promote a complete decellularization of donor rabbit’s cells causing induction of immune response of receptors rabbits. When 10 cycles were used, we observed a better result; therefore, it will be necessary further investigation after group 2 animals’ sacrifice.

Pre-clinical Research

169 - USE OF STEM CELLS AS THERAPY TO REGENERATE DAMAGED TISSUES: AN INTEGRATIVE LITERATURE REVIEW

Francisco A. Klank (Universidade Federal de Sergipe); José Ronaldo A. dos Santos (Universidade Federal de Sergipe/ Campus Lagarto); Thiago dos S. Valença (Universidade Federal de Sergipe/Campus Lagarto); Anne Julie F. de Souza (Universidade Federal de Sergipe/ Campus Lagarto); Emerson T. Fioretto (Universidade Federal de Sergipe)

This study aims to give to our reader, through a bibliographic review, a general view about the advances and perspectives in researches and treatments using stem-cells as therapy to regenerate damaged tissues.
This research was performed in these three data-bases: PubMed, SciELO and BVS. We searched for “tissue regeneration” term, the most recent researches and tests in humans was prioritized as the main criteria. After the articles’ selection, the texts was analyzed, independently, for two reviewers.
The research has returned 1.097 articles on SciELO, 175.459 on PubMed and 337.650 on Biblioteca Virtual em Saúde (BVS), published between 2004 and 2014. The research in these three databases was performed using these keywords: stem cells, stem cells and tissue regeneration. Thirty-six articles of four different themes in stem-cells therapy researches were selected: treatment of cardiac muscle tissue’s damages; osseous and cartilage tissue’s damages; nervous tissue’s damages and treatment of Diabetes Mellitus. In general, we hope soon the medicine field built sufficient knowledge and could regenerate permanently damaged tissues, recovering its cellular mass and the functional capacity. We could visualize in many articles exciting results and several case reports about satisfying tissue regeneration with good recovering of the typical physiology of the treated tissue, after experimental treatments with stem cells therapy.
We could conclude that due to the constants advances, many contemporary researches discuss what are the different types of stem cells that we can use in treatment of tissue’s damages and their different advantages and disadvantages either of discussing the possibility of treatment with stem cells. The scientists raise the possibility of produce in laboratory several cell types from iPS to use as raw material on possible therapeutic use in different diseases. Although we had noticed that there are many barriers that we need to overcome – like several elements that interact with the stem cells and influences the regeneration process, and that we must to better understand – it’s possible that the tissue’s bioengineering could start the beginning of a new medical age, characterized for expands the edges of cure in direction to a more dynamic and personalized medicine, in which the cell therapies will be a great part of our daily lives in health care.

170 - IS THERE CELL CYCLE INTERFERENCE OF YOLK SAC PLURIPOTENT CELLS IN INDUCED-DIABETES MODEL?

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Yolk sac is a source of pluripotent cells (PCs), which might be promising for therapies. PC(s) behavior on physiological disturbances has not been fully understood. Diabetes might trigger malformations like disturbances on endothelial proliferation and vascular growing.
Evaluate cell cycle expression in yolk sac PCs of normal and hyperglycemic rats at gestational day 15th.
Twenty-five female and five male adult Wistar rats weighing 200-250 g. Spermatozoa in vaginal smear determined 1st gestational day (gd). Ethics committee - CEPA 86/2011. Diabetes induced (12 hours of starvation) by single injection of alloxan (37mg/kg, i.v) on 8 gd. Control group received saline solution. Blood glucose level higher than 200mg/dl at 10 gd were considered diabetic. On 15 gd, rats from control (n=5) and diabetic (n=5) groups were euthanized. Five placentas per animal per group were collected; Yolk sac dissected and mechanically dissociated, filtered, added freezing solution (10% DMSO, 60% RPMI-1640, 30% fetal bovine serum) and kept in a -70ºC freezer. Cells were rinsed with PBS, pooled and centrifuged; pellet was resuspended in 5 ml of PBS at a concentration of 106cells/ml. Duplicate samples were submitted to flow cytometry (Scalibur-Becton Dickinson, San Jose, CA). DNA content in the cell cycle phases (sub-haploid, G0/G1, S and G2/M) was analyzed by the Cell-Quest software and by the ModFit LT 3.2 software (Becton, Dickinson, NJ, USA). All data are expressed as means and standard errors of means (SEM). Statistic analysis performed by One-way ANOVA followed by Tukey's Multiple Comparison test (significant value of $p < 0.05$)
Cell cycle analysis in all phases were similar for both groups, excepting for greater number of sub-haploid cells in diabetic group (20.26 ± 4.1 vs 10.37 ± 1.9, $p < 0.05$).
Sub-haploid cells rate, demonstrates loss of cell viability what might be explained by cellular and nuclear fragments, nucleus or micronucleus with normal content of DNA, but containing a different chromatin structure, as well as, apoptotic cells. Altered metabolic environment in maternal diabetes could affect the expression of genes that control the cell cycle. Diabetes might induce high rates of sub-haploid cells in yolk sac, however, more studies are necessary to verify the influence of hyperglycemia on mitotic regulators and cell differentiation pathways in yolk sac cells.

Pre-clinical Research

171 - TOXICITY AND BIOCOMPATIBILITY OF A NEW SCAFFOLD FOR STEM CELLS TRANSPLANTATION IN SPINAL CORD INJURY

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Spinal cord injury (SCI) occurs mainly in young and healthy individuals, causing temporary or permanent motor and sensory impairment which leads to chronic disability. An attractive possibility of treatment for SCI is the use of stem cells to restore neural cells in the spinal cord. Scaffolds for cell therapy must be able to provide structural support and a microenvironment to ensure viability to promote cell integration into the target tissue.
Developed a new polymer and evaluated its cytotoxicity, biocompatibility and safety when used over rats spinal cord injury.
Biocompatible polymeric films composed of polylactic acid-co-acid (PLGA) polymer nanofiber polypyrrole (PPy) were used. Nanofibers and films were synthesized with polypyrrole containing 10% (w/w) PLGA in PPy by solvent evaporation method. Cytotoxicity test were performed using the extraction method. Samples of the polymers were incubated in DMEM culture media according to established by ISO 10993 (2013) during 24, 48, 72 hours, 7 and 14 days. After these periods, NIH3T3 fibroblasts were cultured for 24 hours with extraction media and the cytotoxicity test by MTT assay were performed. The polymer was cut and placed into the laminectomy of the eighth thoracic vertebra of Wistar rats. The motor recovery was assessed 24 hours, 7, 14 and 28 days after injury. The degree of inflammation generated by the polymer in other tissues was performed by placing the biomaterial in the flank followed by histological evaluation.
There was no change in cell viability after cell seeding with the conditioned media exposed to the biopolymer. Cell viability was maintained at 100% when compared to the control. The rats motor functionality show no alteration due to the surgical procedure or possible inflammatory reaction on the spinal nerve tissue due to the biomaterial presence. Histological evaluation did not detect changes suggestive of inflammation or infection of the marrow tissue adjacent to the polymer or the flank in the animal tissues.
The polymer is safe to be used as a scaffold in the SCI rat model because it did not cause any functional, structural or inflammatory alteration in vitro and in vivo.

172 - RECOMBINANT GROWTH FACTORS EXPRESSED IN MAMMALIAN CELLS AS AN ALTERNATIVE APPROACH TO THE USE OF PRP (PLATELET-RICH PLASMA) FOR CELL THERAPY AND REGENERATIVE MEDICINE

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Tissue Repair involves three different phases, namely: blood clotting and inflammation, cell proliferation and tissue remodeling. Exogenous growth factors may be used for wound repair. By manipulating the growth factors composition, it is possible to accelerate or modify the process of regeneration and remodeling of damaged tissues. During inflammation, neutrophils and macrophages are recruited to produce TGF-β1 and G-CSF. Critical roles are played by PDGF and TGF-β1 in recruiting fibroblasts (proliferation phase). VEGF is essential for angiogenesis. During remodeling, epidermal proliferation is mediated by GM-CSF and TGF-β3. While TGF-β1 has a direct involvement in cutaneous scarring, TGF-β3 antagonizes this effect, avoiding excessive scarring. Platelet-rich plasma (PRP) has been employed to facilitate the healing process. Its major components include: PDGF, TGF-βs, FGF and VEGF. By applying PRP at the site of injury, a robust healing response is achieved. PRP also attracts mesenchymal stem cells (MSCs) to the injury site enhancing cell proliferation and migration. PRP is relatively simple and inexpensive to prepare; however, no controlled and reproducible protocol is available, each preparation displaying a different growth factor composition, affecting the clinical outcome. In addition, regulatory guidelines require animal serum replacement for cell expansion in Cell Therapy protocols, leading to the use of allogeneic PRP, which may elicit antibodies formation.

We are committed to producing recombinant growth factors, expressed in mammalian cell systems, to offer an alternative and safe approach to PRP for cell culture media and injuries treatment.

We have produced human PDGF-BB, TGF-β1 and VEGFs in mammalian cells. The cDNAs were amplified from a Human cDNA Bank, constructed in-house, and then cloned into the pGEM®-T-Easy vector. E. coli transformants were screened by colony PCR. Upon DNA sequencing, the cDNA inserts were transferred to an expression vector. HEK293 or 293T cells were transfected with recombinant plasmids containing PDGF-B, VEGF or TGF-β cDNA. PDGF-B and VEGF were co-transfected with a Hygr vector for clone selection, at a 40:1 ratio. Cell clones were selected with 100ug/mL hygromicin.

Overproducing clones were selected and gene expression was assessed by Western blotting and specific in vitro biological activity assays were employed each growth factor. PDGF and VEGF were purified using heparin affinity chromatography. FGF production and TGF-β1 purification are underway. Different concentrations of each factor will be tested to compose a special growth factor cocktail to treat an animal model displaying a tissue injury, in comparison with the PRP treatment.

This safe and alternative approach could greatly contribute to the Brazilian Cell Therapy Network, avoiding the use of PRP in Cell Therapy and Regenerative Medicine.

Support: BNDES, CNPq, FAPESP, FINEP, MCTI, MS-DECITSupport: BNDES, CNPq, FAPESP, FINEP, MCTI, MS-DECIT

173 - IMPROVEMENT OF THE PROTOCOL TO GENERATE DECELLULARIZED PANCREAS BIOSCAFFOLDS

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Type 1 diabetes mellitus (T1DM) results from the autoimmune destruction of pancreatic β cells, leading patients to require lifelong insulin therapy, but, often, this does not avoid the most common complications of this disease. Transplantation of isolated pancreatic islets from heart-beating organ donors is a promising alternative treatment for T1DM, however, this approach is severely limited by the shortage of pancreata maintained under adequate conditions. Recently, pancreatic bioengineering and Regenerative Medicine, have been proposed as potential alternative therapeutic strategies. Upon decellularization, the pancreas, becomes a perfect bioscaffold of extracellular matrix (ECM, retaining its original architecture and microenvironment. This bioscaffold constitutes an attractive 3D template for several studies aiming to restore the pancreas dual role, namely: endocrine and exocrine.
In order to improve the pancreas decellularization process, we modified the most widely used pancreas decellularization protocol, which involves Triton–X and Sodium Dodecyl Sulfate (SDS) detergents.
Cadaveric pancreata were isolated from adult rats (n=5). The hepatic portal vein and the pancreatic duct were canullated to allow detergents and enzymes perfusion and the distal end of the superior mesenteric vein and large branches of splenic arteries and veins were ligated to prevent leakage. Pancreas decellularization was achieved via the pancreatic duct using detergent-enzymatic treatment perfusion. The resulting matrices were analyzed for integrity of the vascular tree using methylene blue injection
The pancreatic tissue was perfused with detergent and enzymatic solutions. Apparently, complete decellularization of the rat pancreas was achieved, in approximately 31h, after only one cycle of this treatment, as compared to other pancreatic decellularization protocols, which employ more than one cycle and take about 48h). Our preliminary results are in accordance with the protocols described in the literature for decelullarization of other tissues. Our next steps include evaluating and characterizing the extracelular matrix integrity.
Rat cadaveric pancreas may be efficiently decellularized paving the way for its reconstitution with both acinar cells and islet cell components aiming at novel alternative treatment for T1DM based on Tissue Engineering.
Support: BNDES, CNPq, FAPESP, FINEP, MCTI, MS-DECIT.

174 - PRODUCTION OF RECOMBINANT ISOFORMS OF HUMAN VEGF-A (VASCULAR ENDOTHELIAL GROWTH FACTOR A) AIMING AT GENERATING BIOPHARMACEUTICALS FOR MOLECULAR THERAPY AND TISSUE ENGINEERING

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In the adult, new blood vessels are formed mainly through angiogenesis from the pre-existing vasculature. In healthy individuals, the vascular architecture is fairly static, but, on the other hand, both the excess and insufficiency of vessels constitute a pathological angiogenic state, to which is atributted the onset and/or progression of several diseases. Therefore, locally controlling the blood vessel density is important for treatment of pathological conditions aiming at prognosis improvement and cure. Among the various known growth factors, VEGF stands out as the major regulator of the angiogenic process, which is mediated through the action of pro- (VEGFxxx) and antiangiogenic (VEGFxxxb) isoforms derived from the VEGF-A gene. Consequently, the proteins encoded by this gene constitute potential therapeutic targets.
We set out to produce the rhVEGF165, rhVEGF165b, and rhVEGF121 recombinant protein isoforms, which originate from the human VEGF-A gene, in order to generate useful biopharmaceuticals for molecular therapy and tissue engineering.
The rhVEGF165 and rhVEGF121 coding sequences were amplified from total cDNA sythesized from human lung total RNA. Conversely, the rhVEGF165b coding sequence was generated by site-directed mutagenesis of the rhVEGF165 sequence. These sequences were cloned into the pGEM®-T Easy cloning vector. These cDNAs were then subcloned into the pLV-eGFP plasmid lentiviral transfer vector, that allows for expression of transgenes in conjunction with the eGFP reporter protein in mammalian cells. Human HEK293 cells cultured under adherent conditions were independently co-transfected with each of the constructs obtained along with the pTK-Hyg vector at a 40:1 ratio, thereby enabling neutral selection of transfectant cells with hygromycin B, independently from detection of eGFP. Cell clones overexpressing the proteins were evaluated expression levels under serum deprivation and adapted to static suspension culture in medium free of animal-derived components, demonstrating that expression of the protein isoforms was possible under these culture conditions. To the best of our knowledge, this is the first report describing the expression of VEGF-A isoforms in HEK293 cells in suspension culture. The rhVEGF165 and rhVEGF165b isoforms were purified by affinity chromatography from media previously conditioned by the overexpressing cell clones.
The biological (pro-angiogenic) activity of rhVEGF165 was demonstrated both in vitro, by the AngioPhaseTM Kit assay, and in vivo, using the CAM (chorioallantoic membrane) assay, both of which are suitable for evaluating the pro- and antiangiogenic activity of different compounds. However, the expected antiangiogenic activity for not observed for rhVEGF165b.
These isoforms were tested in a model of murine tissue-engineered small intestine, indicating their possible therapeutic contribution to Tissue Engineering and Regenerative Medicine.
Support: BNDES, CNPq, FAPESP, FINEP, MCTI, MS-DECIT

Pre-clinical Research

175 - BONE MARROW MESENCHYMAL CELLS IMPROVE HEART FUNCTION IN AN EXPERIMENTAL MODEL OF CARDIOTOXICITY ASSOCIATED WITH RADIATION THERAPY

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The therapeutic options for patients with cancer now include increasingly complex combinations of medications, radiation therapy (RT), and surgical intervention. Many of these treatments have important potential adverse cardiac effects and are likely to have significant effects on patient outcomes. Cell therapy appears to be promising for the treatment of chronic and degenerative diseases, including cardiomyopathy induced by RT, as the current therapeutic options are insufficient.
Evaluate the potential of bone marrow mesenchymal cells (BMMCs) in radioinduced cardiac damage
Female Wistar rats, 3 months old (Ethics Committee 054/14), were divided into 2 groups, non-treated irradiated group (IR n=15) and irradiated and BMMC treated (IRT n=10)). Echocardiography was performed to evaluate heart function. After euthanasia, 3 months post treatment; the left ventricle was removed and prepared for RT-qPCR (VEGF and Pro Collagen I) and histological (picrosirius) analysis.
In both groups, 45 days after irradiation, ejection fraction (EF) was in the normal range for these animals (> 70%). However, the BMMC treated group had EF (83.1%±2.655) while the non-treated IR group showed a significant reduction (76.1%±2.655) in relation to the treated group. In addition, we observed an increase in VEGF gene expression and a decrease in Pro Collagen I in IRT when compared to IR group. We also observed by histology that the collagen deposition was reduced in IRT (10.26%±0.8356) when compared to IR group (25.29%±0.9697).
Treatment with BMMCs was able to prevent ejection fraction reduction and collagen deposition in irradiated animals. The increase of VEGF and the decrease of pro collagen I gene expression might explain, at least in part, the cell therapy benefits.
All authors disclose no financial or personal relationships with individuals or organizations that could be perceived to bias their work.
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176 - OPPOSITE EFFECTS OF BONE MARROW-DERIVED CELLS TRANSPLANTATION IN MPTP-RAT MODEL OF PARKINSON'S DISEASE: A COMPARISON STUDY OF MONONUCLEAR AND MESENCHYMAL STEM CELLS

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The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model is a useful tool to study Parkinson's disease (PD).
This model was used in the present study to investigate the potential beneficial as well as deleterious effects of systemic bone-marrow mononuclear cell (BMMC) or mesenchymal stem cell (BM-MSC) transplantation.
Animals received MPTP (lesioned groups) or saline (sham groups) and were treated with systemic bone-marrow mononuclear cell (BMMC) or mesenchymal stem cell (BM-MSC) transplantation immediately or 24 hours after lesioning. Then, 24 hours later for animals receiving cells immediately or three or seven days after lesion for animals receiving cells 24 hours after lesion, the animals had their motor skills evaluated by means of the open field test and the force swimming test. Following, brain tissues were collected to investigate in the substantia nigra pars compacta (SNpc) the tyrosine hydroxylase-immunoreactivity (TH-ir), the presence of BMMCs or BM-MSCs previously labeled with CM-DiI cell tracker, the presence of inflammatory cells expressing CD45 via immunofluorescence, the morphology of Iba1+ microglial cells via immunohistochemistry and brain-blood barrier breakdown via the extravasation of Evan's blue dye.
MPTP administration resulted in a breakdown of the blood-brain barrier and motor impairment in the open field test 24 h after surgery. Three and 7 days after receiving the lesion, the injured animals showed remaining motor impairment compared to the sham groups along with a significant loss of tyrosine hydroxylase-immunoreactive (TH-ir) cells in the substantia nigra pars compacta (SNpc). The MPTP-lesioned rats treated with BMMCs immediately after lesioning exhibited motor impairment similar to the MPTP-saline group, though they presented a significantly higher loss of TH-ir cells in the SNpc compared to the MPTP-saline group. This increased loss of TH-ir cells in the SNpc was not observed when BMMC transplantation was performed 24 h after MPTP administration. In contrast, in the MPTP animals treated early with systemic BM-MSCs, no loss of TH-ir cells was observed. BMMCs and BM-MSCs previously labeled with CM-DiI cell tracker were found in brain sections of all transplanted animals. In addition, cells expressing CD45, an inflammatory white blood cell marker, were found in all brain sections analyzed and were more abundant in the MPTP-BMMC animals. In these animals, Iba1+ microglial cells showed also marked morphological changes indicating increased microglial activation.
These results show that systemic BMMC transplantation did not ameliorate or prevent the lesion induced by MPTP. Instead, BMMC transplantation in MPTP-lesioned rats accelerated dopaminergic neuronal damage and induced motor impairment and immobility behavior. These findings suggest that caution should be taken when considering cell therapy using BMMCs to treat PD. However, systemic BM-MSC transplantation that reaches the injury site and prevents neuronal damage after an MPTP infusion could be considered as a potential treatment for PD during the early stage of disease development.

Pre-clinical Research

177 - USE OF MAGNETIC RESONANCE IMAGING AND SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY FOR EVALUATION OF CELL THERAPY IN EXPERIMENTAL MODEL OF CHAGAS' DISEASE

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Chagas' disease is a leading cause of cardiomyopathy in Latin America. However, despite all the advances in the treatment of cardiovascular diseases, there is no effective therapy for chagasic cardiomyopathy. In previous work we observed that the injection of adipose tissue-derived mesenchymal cells (mASC) in acutely infected mice prevented right ventricular dilation and promoted a marked reduction in myocardial inflammation and fibrosis after infection with Trypanosoma cruzi (T. cruzi). For further inquiries about cardiac function and perfusion, it is essential to use in vivo analysis.

The objective of this work was to demonstrate whether Magnetic Resonance Imaging (RMI) and Single Photon Emission computed Tomography (SPECT) were able to evaluate mouse's heart infected with T. cruzi treated with mASC.

mASC were isolated from subcutaneous adipose tissue of transgenic mice expressing the green fluorescent protein gene under the control of the β-actin promoter through enzymatic digestion and maintained until third passage. Three days post-infection (3dpi) 106 cells were injected ip. Parasitemia was evaluated between 5 and 34dpi by parasite count in the peripheral blood. RMI studies measured ejection fraction (EF), end diastolic volume (EDV), end systolic volume (ESV) and ventricular area (Va) in both ventricles; and the cardiac perfusion was assessed by SPECT. Two-way ANOVA and Student's t test was used for statistical analysis.

Treatment significantly decreased the amount of parasites in Infec + mASC group compared to Infec + PBS (3.94±3.6 vs 10.37±7.06 x105 Trypomastigotes/mL of blood). MRI analysis showed that Infec + mASC group showed reduction Va, EDV and ESV right ventricle in comparison to the Infec + PBS group values (11.93±0.84 vs 16.04±0.84mm2; 43.06±3.97 vs 59.41±3.52µL; 24.62±3.66 vs 39.77±2.83 µL, respectively). In scintigraphy a discreet reperfusion was found. However, when we look for these fluorescent cells in the cardiac tissue, we found no sign of them.

In conclusion, MRI and SPECT were able to evaluate cardiac function and perfusion respectively. The images acquired showed that mASC treatment was able to prevent right ventricular dilation caused by the infection as well as increase myocardial perfusion.

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178 - BONE MARROW MONONUCLEAR CELL THERAPY IN GLOBAL CEREBRAL ISCHEMIA IN RATS: FUNCTIONAL ANALYSIS AND EFFECT IN NEURODEGENERATION, NEURONAL SURVIVAL AND REACTIVE MICROGLIOSIS

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The aim of our study was to investigate whether the object recognition test can be used to assess memory deficits observed in rats subjected to global cerebral ischemia (GCI) by four vessels occlusion (4VO) method. In addition, we investigated whether the bone marrow mononuclear cells (BMMC) treatment could increase neuronal survival and reduce the neurodegeneration and the inflammation observed in CA1 layer of ischemic animals.
Adult male Wistar rats were utilized. All procedures were carried out in accordance with the National Institutes of Health Guide lines for the Care and Use of Laboratory Animals and approved by the Institutional Committee for the Use of Experimental Animals (IBCCF192-06/16). For functional analysis, three experimental groups were evaluated: non-operated group; sham group and ischemic animals group. The test consisted of two sessions, in the first session two identical objects were placed in the arena and the rats had 10 min to exploit them. After 1 h, the animals returned to the arena, this time with a familiar object and a new object, which could be exploited by 5 min. The total time and percentage of exploration of objects were assessed. To establish the time course of CA1 neurodegeneration the rats were transcardially perfused 3, 7, 14, 21 and 90 days after ischemia (DAI). To analyze the effect of BMMC therapy in neurodegeneration, neuronal survival and reactive microgliosis in CA1 layer, ischemic animals received $3x10^7$ BMMC 3 DAI, in the left carotid, and were sacrificed different DAI. For quantification of neuronal degeneration, survival and reactive microgliosis, we counted Fluoro-Jade C, NeuN and ED-1 positive cells in CA1 layer, respectively.
Non-ischemic rats spent more time exploring the new object in the second test session. However, rats subjected to global ischemia showed no difference in the percentage time spent exploring the new object compared to the familiar object. We observed a greater number of FJC positive cells in CA1 layer of ischemic animals 7 DAI when compared with 3, 14, 21 and 90 DAI. The time course of neuronal survival shows a reduction of pyramidal neurons 7 DAI and in the days after. In the analysis of the effect of BMMC in neurodegeneration and neuronal survival, we observed a significant number reduction of FJC positive cells and increase of NeuN positive cells in CA1 of animals injected with BMMC when compared with ischemic animals injected with saline and sacrificed 7 DAI. Furthermore, we observed a lower number of ED-1 positive cells in ischemic animals treated with BMMCs compared with ischemic animals that received only saline.
We suggest that 4VO method induces recognition memory impairment in short-term assessed by the assay used. Furthermore, BMMC therapy in transient global ischemia has a neuroprotective effect in CA1 layer that was followed by reduction of microgliosis in this region.

179 - MESENCHYMAL STEM CELLS PRIMED WITH ENDOTHELIAL BASAL MEDIUM REDUCE ARTERIAL BLOOD PRESSURE IN A RAT MODEL OF SYSTEMIC ARTERIAL HYPERTENSION

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Previous data from our laboratory have demonstrated that syngenic transplantation of bone marrow mononuclear or mesenchymal stem cells (MSC) into spontaneously hypertensive rats (SHR) has anti-hypertensive effect, which seems to be related to endothelial dysfunction improvement.
The central aim of the present study was to evaluate whether priming of MSC for 72 hours with growth factors present in endothelium basal medium (EBM-2) was able to increase their anti-hypertensive effects in SHR.
Adult female SHR were treated with vehicle solution (n = 6), MSC cultured on conventional medium (DMEM plus 10% FBS, n = 11) or MSC cultured on conventional medium followed by 72 hours in EBM-2 medium (primed MSC, n = 10). The animals of both MSC groups have received an intra-peritoneal injection (i.p.) of 5 x 106 MSCs (expanded until 5th passage, primed or not) while control group have received physiological saline. Systolic arterial pressure (SAP) was monitored by means of tail occlusion method 5 days before and during 10 days after treatment. Following, for direct recordings of hemodynamics, all animals were anesthetized with tribromoethanol (250 mg/kg, i.p.) and had their right femoral artery catheterized. After 24-48 hours of surgical recovering, arterial blood pressure (ABP) was directly recorded during 60 minutes. At the end, under anesthesia (sodium thiopental, 40 mg/Kg, i.p.), intra-carotid injections of acetylcholine (3-25ng/Kg) or sodium nitroprusside (0,5-4µg/Kg) were performed in order to evaluate systemic endothelial function. At the end of the experimental protocol, heart weight was measured.
Indirect measurements of SAP have shown long lasting reduction (10 days) of tensional levels (around 10 - 15 mmHg), after i.p. administration of MSC cultured for 72 hours in EBM-2 medium. In addition, MSC cultured in EBM-2 transplantation have also provoked a decrease in direct measurement of mean arterial pressure compared to vehicle or MSC cultured in DMEM (147±14mmHg vs 165±12mmHg and 161±7mmHg, respectively, $p<0.05$), although the cardiac hypertrophy was not significantly reduced. The endothelial function tests showed an improvement in vasodilation response in animals treated with MSC primed with EBM-2 medium compared to vehicle or MSC cultured in DMEM injections (19.0±7.0% vs 12.6±2.4% and 12.1±5.0% for Ach 3.125ng/Kg, $p<0.05$; 22.1±5.3% vs 16.0±2.6% and 13.6±4.9% for Ach 6.25ng/Kg, $p<0.05$; 26.6±5.4% vs 19.4±3.5% and 20.1±3.9% for Ach 12.5ng/Kg, $p<0.05$; 29.6±6.0% vs 27.6±5.3% and 24.5±2.3% for Ach 25ng/Kg).
Taking all together, our findings seem to indicate that priming MSCs with endothelial basal medium boosts stem cell therapy to treat systemic arterial hypertension. This functional improvement of MSC could be relevant and desirable in a context of autologous transplantation since previous data from our laboratory have indicated that mesenchymal stem cells harvested from SHR donors present some biological dysfunctions.

Pre-clinical Research

180 - FUNCTIONAL EFFECTS OF CELL THERAPY WITH MESENCHYMAL STEM CELLS IN ELASTASE-INDUCED EMPHYSEMA IN MICE

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Chronic Obstructive Pulmonary Disease (COPD) can be described as progressive airflow limitation due to increased inflammatory response of pulmonary tissue to noxious particles. In the COPD spectrum, lung emphysema has as main feature the destruction of alveolar septa, with consequent air entrapment, breathlessness, diminished expiratory capacity and elastic recoil. Once a curative treatment is yet to be achieved, different animal models of emphysema have been employed in the study of new therapeutic approaches, such as mesenchymal stem cell therapy.
Functionally evaluate the effects of cell therapy with mesenchymal stem cells for elastase-induced emphysema in mice by means of plethysmography.
Female mice (25g, 8 weeks) were randomly divided into the following groups: a) Saline (n=4)– submitted to intranasal instillation of 3 doses of 50 µL of saline; b) Elastase (n=4) – submitted to intranasal instillation of 3 doses of 1.3 units of porcine pancreatic elastase in 50 µL of saline; c) Saline/Medium (n=4)– submitted to intranasal instillation of 3 doses of 50 µL of saline and, 21 days after, retro-orbital infusion of 100 µL of RPMI culture medium and d) Elastase/MSC (n=4)– submitted to intranasal instillation of 3 doses of 1.3 units porcine pancreatic elastase in 50 µL of saline and, 21 days after, retro-orbital infusion of 100 µL RPMI culture medium containing 2,6 x 107 mesenchymal stem cells/mL derived from male EGFP+ mice. At the 21th day after infusion, the pulmonary ventilation was analyzed via whole body plethysmography. Were measured the tidal volume (VT) and the respiratory frequency (FR) which possibility the calculation of pulmonary ventilation (VE). The mice were then euthanized by cervical dislocation for hystological analysis of the pulmonary tissue.
The animals subjected to emphysema induction developed morphologic and functional patterns consistent with pulmonary emphysema [Elastase – VE = 1451 ± 109 ml.kg-1.min-1; VT = 8.92 ± 0.9 ml.kg-1 and FR = 164 ± 11.0 breaths.min-1], and, after cell therapy with mesenchymal cells [Elastase/MSC – VE = 2012 ± 193.3 ml.kg-1.min-1; VT= 9.62 ± 0.79 ml.kg-1 and FR = 210 ± 24.5 breaths.min-1], the patterns became similar to those exhibited by the control groups [Saline – VE = 2062 ± 379.4 ml.kg-1.min-1; VT= 8.95 ± 1.5 ml.kg-1 and FR = 232.5 ± 41.1 breaths.min-1] and [Saline/Medium – VE = 2075 ± 364.5 ml.kg-1.min-1; VT = 9.82 ± 1.0 ml x kg-1 and FR = 210 ± 18.2 breaths.min-1].
The histomorphological pattern recovery of elastase-induced emphysema after infusion of adult stem cells has been previously reported by different authors, although no functional evaluations were reported. In this work, we show that mesenchymal stem cell therapy promoted hystological and functional recovery of pulmonary tissue after elastase-induced emphysema.

181 - EFFECT OF AGE AND GENDER IN THE SENSORIMOTOR RECOVERY PROMOTED BY THE TREATMENT OF FOCAL CEREBRAL ISCHEMIA WITH BONE MARROW MONONUCLEAR CELLS IN RAT

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Stroke is a disease of the elderly. However, most of the preclinical studies about the treatment of stroke with bone marrow-derived cells have used animals with age equivalent to young human, when the incidence of stroke is low. Thus, the studies with young animals might not be appropriated to design therapeutic approaches for middle-aged and old humans. Moreover, there are evidences that the gender is also a relevant factor on the level of injury, outcome and effect of therapies.
This study aimed to assess whether the sensorimotor recovery promoted by the treatment of focal cerebral ischemia with bone marrow mononuclear cells (BMMCs) is influenced by age and/or gender.
Male and female Wistar rats aged 2-5 months (young) and 12-17 months (middle-aged) were divided into eight groups: YM/BMMCs group, ischemic young male (YM) that received BMMCs from YM donors; YM/control group, ischemic YM that received vehicle; MAM/BMMCs group, ischemic middle-aged male (MAM) that received BMMCs from MAM donors; MAM/control group, ischemic MAM that received vehicle; YF/BMMCs group, ischemic young female (YF) that received BMMCs from YF donors; YF/control group, ischemic YF that received vehicle; MAF/BMMCs group, ischemic middle-aged female (MAF) that received BMMCs from MAF donors; MAF/control group, ischemic MAF that received vehicle. The animals were subjected to unilateral cortical ischemia by thermocoagulation of the superficial vascularization of the cerebral cortex. Twenty-four hours after ischemia, the animals received an intravenous injection of 3x107 BMMCs or vehicle (PBS). Survival rate was evaluated, and the cylinder and adhesive tests were conducted to evaluate the sensorimotor recovery. Additional YM, MAM, YF and MAF animals were euthanized approximately 24 h after ischemia to verify the cortical lesion extension.
The results showed that treatment with BMMCs resulted in sensorimotor recovery of young and middle-aged ischemic rats. No significant main effect of gender was found in the survival rate, ischemic lesion size and sensorimotor recovery promoted by the BMMCs. The effect of gender was only seen in the interaction with age in the cylinder test, where the group of middle-aged males had the worst performance. Moreover, the results showed that age was a significant factor, since the middle-aged animals had mortality rate and lesion volume higher than the young animals. In the cylinder, the promotion of sensorimotor recovery by the BMMCs was not influenced by age. However, age influenced the performance in the adhesive test, since the recovery promoted by the BMMCs was significantly higher in the young animals. Thus, despite the effect of aging on mortality and lesion size, it did not avoid the induction of sensorimotor function recovery by the BMMCs.
The results suggest that the treatment of stroke with BMMCs can be beneficial, irrespective of age or gender.

182 - TRANSPLANTATION OF MESENCHYMAL STEM CELLS IN A MURINE MODEL OF TRAUMATIC SPINAL CORD INJURY

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Spinal cord injury (SCI) affects millions of people and no effective treatment is available. SCI progression is characterized by changes in lesioned microenvironment, beginning with an inflammatory response and evolving to a fibrotic glial scar. Cellular therapy is a potential treatment option that could reconstitute the severed neuronal circuitry.
The aim of this study is to evaluate the effects of mesenchymal stem cells (MSC) transplantation in a SCI murine model, using bone marrow MSC over-expressing or not insulin-like growth factor 1 (IGF-1), a factor linked to neuronal functional repair.
Female C57Bl/6 mice (8 weeks) received a standardized contusion spinal cord injury (70 kDyn, 10 sec) at T9 following laminectomy. Paraplegic-state was confirmed by complete/near-complete loss of hind limb function. Body weight and BMS (Basso Mouse Scale) functional analyses were performed weekly, accompanying SCI progression. Mouse MSC were obtained from femurs and tibiae of GFP transgenic mice and were transduced or not to overexpress human IGF-1. Mice were transplanted with MSC-GFP+ (1×10^6) at three time points: immediately following SCI, 2 or 16 weeks post-injury. Transplanted cells were introduced directly into the injured site. Two days post-transplantation, SCI mice were euthanized and spinal cords were removed and analyzed to quantify and assess cell survival, measured by fluorescent microscopy.
Injured mice had loss of body weight and showed diferente degrees of functional recovery, as assessed by the BMS score. At the site of impact, spinal cord presented area of gliosis, as shown by histopathological analysis. We observed the presence of transplanted MSC into the injured site, showing that dynamic-injured microenvironments were receptive to transplanted cells. GFP+ MSC were observed within injured spinal cords when adminstered at the different time points evaluated, and presented a spherical phenotype. The number of cells found into the injured area decreased with time.
We demonstrate that the different time-dependent microenvironments observed following SCI appear to be receptive to transplanted MSC. Continued improvement in technical application of MSC should greatly enhance the quantifiable presence of transplanted cells and potentially improve the injured spinal cord.

183 - QUALITY ASSESSMENT OF SCAFFOLDS OBTAINED FROM BLOOD VESSELS DECELLULARIZED OF RABBITS

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Cardiovascular disease is the leading cause of mortality in the western population. The Peripheral Arterial Disease (PAD) is part of this group of diseases and is present in 5% of the population. In about 20-30% of these cases progress to amputation of the affected lower limb occurs. Have proposed the use of biological media derived from decellularized organs and tissue engineering to regenerative. The use of these natural materials have advantages: biocompatibility, maintaining conducive to development and cell differentiation microenvironment, are resistant to physiological absorbable and tensions. To this all cellular and nuclear material has to be removed, preserving the composition, biological activity and mechanical integrity of the extracellular matrix, without stimulating the rejection reaction. To achieve these goals it is necessary a thorough preparation of the material for the removal of cell debris potentially causing immune reaction in allogeneic recipient. Moreover, substances used in this process have yet to have minimal residual effects, not to inhibit cell growth after the scaffold implantation.
To produce decellularized vascular scaffolds obtained inferior cava vein of rabbit. Then, the comparison test scaffolds produced by histological analysis, immunohistochemistry, electron microscopy and molecular biology. Establishing this way, the safety of the use of scaffolds for use as re-cellularized graft.
The samples consist of inferior cava vein of 20 female rabbits, adult, non-pregnant, weighing between 2.5 and 3 kilogram. The decellularization protocols previously defined as best to promote the decellularization and now analyzed for quality were: 1) Deoxycholic sodium (DS) 2% with exposure time of 1 hour; 2) Sodium dodecyl sulfate (SDS) to 1% with exposure time of 2 hours; to a control veins in natura. Each treatment has three samples. And these were subjected to analysis by histology, H & E, Picrossirius, Masson's Trichrome, Calleja, Masson & Calleja; immunohistochemistry for Collagen Type III and IV. Similarly, were analysis by electron microscopy to investigate the integrity of the extracellular matrix. Furthermore, quantitative analysis of residual DNA was performed.
Histological analysis revealed elimination of cellular components and preservation of the extracellular matrix. Immunohistochemistry showed a significant reduction in collagen IV fibers and a more mild decrease of collagen III fibers in both treatments. Microscopy analysis confirmed that the collagen fibers maintained their integrity. Quantitative DNA analysis is underway.
The methods of decellularization of rabbit vena cava were effective in removing the cells and preserve the structure of the extracellular matrix in the protocols analyzed. Thus, the scaffolds produced are safe for use in tissue engineering.

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184 - INTRATRACHEAL INJECTION OF HUMAN MESENCHYMAL STEM CELLS IN AN ACUTE ASTHMA MODEL IN BALB/C MICE

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Asthma is a chronic inflammatory disease characterized by variable airflow obstruction, pulmonary eosinophil infiltration, mucus secretion and increased airway remodeling. In this context, cell therapy from mesenchymal stem cells (MSCs), could act in the inflammatory process and minimize tissue changes resulting from this disease.
To evaluate the effects of the intratracheal injection of human bone marrow-derived mesenchymal stem cells (MSCs) in the inflammatory process in an acute asthma model using BALB/c mice.
This study was approved by the local Ethics Committee (n.04425212.6.0000.0020 and 724). Twenty five male mice were divided into five groups: Control saline (CS), Control asthmatics (CA), 7 days saline (7dS), 7 days asthmatics (7dA) and 7 days treated asthmatics (7dTA). CA, 7dA and 7dTA groups were immunized with intraperitoneal injections of 10 µg of ovalbumin (OVA) on days 0, 2, 4, 7, 9 and 10. CS and 7dS received saline. On days 15, 18 and 21, CA, 7dA and 7dTA groups received intratracheal instillation of 20 µg of OVA, and CS and 7dS received saline. Twenty-four hours after the last challenge, 7dTA group was treated with an intratracheal injection of 1×10^6 MSCs, as well as 7dS and 7dA received the same volume of saline. CS and CA groups were euthanized on day 22 in order to ensure the model of asthma induction. On the seventh day after transplant, 7dS, 7dA and 7dTA were euthanized. The lungs were removed for histopathology. Histopathology analysis was carried out investigating 30 bronchioles, 15 of each lung, randomly selected. The analyses were semi-quantitative, applying a grading system used to calculate the ratio of inflammation severity (0%: absent or normal, 1-33%: mild, 34-66%: moderate and 67-100%: severe) according to the following criteria: thickening of the epithelium and epithelium desquamation, inflammatory infiltration, thickening of muscle layer, increased collagen fibers and mucus-production. Statistical analysis was performed with Kruskal-Wallis test followed by Dunn's test for multiple comparisons.
According to the results, CA group confirmed the presence of pulmonary inflammation on the day of transplantation, in mice subjected to the allergen, compared with the CS group. On the 7^{th} day after MSCs transplant, animals of the 7dTA group showed a significant improvement of lung histological architecture compared with the 7dA group: thickening of the epithelium (1.30±1.09vs.2.39±0.93, $p<0.0001$) and epithelium desquamation (0.66±0.7vs.1.36±0.92, $p<0.0001$), inflammatory infiltration (0.68±0.72vs.1.38±1.01, $p<0.0001$), thickening of muscle (0.79±0.95vs.1.86±1.08, $p<0.0001$), mucus-production (0.80±1.14vs.1.77±1.17, $p<0.0001$) and increased collagen deposition (1.06±0.9vs.1.79±1.0, $p<0.0001$).
The results demonstrated a reduction of inflammatory process in the asthmatic mice seven days after intratracheal injection of human MSCs, mainly concerning airway remodeling, suggesting a potential therapeutic use of MSCs in acute asthma.

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185 - IN VIVO ADIPOSE TISSUE-DERIVED STEM CELLS TRACKING ADMINISTERED BY DIFFERENT ROUTES IN WISTAR RATS

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Cell therapy with mesenchymal stem cells (MSC) has become the focus of numerous studies for providing clinical promising prospects in tissue recovery and it is easily isolated and cultivated. Their use in preclinical and clinical studies has shown good results, but it is necessary to optimize the therapeutic strategies. To complement MSC research, tracking of transplanted cells in vivo has been used. MSCs are transduce with luciferase gene, which protein product degrades D-luciferin and emits light that is captured by a high sensitivity camera.
The aim of this study was to track human adipose tissue-derived stem cells (ADSC) in healthy Wistar rats 0, 2 and 7 days after injection using different administration routes: intramyocardial, intraperitoneal and caudal.
All samples were collected after informed consent had been obtained in accordance with guidelines for research involving human subjects, and with the approval of the Ethics Committee of PUCPR (approval number 825 and 536842). The ADSCs were isolated by enzymatic digestion (collagenase type I). Cells were transduced with viral particles to express luciferase. An aliquot of the cells was evaluated in vitro (Lumina Ivis II) in the presence of D-luciferin (150µg/mL) to quantify the emission of the light signal. For in vivo evaluation (Ivis Lumina II) 1E+06 cells were injected per animal (n=3) for each administration route. For each evaluation, the animals received D-luciferin (150mg/kg) on intraperitoneal region 5 minutes before analysis. Luminescence was quantified in the region of interest using Living Image software.
Analysis in vitro has shown successful transduction of cells. In the in vivo analyses, the cells remained at the target site without dispersing to other organs or regions when injected by intramyocardial and intraperitoneal routes. When using caudal injection, cells were located in the tail and in the lung. Analysis of all administration routes showed a decrease of the light signal during evaluations, with nonappearance of light signal on day 7.
All routes of administration allowed the tracking of the injected cells. There was a decrease of the light signal during assessments in all animals.

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186 - BONE MARROW-DERIVED MESENCHYMAL STEM CELLS PRESENT REDOX-REGULATORY CAPACITY AND IMPROVE INJURY IN HEPATOCYTES

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Cell therapy using mesenchymal stem cells (MSC) has been extensively investigated for treatment of liver diseases. Although positive results have emerged, the number of viable transplanted cells present in liver tissue is frequently very low. Oxidative stress is associated with liver diseases in experimental models and clinical practice and may negatively impact on survival and effectiveness of transplanted cells.
Therefore our principal aim is develop a standardized protocol to prepare MSC for better adaptation into stressful hepatic environment and to overcome oxidative stress damage.
To verify the ability of MSC challenged to stressful conditions, we used culture medium with serum deprivation in the presence or absence of thioacetamide (TAA), widely used for oxidative stress-induced experimental chronic liver disease , or tert-Butylhydroquinone (tBHQ), an agent known to promote Nrf2, a transcriptional factor which has a crucial role in oxidative stress resolution. We evaluated levels of reactive oxygen species (ROS), necrosis, apoptosis, and mRNA production from several genes related to oxidative stress resolution. In order to check whether transplantation of these conditioned cells would provide positive gains in liver disease, we co-cultured hepatocytes with pre-conditioned MSC or their exosomes. Moreover, we investigated the role of miR-200a-3p in this phenomenon.
MSC from different sources demonstrated a high capability to the challenge oxidative stress conditions in vitro by decreasing of ROS in the presence of TAA or tBHQ. There were no significant differences in necrosis/apoptosis levels among the groups until 48 hours. Importantly, MSC submitted to these conditions showed higher amount of mRNA from genes induced by the antioxidant response element such as GST, Gclc and HO-1. Finally, Hepatocytes co-cultured with not only MSC, but also their exosomes, showed lower levels of ROS. Hepatocytes in which the miR-200a-3p target site was blocked showed significantly higher levels of ROS, despite supplementation with exosomes of MSC in vitro.
These results suggest that MSC have a great ability to face oxidative stress and can protect hepatocytes from injury in vitro by secreting exosomes including miR-200a-3p. We speculate that pre-conditioned MSC may be more compatible for engraftment into damaged tissue and improve liver injury by stabilizing redox homeostasis. These results open new perspectives into the development of a less invasive liver regeneration therapy for liver cirrhotic patients by using pre-conditioned autologous MSCs.

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187 - CELL THERAPY USING BONE MARROW MESENCHYMAL STEM CELLS (MSC) IN A CANINE MODEL OF CHAGASIC CARDIOMYOPATHY

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To investigate cardiac histology and cardiovascular functional parameters of Trypanossoma cruzi (T. cruzi)-infected dogs treated with bone marrow mesenchymal stem cells (MSC) in the chronic phase of the disease.
Mongrel dogs (4 months age) were infected with 2000 parasites/kg of VL-10 T. cruzi strain. Treatment was performed with 1-6x106 cells/kg of autologous MSC (auto-MSC) or non-infected allogeneic MSC (alo-MSC) with matched age. Cells were injected in peripheral vein 6-9 months post-infection. Left ventricle ejection fraction was assessed by echocardiography before treatment, 45 and 90 days post-treatment (dpt). After 180 dpt animals were euthanized and samples from different parts of the hearts were collected. Inflammation and fibrosis were evaluated using histological Haematoxilin & Eosin and Sirius Red staining, respectively.
Considering animal growth in our experimental model, we used the weight as normalization parameter for functional data. Placebo-treated animals had a significantly reduced ejection fraction when compared to non-infected animals in all time points analyzed. (Non-infected before therapy: 69.00±3.00; 45 d follow-up: 70.67±0.577; 90 d follow-up: 69.67±1.52%, N=3; Placebo before therapy: 49.17±3.54; 45 dpt: 50.33±5,75; 90 dpt: 53.83±9.76%, N=6). Auto-MSC intra-group analysis shows an increase of LVEF at 45 and 90 dpt when comparing the same animals before the treatment (Auto-MSC before: 49.40±10.53; 45 dpt: 66.60±11.67; 90 dpt: 71.00±9.823%, N=5). Values at 45 and 90 dpt were also significantly increased when compared to the placebo in the corresponding time points. Interestingly, in alo-MSC group no difference was observed when compared to placebo. Inflammation and fibrosis were present in all parts collected from the hearts and in variable degrees. Total heart fibrosis was not different in auto-MSC treated when compared to placebo animals (Placebo 9,013±3,09; auto-MSC 6,510±1,27%, N=3).
We demonstrated that autologous bone-marrow mesenchymal stem cell treatment of chronic phase chagasic dogs resulted in an increase of LVEF 45 and 90 dpt, but the same was not observed for allogenic cells. Fibrosis quantification shows a tendency towards reduction in MSC-treated animals, although the n still needs to be increased. More experiments need to be performed, but it seems plausible that cell therapy using bone-marrow mesenchymal stem cells is promising for clinical trials in chagasic cardiomyopathy patients.

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188 - MESENCHYMAL-DERIVED STEM CELLS ATTACHED TO SUTURE MATERIAL ENHANCE THE CLOSURE OF ENTEROCUTANEOUS FISTULAS IN A RAT MODEL

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Surgical treatment for Enterocutaneous fistulas (EF) frequently fails. Cell therapy might represent a new approach. Mesenchymal stem cells (MSCs) have high proliferative and differentiation capacity.
This study aimed to investigate whether MSCs could adhere to suture filament (SF) promoting better EF healing.
MSCs, $1x10^6$, from adipose tissue (ATMSCs) were adhered to a poly-vicryl SF by adding a specific fibrin glue formulation. Adhesion was confirmed by confocal and scanning electron microscopy (SEM). A cecal fistula was created in 22 Wistar rats, by incising the cecum and suturing the opening to the surgical wound subcutaneously with four separated stitches. The animals were randomly allocated to 3 groups. Control (CG): 5 animals EF performed. Injection (IG): 8 animals $1x10^6$ ATMSCs injected around EF borders. Suture Filament (SG): 9 animals, sutured with $1x\ 10^6$ ATMSCs attached to the filaments with fibrin glue. Fistulas were photographed on the operation day and every 3 days until the 21st day and analyzed by two different observers using ImageJ Software.
Confocal and SEM results demonstrated ATMSCs adhered to SF (ATMSCs-SF). The average reduction size of the fistula area at 21st day was greater for the SG group (90.34%, $p<0.05$) than the IG (71.80%) and CG (46.54%) groups.
ATMSCs adhered to SF maintain viability and proliferative capacity. EF submitted to ATMSCs-SF procedure showed greater recovery and healing. This approach might be a new and effective tool for EF treatment.

189 - EVALUATION OF DIFFERENTS PROTOCOLS OF TREATMENT WITH GUANOSINE ON NEURONAL SURVIVAL IN A MODEL OF OPTIC NERVE LESIONS

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Optic nerve lesions can lead from partially loss of vision to complete blindness. These types of lesions affect especially the retinal ganglion cells (RGCs). One potential therapy for this case is the administration of the nucleoside guanosine. The guanosine treatment has demonstrated neuroprotective effects in several models.
This work invesgated the putative neuroprotective effect of systemic administrations of guanosine in a model of optic nerve lesion.
Lister-Hooded rats were submitted to crushing of the optic nerve, followed by intraperitoneal injection of guanosine or vehicle. The treatment was evaluated in three distinct protocols. In Protocol 1the groups received just only one guanosine injection (7,5 mg/kg). In Protocol 2, the animals received a daily injection of guanosine for seven days (7,5 mg/kg). In Protocol 3, the animals received 1 injection every 12 hours for seven days (60 mg/kg). The experimental groups used in Protocols 1 and 2 were evaluated fourteen days after surgery, while the groups were analyzed after seven days. The RGC survival was assessed by Brn3a immunostaining in whole mount retinas. The number of RGCs in the fellow contralateral eye was used as control.
There was no significant difference in the number of RGCs in guanosine groups when compared to the vehicle groups in all experimental protocols tested. The number of extended axons beyond the lesion site in guanosine-treated animals was similar to the vehicle-treated ones.
We conclude that the guanosine systemic treatment using these doses and therapeutic windows was not effective to promote the survival and axonal extension in RGCs after optic nerve lesion.

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190 - ENCAPSULATION OF MULTIPOTENT STROMAL CELLS IN CARRAGEENAN-BASED HYDROGELS FOR SKIN TISSUE ENGINEERING

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Natural hydrogels have received much attention because of their potential applications for tissue engineering scaffolds. They are used due to its similarity to extracellular matrix, chemical variability and biological compatibility. Thus, natural hydrogels allows dynamic three-dimensional (3D) environment for cell encapsulation.
In this study we analyzed the potential of the carrageenan hydrogel (CH), thermo reversible polymer of natural origin, to create a 3D environment for the growth of multipotent stromal cells (MSCs) derived from human dermis. We also evaluated if this association could be efficient to promote mouse skin wond healing.
Human skin samples were obtained from patients submitted to facial-lift (approved by the UFSC Human Ethics Committee). The cells were encapsulated in two distinct CH: native carrageenan (obtained from red algae cultivated in Florianópolis – SC, Brazil) and commercial carrageenan (control condition) purchased from Sigma®. MSCs were maintained in DMEM plus 10% FBS for 14 days and then morphology, survival and adherence were analyzed. Structural analysis of the CH was performed using CryoSEM, chemical analysis by FT-Raman spectroscopy and determination of sulfate. Evaluation of mouse skin wound healing (approved by the UFSC Animal Ethics Committee) was carried out by three steps: excisional wound in the dorsal region, treatment application and coating with Tegaderm® dressing. The conditions of treatment were: (1) control (only injury), (2) CH, (3) MSCs and (4) MSCs encapsulated in CH. After three and seven days we performed histological analyzes by HE staining.
As result we observed a uniform distribution of MSCs inside the CH. The cells did not adhere to CH along all the culture period since they displayed a round shape. When analyzed by MTS colorimetric assay, which indicates the cell metabolic activity, MSCs encapsulated in hydrogels showed good viability until the seventh day of culture. In the fourteenth day of culture we detected a huge decrease in cell viability. The structural analyses of the both carrageenan hydrogels tested by CryoSEM showed an interconnected porosity, with pores displaying diameters around 1µm. The chemical analysis showed that both carrageenan are the type kappa, and the samples had high molecular weight with percentage of sulphate close to 25%. Histological analysis in both periods showed that CH allows the inflammatory cells infiltration, especially neutrophils. The animals treated during 7 days with MSCs encapsulated in CH showed an decrease of fibroblasts when compared to control.
Both, native and comercial CHs were chemically equivalent and able to support MSCs encapsulation. Native carrageenan was an excelent scaffold to administrate MSCs to mice lesions promoting an increase in skin wound healing. This new scaffold may serve as a vehicle for cell delivery with potential applications in tissue engineering.
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Pre-clinical Research

191 - CHITOSAN MEMBRANE INCORPORATED WITH HORMONE DERIVADED OF PLATELET AS A SCAFFOLDS FOR GROWTH OF IN VITRO CELLS

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Cell culture had a significant jump on the development of scaffolds. This technology opens new gates to generate functional transplants. Platelet secrets and stores several important platelet derived hormones (PDH) and grow factors. This milieu has been used instead of bovine fetal serum (BFS) in order to cultivate cells to therapeutic utilization, since this platelets are not xenocompounds as BFS. In this work we used PDH as a gel scaffold for the cultivation of adipose derived stem cells (ADSCs). Basing on the fact that α, β e denses granules are storages of more than 15 different grow factors linked with adhesion, healing and neovascularization tissue process. Chitosan is widely used in biomaterials engineering, for this, we chose it because their anti-microbial, biocompatibility properties, wound healing capacities and nontoxic metabolites as well.
The aim of this study was to compare chitosan membrane assembled with PDH to pure chitosan membrane and the platelet gel as well.
Chitosan membranes was prepared 2,5% per volume according to our standard operational procedures previously established as well as platelet gel. PDH incorporation process was accessed by immersion of chitosan scaffold with known and rated volume of a pool of hormones and grow factors previously quantified in other studies. Platelet gel was obtained from PDH added by calcium gluconate and purified human thrombin prepared following the same standard operational procedures. ADSCs were seeded onto three types of scaffolds and apoptosis/necrosis assay was carried out using flow citometry assay FACS Calibur® and results processed by CellQuest® software.
Control culture had apoptosis/necrosis index fixed in 9,09%, while chitosan added with PDH was 6,13%, so, smaller than the standard control. For platelet gel, this index was 11,65% and 4,5% for pure chitosan. Even 10% apoptosis/necrosis index is a normal cell rate. What was observed was that pure chitosan scaffold was the only one that didn´t promote cell spreading and amplification, so, the cells seeded remained at the same number as plated initially; and we conclude that apoptosis/necrosis was less than 10%, because the number of cells was smaller than the others.
Apoptosis/necrosis index presented expected normal percentages. Chitosan scaffold added with HDP and platelet gel scaffold showed to be livable to cells allowing them to adhere, spread and amplify, while pure chitosan prevented cells to exhibit normal behaviors.

Pre-clinical Research

192 - CELL THERAPY IN AMYOTROPHIC LATERAL SCLEROSIS MURINE MODEL: FUNCTIONAL AND HISTOLOGICAL APPROACH

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Amyotrophic lateral sclerosis is a fatal neurodegenerative disorder which affects motorneurons primarily leading to axonal retraction and cell death. The symptoms are tremors, spasms, loss of muscular strength and tonus until total loss of movement. Patient death occurs from 3 to 5 years after diagnosis. About 90% of the cases are sporadic, with unknown causes, and 10% have a genetic origin.
In this study, we used the animal model of the disease and tested a possible therapy using bone-marrow mononuclear cells injected intravenously and/or intramuscular aiming to see if there is any effect on the disease course and the survival of these animals.
These animals were evaluated in order to assess their motor capacity using the rotarod, hanging wire test and the motor score. Their survival was noted and we performed imunohistochemical analyses to quantify the number of motorneurons, microglia and functional neuromuscular junctions at different time points.
The cells remained on the muscle at least until seven days after the procedure, when a mild injury was made on the target muscle. The protocol combining on intravenously and intramuscular injections was able to delay the motor deficit and to reduce the motor plate denervation and microglial cell numbers, although it did not had any effect on neuronal survival. The intramuscular injection alone did not show any functional outcome.
We concluded that the administration of bone-marrow mononuclear cells both intravenously and intramuscular is necessary to delay the progression of the disease, possibly by reducing inflammation and delaying muscle denervation. However, more studies need to be conducted in order to investigate whether the intravenous injection is required for a these results and to improve the therapy efficacy aiming to increase animal’s lifespan.

193 - TEMPORAL EVALUATION OF HEMATOLOGICAL PARAMETERS OF HEALTHY HORSES AFTER TRANSPLANTATION OF MESENCHYMAL STEM CELLS DERIVED FROM ALLOGENEIC UMBILICAL CORD MATRIX

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The mesenchymal stem cells (MSCs) mediate immunoregulatory effects on innate and adaptive immunity indirectly through soluble factors or by direct physical contact. The influence of allogeneic MSCs can be evaluated by leukocyte response, which in acute inflammation can result in changes in leukocyte counts.
This study aimed to evaluate the effect of intramuscular transplantation of allogeneic MSC obtained from umbilical cord matrix (MSC-UCM) on hematological parameters in healthy horses at different time points.
Cryopreserved MSC-UCM from a single donor were used for allogeneic transplantation, after previously immunophenotypic characterization and response to adipogenic and osteogenic differentiation. After thawing the cells were expanded in culture bottles before transplantation. Cell transplantation was performed in the right and left gluteus medium muscle at two different regions. In each region was inoculated 0.9 µL, divided into nine points containing 3 million cells resuspended in Hank's Balanced Solution. Blood samples were collected from each animal immediately before transplantation; after 48 hours; and after 7 days. Blood count, platelet count, fibrinogen and leukocyte count were performed. Statistical analysis of the effect of allogeneic transplantation on hematological parameters was performed with the software Sigma Plot for Windows version 11.0 software (copyright ® 2008. Systat Software, Inc, Germany). Variables that did not pass the normality (Kolmogov-Smirnov test) were analyzed by One Way Repeated Measures Analysis of Variance and the other by One Way Repeated Measures Analysis of Variance. The Tukey test was used for all pairs of multiple comparison, adopting P 0.05) between studied times before and after transplantation of MSC-UCM.
In experimental conditions, allogeneic MSCs-UCM had no systemic effect based on hematologic variables studied. Is worth mentioning that the analysis of immunoglobulin and subpopulations of CD4 and CD8 lymphocytes will still be conducted in this study aiming a more accurate assessments of possible systemic effects of allogeneic MSCs.
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Pre-clinical Research

194 - HUMAN ADIPOSE-DERIVED STEM CELL TRANSPLANTATION IN WISTAR RATS SUBJECTED TO SPINAL CORD INJURY: MONITORING, IMMUNOMODULATION ASSESSMENT AND NEURONAL MARKER EXPRESSION

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Spinal cord injury (SCI) is sudden and unexpected event and it is a relevant cause of mortality. Glucocorticoids have been used for treating in order to reduce edema, as well as vascular inflammation and lesions. Adipose-derived stem cells (ADSCs) have shown differentiation potential among other cell types, and they release factors that stimulate stem cells present in the tissue to recover the injured area through paracrine action. They also have immunomodulating action over the immune system cells. Due to those characteristics, the ADSCs are known as a promising treatment for patients with spinal cord injury. Preclinical studies on ADSCs have shown that there is functional recovery after transplant with those cells.
Therefore, the aim of this study was to monitor the transplanted human ADSCs, evaluate their immunomodulating action and their neuronal markers on spinal cord injury in Wistar rats, whether treated or not treated with corticosteroids, 30, 60 and 90 days post spinal compression injury.
All samples were collected after informed consent had been obtained in accordance with guidelines for research involving human subjects, and with the approval of the Ethics Committee of PUCPR (approval number 601 and 67978). The ADSCs were isolated and cultured up to the third passage. The spinal cord injury was made through compression, animals were divided into three groups and transplanted as follows: culture medium, group A; ADSCs, 7 and 14 days post injury, group B; methylprednisolone sodium succinate injection, three hours post injury, and ADSC transplantation, 7 and 14 days post injury, group C. Two animals from each group were transplanted with PKH26 stained cells (1^{st} transplantation) and PKH67 (2^{nd} transplantation). Cytokines (IL1-α, MCP-1, TNFα, IFN-γ, IL-4) were quantified from the animals' blood sera soon after injury, after the second transplantation, and 30 days post injury. In order to evaluate the expression of neuronal markers, spinal cord were marked with antibody anti-NF200 and anti-βtubulin III.
After the euthanasia, it was possible to verify the presence of PKH26 and PKH67 stained ADSCs on groups B and C, after 30, 60 and 90 days of SCI. The quantification of cytokines did not show significant difference from the comparison among groups A, B and C, neither at the moment of transplantation nor 30 days post SCI. Concerning neuronal marker expression, it was possible to verify the expression of NF200 and βtubulin III at the injured spot, even 90 days post SCI, which demonstrates the cell differentiation potential on neuronal lineage.
PKH26 and PKH67 dyes are a simple and quick staining method that allows stable and effective results, which allows monitoring the ADSCs after transplantation. In addition, the transplanted ADSCs into the liquor showed the NF200 and βtubulin III markers, which demonstrates the cell differentiation potential on neuronal lineage.

Pre-clinical Research

195 - CORD BLOOD HEMATOPOIETIC STEM CELLS EX VIVO EXPANSION PROMOTED BY MESENCHYMAL STEM CELL CO-CULTURE

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Umbilical cord blood (UCB) hematopoietic stem cells have been successfully used for the treatment of both malignant and non-malignant diseases. Nevertheless, some UCB units could have low total nucleated cells (TNC) dose; this fact has limited their use to children since adults need a higher doses. Several approaches have been suggested to avoid inadequacy problems of hematopoietic stem cells (HSC) number for transplantation, such as administration of two UCB units to the patient and HSC ex vivo expansion.
Evaluate UCB ex vivo expansion proliferative rates in a high and low MSC confluence feeder layer obtained from different MSC sources adding or not cytokines cocktail into the media.
This study was approved by the Research Ethic Committee (CAPPESQ) of Hospital das Clínicas da Faculdade de Medicina da USP. The collection of UCB (n=10) was made after delivery of the infant and the expulsion of placenta. Processing was performed using volume reduction method which consists in red blood depletion. Units were cryopreserved using a controlled-rate freezer. MSC samples from umbilical cord endothelium and adipose tissue were obtained each from LIM31's pattern inventory. The TNC, expression of hematopoietic surface markers such as CD34+ and CD133+ were observed after seven days of culture. Beyond that, colony forming unit assay (CFU) was performed before and after UCB expansion. The expansion by co-culture method was separated in two groups (Group I – co-culture with cytokines cocktail added/ Group II- Co-culture without cytokines cocktail) for both MSCs sources.
After seven days, analysis of confluent co-culture showed that TNC proliferation rate ware almost 2,5 times higher (40-fold) than in subconfluent co-culture (17-fold) in group I. Flow cytometry also revealed higher proliferative rate in CD133+ (15-fold confluent/ 8-fold subconfluent) and CD34+ (7-fold confluente/ 2-fold subconfluent) cells, considering group I. CFU showed similar enhance after seven day of culture in comparison of day 0 (25-fold), also in group I. Subconfluent co-culture for both umbilical cord endothelium and adipose tissue showed lower yield compared with those with high MSC confluence and there was not remarkable difference in group I and II. However, confluent co-culture from umbilical cord endothelium showed 2 times lower proliferation in group II than group I.
Intercellular cytosolic transfer between MSC and UBC and the exchange of some factors contributes for UCB viability during the culture. Based on that, we inferred that this exchange should play a role in ex vivo HSC expansion as well in cell viability. This study showed that some co-culture system may require adding cytokines cocktail in the media, but it depends on the MSC source used. MSC from adipose tissue seems to release more endogenous cytokine which it improves the yield of UCB expansion

196 - CARDIOMYOCYTES DERIVED FROM MOUSE EMBRYONIC STEM CELLS IMPROVE CARDIAC FUNCTION IN DOXORUBICIN-INDUCED CARDIOMYOPATHY

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Doxorubicin (Dox) is an effective antineoplastic agent used for the treatment of a variety of cancers. However, its use is limited because of the risk of severe, dose-dependent, cardiotoxicity that may progress to heart failure.
This study aims to establish mouse model of Dox-induced cardiomyopathy (DIC) and evaluate the role of cardiomyocytes derived from mouse embryonic stem cells (CM-mESC) in the treatment of this chronic disease.
mESC line E14TG2A was cultured and characterized according to expression pluripotency-associated genes by RT-PCR and Immunofluorescense. Karyotype analysis was performed. mESC were submitted to cardiac differentiation protocol and efficiency was evaluated by flow cytometer for Troponin T. CD1 mice were given Dox 7.5mg/kg/injection IC (once weekly for 3 weeks). 26 days later, mice were submitted intramyocardial injections of CM-mESC (8x105 cells). Functional parameters were evaluated at different time points by echocardiogram. The mESC were stably transduced with a viral construct expressing luciferase under the control of the Murine Stem Cell Vector (MSCV) promoter and they were used in a bioluminescence assay.
mESC expressed transcription factors Oct3/4, Sox2 and Nanog by RT-PCR and SSEA-1 and Oct3/4 by immunofluorescence assays. mESC had a normal Karyotype with 40 chromosomes. After 8 days of cardiac differentiation protocol, beating cells were observed. On 14 day, differentiated cells had a high expression of Troponin T (76.925±11.61, n=4). 21 days after Dox-treatment, statistical difference was observed in the efection fraction (EF) of Dox (n=23) and placebo-treated groups (n=9) (38.87±1.229% versus 58.15±1.954%, respectively). After that, the Dox-group that received CM-mESC showed a significant increase of the EF on 5 and 30 days after the beginning of treatment [(EF5d: CM-mESC-Dox: 51.57±1.910% versus Placebo-Dox: 39.42±1.649%; $p<0.05$) (EF30d: CM-mESC-Dox: 54.66±4794% versus Placebo-Dox: 41.89±2.921%; $p<0.05$)]. No difference was observed in the EF between Control and CM-mESC-Dox-groups on 5 and 30 days after the beginning treatment [(EF5d: Control: 53.86±2.683% versus CM-mESC-Dox: 51.57±1.910%, $p>0.05$) (EF30d: Control: 54.92±1.765% versus 54.66±4794%, $p>0.05$)]. Transduced CM-mESC was detected until 11 days after intramyocardial injection.
The study results show that the mouse model of Dox-induced cardiomyopathy was established and CM-mESC transplantation contributes to improvement of cardiac performance in heart failure.

197 - STEM CELLS FROM HUMAN ESFOLIATED DECIDUOS TEETH (SHED) IN MURINE BONE DEFECT MODEL

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The dental pulp stem cells are a recently source of mesenchymal cells that can be used in regenerative medicine. Their great proliferation in cell culture, high plasticity and the ease of obtaining are characteristics of a good source of stem cells to use in cell therapy.
In the search of a therapy that can promote bone repair, there were evaluated the xenologous transplantation of human stem cells from exfoliated deciduous teeth (SHED) on murine bone defect model regeneration.
A non-critical bone defect was made in the tibia of 45 non-immunocompromised mice, and these were filled with: blood clot, absorbable collagen sponge, and SHED seeded in resorbable collagen sponge. After seven, 15 and 30 days the animals were euthanized and the parts subjected to histological and immunohistochemical processing. The animals were followed radiographically and evaluated by the percentage of lesion filling.
The histological evaluation was performed semi-quantitatively, with assigned scores according to the stage of bone repair and inflammatory infiltration process. For immunohistochemical analysis, scores were assigned by the intensity of expression of osteopontin. Radiographically, all groups demonstrated improvement in repair and the SHED treated group showed the highest percentage of reconstruction (97%-100%). The inflammatory infiltrate decreased during the experimental period, and there was no significant difference between groups ($p>0,05$). Regarding the bone repair process, a significant difference at days seven ($p=0,022$) and 30($p=0,016$) days. The treated group with SHED had total cortical repair with the presence of primary bone tissue. This result was confirmed by the increased expression of osteopontin ($p=0,022$) at 30 days, showing a greater restorative activity compared to the other groups.
Despite that this is an autologous transplant, no animal exhibited signs of rejection. In conclusion, SHEDs favored the process of bone defect repair in an animal model, providing evidence that these cells can be used in human therapies.
(UFPI /CEEA nº 0218.0.045.000-11)

Pre-clinical Research

198 - COLLARED PECCARY (*Tayassu tajacu*) AS AN MODEL FOR STEM CELL THERAPY IN RENAL DISEASE: INDUCTION OF ISCHEMIC RENAL INJURY

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Collared Peccary (Tayassu tajacu) is an ungulate mammal of the Tayassuidae family. The species resemble pigs because they belong to the same order, Artiodactyla, although the pig belong to the Suidae family.
This study proposes the use of the collared peccary as an experimental model for ischemic renal injury that could be used in stem cell therapy.
Collared peccary bone marrow mesenchymal stem cells (cp-BMMSC) were previously isolated and characterized by our research group. Ischemic nephropathy was induced surgically by partial occlusion of the left renal artery. The disease course was assessed by hematological tests, serum chemistry , urinalysis, ultrasound (US) and doppler ultrasound function of the renal artery before induction, and at 5, 10, 15 and 20 days after surgery. Twenty days after the occlusion, unilateral nephrectomy and histopathological examination were performed to assess renal morphology. The results will be evaluated by Fischer's test.
The histopathological examination showed glomerular, tubular and interstitial lesions. In the experimental group, 83.3 % (5 /6) showed moderate renal lesions and only 16.7% (1/6) were classified with no lesions. The ultrasound examination of the right kidney presented statistical difference between day 5 and day 10 post occlusion. Statistical analysis by Fischer's test showed a significant difference ($p < 0.05$) between the control group and the experimental group.
This study suggested the collared peccary as a good experimental model for ischemic renal disease, because it could be manipulated during the research time without death, with health conditions that permit any subsequent procedure for disease therapy with stem cells.
(UFPI /CEEA nº 041/11; SISBIO nº 33058)
Financial source: CAPES

Pre-clinical Research

199 - GENERATION OF TRANSGENIC AMNIOTIC MSCs EXPRESSING GFP USING VIRAL VECTOR FOR FUTURE CELL TRACKING AFTER TRANSPLANTATION INTO CAT KIDNEY

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Amnion-derived mesenchymal stem cells (AMSCs) are multipotent cells with an enhanced ability to differentiate into multiple lineages. AMSCs can be acquired through non-invasive methods (the amnion is discarded after birth), and therefore are exempt from the typical ethical issues surrounding stem cell use. Due to their plasticity, non-tumorigenicity and immunomodulatory properties, AMSCs are the most widely studied for pre-clinical and clinical trials. The key point in the field of cell-based therapies is the engraftment of the cells in the specific injured tissues. It is known that the cellular microenvironment, soluble growth factors and cytokines directs the cell homing and cell differentiation.
This study is aimed to generate transgenic AMSCs though viral vector for future cell tracking after cell transplantation into kidney of cats.
Feline amniotic-derived cells were transduced with a lentivirus containing the eGFP gene driven by the ubiquitin-C promoter and a post-transcriptional regulatory element. It does not have replication sites, for safety reasons. For transduction, 293FT cells were lipotransfected overnight with the eGFP and auxiliary vectors. Fouty eight hours after transfection, the supernatant was collected, filtered through a 45mm syringe filter, supplemented with 8ug/ml of polybrene and used for transduction of 3x104 amniotic cells previously plated in 6 well dishes overnight. Three days after transduction eGFP-positive amniotic cells were analyzed by flow cytometry (FACSAria and FACSDiva software, BD Bioscience) and positive cells were sorted and cultured in vitro until use or frozen.
In this study we successfully generated transgenic AMSCs. In flow cytometry 45,8% were positive for GFP. In negative control group (performed to validate this result) only 0,2% expressed GFP. When examined by immunofluorescence microscopy, these cells expressed GFP green fluorescence throughout its length. None cells of negative control expressed green fluorescence in immunofluorescence microscopy. These findings confirm that transgenic amniotic mesenchymal stem cells were successfully generated using this protocol. Positive cells were sorted, cultured and frozen for future application.
In our current studies we aim to assess whether the injection of amniotic derived mesenchymal stem cells (AMSCs) could reduce or stabilize the rate of progression and the clinical features of Chronic Renal Failure in the feline model. Transgenic cells expressing GFP will be used for cell tracking after cell transplantation into kidney of cats.

200 - CHARACTERIZATION OF CARDIAC PROGENITOR CELLS AND EVALUATION OF THEIR THERAPEUTICAL POTENTIAL IN A MURINE OF HEALED MYOCARDIAL INFARCTION

Tais H. K. Brunswick (UFRJ); Andrea Costa (UFRJ); Fernanda Cristina P. Mesquita (UFRJ); Raiana A. Q. Barbosa (UFRJ); Isalira P. R. de G. Freitas (UFRJ); Grazielle S. Dias (UFRJ); Guilherme V. Brasil (UFRJ); Sandro T. da Cunha (UFRJ); Juliana do A. Passipieri (UFRJ); Bruna Farjun (UFRJ); Adriana B. Carvalho (UFRJ); Antonio Carlos C. de Carvalho (UFRJ)

The recognition that the adult heart possesses a stem cell compartment capable of regenerating myocytes and coronary vessels after myocardial infarction (MI) has accelerated the development of cardiac regenerative therapies. We have isolated, characterized and evaluated the role of cardiosphere-derived-cells (CDC) derived from human and rat in the cardiac regeneration after MI. The therapeutical potential was evaluated by echocardiogram, morphometry and mechanic development of isolated treated hearts. The time of engraftment was evaluated by bioluminescence assay.

The cardiac-stem cells were obtained from human discarded myocardial tissue (n=40) and rat hearts (n=9) by enzymatic digestion with collagenase II. 10-15 days after isolation, small, round, phase-bright cells (PBC) appeared on top of the adherent fibroblast-like cells. The PBCs were collected and placed in a non-adherent plate for 2 days where they formed cardiospheres, which were then transferred to adherent plates, giving rise to CDCs. These cells are adherent to plastic, with a spindle-shaped morphology and expressed mesenchymal markers – CD105, CD90, CD73 – adhesion molecules - CD54, CD146, CD166 and presented low expression (<5%) of hematopoietic and endothelial markers – CD34, CD45, CD31, CD133 – when analyzed by flow cytometry. Wistar/Kyoto rats were submitted to MI through permanent occlusion of the anterior descending coronary artery. After 60 days, they were immunosuppressed with Cyclosporine A (10mg/kg) during 10 days. In the third day the animals were treated with 5x105 human CDC (hCDC) or placebo through intramyocardial injection guided by echocardiogram. Another group of animals was treated with rat CDCs (rCDCs) without immunossuppression. The hCDCs (n=3) and rCDCs (n=3) were stably transduced by a viral construct expressing luciferase under control of a constitutive promoter. CDCs were then used in a bioluminescence assay. Transduced CDCs were detected only in the thoracic cavity and remained at the injection site for two days (rCDCs) and 6 days (hCDCs). Functional parameters were evaluated by echocardiogram 30 and 60 days after treatment and by Langendorff at the 60 days. 60 days after treatment no differences were observed in the cardiac function of hCDCs and rCDCs when compared to placebo group (Ejection fraction (%): hCDCs: 30,89±1,93%; rCDCs: 28,58±2,43; placebo:34,62±3,69/ End diastolic volume (µL): hCDCs: 830±73,54; rCDCs: 840,2±2,43; placebo: 736,2±53,88/ End sistolic volume (µL): hCDCs: 551±64,81; rCDCs: 591,4±85,8; placebo: 492,5±54,53). Histological analyses of the hearts were used to quantify the infarcted area and no reduction was observed in cell-treated animals, compared to placebo (hCDCs: 35,74 ± 3,65%; rCDCs: 35,83±3,71%; placebo: 35,54±2,99%).

We isolated and cultivated CDCs from human and rats, but the treatment with CDCs did not contribute to heart function, neither prevented heart chamber dilatation in rats with MI and cardiac remodeling.

Pre-clinical Research

201 - CHARACTERIZATION AND EFFECTS IN VIVO OF EXTRACELLULAR VESICLES DERIVED FROM HUMAN MULTIPOTENT BONE MARROW STROMAL CELLS AND CD133+ CELLS FOR TREATMENT OF ACUTE CARDIAC IFARCT MODEL

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Human multipotent bone marrow stromal cells (hMSCs) and endothelial progenitor cells (EPCs) are able to secrete extracellular vesicles (EVs). Recent findings suggest that extracellular vesicles (EVs) released by these cells are able to mediate several biological responses by transferring proteins, lipids and various forms of RNA to neighboring cells. Among these responses are the properties of repair and protection of the injured tissue.
Thus, the aim of this study is to characterize EVs obtained from both sources and evaluate the effects on cardiac and renal function of infarcted rats.
To obtain the EVs, hMSCs and CD133+ cells (EPC–enriched population) were grown in culture medium with 2% fetal bovine serum depleted of vesicles and the conditioned media of these cells were collected and submitted to cycles of centrifugation and ultracentrifugation. To confirm the isolation, the EVs were analyzed by flow cytometry, nanoparticle tracking analysis (NTA) and immunogold labeling/transmission electron microscopy (TEM). The induction of myocardial infarction (AMI) in the rats was performed by occlusion of the left coronary artery, in order to generate rats with a left ventricular ejection fraction (LVEF) less than 45%. After AMI, three groups were established: control group (PBS1x only); group transplanted with hMSC derived EVs and group transplanted with EPC derived EVs. The rats received a single application of 50 µg (equivalent to $7{,}8x10^8$ EPC-EVs and $14x10^8$ hMSC-EVs) of EVs 24 hours after AMI. The ventricular function of the animals was determined by echocardiograph 28 days after treatment. The protein content of EVs was analyzed by proteomic assay with the LQT-Orbitrap XL mass spectrometer.
Through this project it was possible to standardize the methodology for EVs isolation from hMSCs and EPCs. The EVs evaluation by NTA showed EVs with size ranging from 50 to 800 nm (EPC-EVs 50-500 nm and hMSC-EVs 50-800 nm). Analysis by TEM showed that EPCs derived EVs exhibit double membrane, size between 70-400 nm and the membrane markers CD31 and CD63, demonstrating the presence of microvesicles and exosomes. The results of a preliminary assessment showed that 29 days after infarction there was a significant improvement in LVEF of rats treated mainly with EPC derived EVs. Also, there was an important reduction of the infarcted area. Our proteomic analyses of EVs revealed a total of 170 proteins in EPC-EVs and 49 proteins in hMSC-EVs. GO analyses using G: profiler demonstrated that these genes are involved in negative regulation of apoptotic processes, response to injury and exosome biogenesis pathway.
In conclusion, the results obtained with NTA and TEM validated our method for isolation of EVs from hMSCs and EPCs. Furthermore, the preliminary results from in vivo assays suggest a surprising beneficial effect of EPCs derived EVs in the improvement of cardiac function. We are currently increasing the n of our studies to confirm this encouraging effect.

202 - EFFECTS OF BONE MARROW-DERIVED MESENCHYMAL CELLS AND CONVENTIONAL PHARMACOLOGICAL TREATMENT ON EXPERIMENTAL NEUROPATHIC PAIN: A COMPARATIVE ANALYSIS

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Neuropathic pain is a type of chronic pain caused by injury or dysfunction in the nervous system. Considered a syndrome, this chronic condition diminish patients' quality of life and increase healthcare utilization and costs. Currently there is no gold standard for the treatment of neuropathic pain. Based on the potential of stem cells for functional reestablishment of the damaged nervous system, the cell therapy represents a promissory alternative to the neuropathic pain control. In fact, the antinociceptive effects of cell therapy on neuropathic pain models have been demonstrated.

In this study we used a nerve constriction model to compare the effects of bone marrow mesenchymal cells (BMMC) and gabapentin, a gold standard drug to clinical control of neuropathic pain, on behavioral neuropathic pain.

The neuropathic pain model induced by the sciatic nerve chronic constriction in male C57Bl/6 mice (20-23 g) was used. The paw mechanical and thermal nociceptive thresholds were evaluated before and after treatments by using von Frey filaments and Hargreaves, respectively. Bone marrow mesenchymal cells (BMMC) were obtained and characterized as previously described by Krampera (2003). Seven days after the sciatic constriction, mice were treated with 106 BMMC or saline by lateral tail vein (100 µL). Gabapentin (70 mg/kg) was administered twice a day for 6 days, oral route. Animal care and handling procedures were in accordance with the Institutional Animal Care and Use Committee FIOCRUZ (L-IGM-025/09).

One day after sciatic constriction procedures the nociceptive thresholds were significantly lower ($p<0.001$) for the operated groups relative to the control group, indicating the development of a marked mechanical and thermal hyperalgesia. Six days after the transplantation, the nociceptive threshold of BMMC-treated mice was similar to that observed before the sciatic constriction procedure. This antinociceptive effect was maintained throughout the testing period. On the other hand, gabapentin-treated mice increased nociceptive thresholds only during the treatment period.

A single administration of bone marrow mesenchymal cells was able to reverse the behavioral neuropathic pain, while the pharmacological approach produced just a transient effect.

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203 - A SYSTEMATIC REVIEW OF PRE-CLINICAL TRIALS USING STEM CELL THERAPY UPON DIABETIC FOOT ULCER

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Describe the profile of preclinical studies on this topic.
A Pubmed and Scielo searching, between 2010 and June 2014, revealed about two thousand and five hundreds articles investigating diabetic foot, however, its relation to stem cells, found less than 0.5 percent, and only four in 2014. Most recent review dated in 2012, considering papers until 2010. Scielo is a Brazilian Scientific Electronic Library, supported by governmental sponsorship agencies. Main criteria for this review included only papers discussing about stem cells application for diabetic foot healing.
Pre-clinical trials have been investigating stem cells behavior for alternative therapies. Streptozotocin-diabetic induced-rat models submitted to dorsal surgical wounds were tested for human umbilical cord blood-mononuclear cells (HUCB) and calves' blood haemodialysate (HUCB-HD) applications. Systemic administration of multinational HUCB and topical application of newly prepared HUCB-HD or HD 'calves blood significantly accelerated the rate of diabetic wound healing and opening up the possibility of its future use in regenerative medicine1.
Another study in the same model, analyzed the vascular endothelial growth factor on the treatment of diabetic foot using mesenchymal stem cells from human umbilical cord. Surgical foot ulceration was induced and combined to staphylococcus aureus. The graft of mesenchymal stem cells proved to be effective in promoting and recovery of ulceration of the foot in diabetic rats3.
Evaluation of therapeutic efficacy of injecting mesenchymal stem cells from bone marrow was performed on feet wounds. Results demonstrated an increase on feet wounds reepitelization when compared to those on tights. They conclude healing improvement was achieved by changes on keratinocytes caused by stem cell2.
All the studies demonstrated similar results despite the different protocols for the stem cell application and stem cell origin, human umbilical cord or rat bone marrow. The studies were carried out in diabetic induced rat model and conclusions were driven to a regenerative capacity of stem cells therapies for diabetic foot.
We believe stem cell therapy can be an effective treatment, additionally, pre-clinical trials in other species and in human might be continued investigated.
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Pre-clinical Research

204 - IN VIVO BONE REGENERATION MEDIATED BY HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS PARACRINE FACTORS

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Mesenchymal stem cell (MSC) transplantation has proved to be a promising strategy in cell therapy and regenerative medicine. Although their mechanism of action is not completely clear, it has been suggested that their therapeutic activity may be mediated by a paracrine effect.
The purpose of this study was to evaluate the ability of mesenchymal stem cells derived from human adipose tissue (Ad-MSC) and their conditioned media (CM), to repair bone lesions in a model in vivo in rabbit mandibles.
Using the Human Blood Plasma Hydrogels (HBPH) as a delivery system, proliferation and osteogenic differentiation of Ad-MSC into HBPH in vitro was demostrated. Critical-size defects were surgically created in the mandible of rabbits. Rabbits with these defects were divided into groups that were treated with: 1) HBPH alone, 2) HBPH with MSC, 3) HBPH with CM and 4) a last group where no treatment was performed and allowed the healing by second intention. Radiographic, histological and morphometric studies were performed 15, 30, 45 and 60 days postoperatively.
The results demonstrated that both, Ad-MSC and CM, induce bone regeneration in surgically created lesions in rabbit's jaws. Morphometric, radiographic and histological data reported here demonstrate that the amount and quality of neoformed bone, repaired area, bone density, arrangement of collagen fibers, maturation and inorganic matrix calcification are very similar between Ad-MSC and CM treated groups, suggesting that Ad-MSC improve the process of bone regeneration mainly by releasing paracrine factors.
This study demonstrated that the Ad-MSC improve the process of bone regeneration in an animal model, mainly by releasing their paracrine factors, that when collected and applied as conditioned media, have the same effect in tissue regeneration than Ad-MSC. This fact, supports and reinforces the possibility of developing a new therapeutic strategy for the application of MSC and MC in the treatment of certain bone disorders.

205 - MESODERMAL DIFFERENTIATION OF MESENCHYMAL STEM CELLS FROM UMBILICAL CORD AND BONE MARROW

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In the last years, studies with stem cells have generated great expectations regarding new therapeutic strategies, especially due to the possibility of tissue repair and regeneration, and for its instrinsic capacity for self-renewal and differentiation in functional tissues. The cryopreservation has become an excellent form of stocking these cells, allowing its viability and regeneration capacity even after the frozen period.
This study aimed to evaluate the differentiation capacity, plastic adherence and expression of surface antigens of mesenchymal stem cells from cord blood and bone marrow cryopreserved, belonging to a cell bank of a Higher Education Institution in Porto Alegre, RS.
Stem cells cultures were performed using frozen material from the Laboratory of Cellular and Molecular Biology of the Institute of Biomedical Research – Pontifícia Universidade do Rio Grande do Sul, where were used three cryopreserved mesenchymal stem cells from bone marrow and three samples of umbilical cord, stored and frozen in liquid nitrogen from different patients. The stem cells from the umbilical cord and bone marrow were cultured during four weeks through supplemented means to differentiate into adipogenic and osteogenic. Another evaluation was about the culture adherence to the plastic bottles and the expression of its surface antigens.
It was possible to observe that even after cryopreservation the cells didn't lose their adherence capacity to the plastic bottles used in culture. It also keep its potential of differentiation, originating specialized tissues and even expressing the surface antigens specific to the mesenchymal lineage.
With this work we can conclude that mesenchymal stem cells do not lose their characteristics of plastic adherence and leads specialized tissues after periods of cryopreservation, showing even after freezing important plasticity and expression of specific immunophenotypic markers for these cells. In this way cryopreservation is a good method of choice of mesenchymal stem cells storage. In the present study, we observed differences in the potential for adipogenic and osteogenic differentiation of bone marrow and cord blood cells, and the bone marrow show up with greater capacity for differentiation. However, further studies are needed to ascertain other characteristics of these cells as important in the process of regeneration.

206 - EVALUATION OF GENETIC STABILITY OF HUMAN MESENCHYMAL STEM CELLS BY FLUORESCENCE IN SITU HYBRIDIZATION – FISH

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The use of human mesenchymal stem cells (MSC) has been shown to be a promising strategy for cell therapy in recovery of injured tissues. However, it has been described in the literature that MSCs cultured in vitro can acquire genetic changes and therefore would be more likely to undergo neoplastic transformation. Thus, the application of cytogenetic techniques is needed to evaluate and ensure the integrity of the genetic material of MSCs used in cell therapy. The FISH technique is able to detect low frequency genetic changes, allowing the identification of specific sequences in the DNA molecule. A recurring change in tumoral cells is the loss of one allele of TP53 and RB genes. These genes are in the center of two major tumor suppressor pathways that control cellular responses to potentially oncogenic stimuli. Therefore, alterations associated with these genes are closely related to the development of the cancerous process.
To evaluate the maintenance of genetic stability MSCs kept in culture, through the investigation of the presence of TP53 and RB genes.
In total 14 experiments were performed, with 8 MSCs samples evaluated for the presence of TP53 and 6 evaluated regarding the presence of the RB gene. The FISH technique was performed in two steps. In the first stage was added RB and TP53 probes(Cytocell-Aquarius, LPS011 and LPH017) in slide preparation, which were co-denatured at 75 ℃ for 2 minutes. In the second step, after incubation at 37 ℃ and washing the slides,th e DAPI antifade solution was added.
All samples showed the expected signals for normal cells, it was possible to identify the presence of both alleles, maternal and paternal, of both genes, TP53 and RB. The frequency of the signals expected (2 reds and 2 greens) was 91,53% and 87,42%, for the TP53 and RB genes, respectively. Some of the cells showed signs corresponding to monossomies and tetraploids, confirming what had been detected on conventional cytogenetic. The importance of detecting these cells lies in the fact that they may represent the loss of genetic stability, expected for long-term cultured cells.
The test detected the presence of TP53 and RB genes in all samples. All samples showed the expected for normal cells. These results demonstrate the possibility of using this test to assess the genetic stability in MSC, complementing conventional cytogenetics.

207 -GENETIC INSTABILITY TEST IN MESENCHYMAL STEM CELLS

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The mesenchymal stem cells (MSC) cultivation must be followed by a periodic cytogenetic evaluation, because instabilities can arise during cultivation and can explain, at least partially, the possibility that these cells become tumorigenic. One of the techniques to evaluate the genetic instability is the test of chromosomal instability induced by alkylating agents, for example, diepoxybutane (DEB, 1,3-Butadiene Diepoxide). This test exposes the chromosome fragility inducing chromatid or chromosome breaks in cells cultured in the presence of these agents.
To evaluate the genetic instability of MSCs using the DEB test
For this study we used 7 samples of human bone marrow-derived MSCs. Approximately $2x10^5$mononuclear cells were plated in T-25 flasks at 37ºC in a humidified atmosphere containing 5% CO2. When culture reached a confluence of 80% it was sub cultured and were replaced to new flasks (passage 1). In the second passage, the DEB solution was added to the culture, in the 0,01 and 0,001 µl/mL dilutions in different flasks. After 48 hours in 37℃, was added arresting solution, the culture was detached with 0.25% trypsin/EDTA, the cells were transferred to a 15ml tube, centrifuged and ressuspended in warm hypotonic solution. After that cells were fixed with 3:1 fixative solution (methanol/glacial acetic acid) and washed twice with 2:1 fixative solution. The slides were prepared in a humid and warm place and the cells were stained using a Giemsa solution. The metaphases analyzed were found and captured, noting the frequency of breaks, gaps, acentric fragments and figures in each cell. To calculate the rate of breakage and radial figures it was used the formula: total number of failures/total number of cells analyzed.
330 metaphases of MSCs were evaluated, analyzing 20 metaphases whenever it possible of each sample (without and with DEB at different dilutions). Where observed only 7 breaks in the samples without DEB, while the samples exposes to the DEB showed 19 breaks, 8 fails and 1 ring chromosome, but any of them reached the index of 1.06 breaks per cell, which would indicate a positive DEB result.
There is possibility to evaluate chromosomal instability in CTMs using the DEB test. Even without reaching the index, the fact that they show breaks and gaps demonstrates the importance of this test as a complement to conventional cytogenetic examination.

208 - GENETIC EVALUATION OF MESENCHYMAL STEM CELLS BY G-BAND KARYOTYPING IN CELL A TECNOLOGY CENTER

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Cultived stem cells (SC) have shown great potential for use in several areas of cell therapy, however it has been reported that they can acquire genetic changes and therefore would be more likely to undergo neoplastic transformation. For this reason, in order to use SC in a therapeutic context, they must require guarantee of their quality and safety, with the implementation of exhaustive quality control programs, including chromosomal instability analysis. Unlike the embryonic SC, the adult SC, like mesenchymal stem cells (MSCs), have been maintaining genomic stability in culture. In Brazil, the Ministry of Health created in 2008 eight Cell Technology Centers (CTC) where the main goal is the provide stem cells under conditions of GMP, to the National Network for Cellular Therapy. One of the requirements of the Brazilian National Health Surveillance Agency (ANVISA) is the implementation of genetic control as a release criterion for use of these cells in humans.
The aim of this work is to show how the quality control by G-banding karyotyping is performed and evaluated in our CTC.
We analyzed bone marrow-derived MSCs from 33 patients (mean age = 51,9 ±9,9 years). To the G-band karyotype, metaphases were analyzed before (bone marrow) and after (MSCs) cultivation, until the second passage (P2). We used LUCIA image analysis systems for cytogenetic and the results were described following the guidelines to International System for Human Cytogenetic Nomenclature (2013).
Metaphases were successfully obtained in 32 cases. Twenty two (68.75%) presented instability chromosomal signs: cromatidic gaps, chromosome breaks, cromatidic breaks and tetraploidy, which could indicate an intermediate step for tumorigenesis. A total of 1.414 metaphases analyzed, 57 (4.03%) presented some of these signs. No clonal chromosomal rearrangements were detected. All the samples were approved by the cytogenetic quality control to therapeutic use.
Our results confirm the importance of G-band cytogenetic study, since this technique is able to detect both numeric and structural alteration, including balanced rearrangements and mosaicism, other than evidencing instability chromosomal signs.

209 - THE PRESENCE OF STEM CELLS ON THE EPIDERMOID CARCINOMA

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The epidermoid carcinoma (ECC) is a comum lesion among oral malignant lesions, it occurs in approximately 94 % cases. Such risk is increased significantly in older men. Heredity is not a primary factor of ECC occurrence, which is multifactorial disease, consisting of intrinsic factors such as systemic state and extrinsic factors that include tobacco, alcohol, and sunlight. ECC has different clinical characteristics and may be exophytic, endophytic, leukoplasic, eritroplasics and eritroleukoplasics. More rarely, ECC can lead to destruction of the underlying bone, which can be painful for patients. Metastasis occurring in ECC is not early event and it may happen mainly in lymphatic vessels. However, due to delay in diagnosis, the 21% of patients show the cervical metastases and at least 2 % demonstrated the distant metastases. Studies suggested the existence of subpopulation of stem cells (SC) within ECC resistant to anticancer treatments, which theoretically promote tumor growth and invasion. It is necessary to identify these cells in ECC in order to understand their role in tumor formation and progression.
Thus the present study aimed to evaluate the expression of the markers of pluripotent stem cells in ECC, such as Oct ¾, Nanog and Sox2.
The blocks of biopsies with ECC were obtained from the collection of the Department of Pathology, School of Dentistry of Ribeirão Preto, SP, Brasil. Immunohistochemistry assay has been performed. Simultaneously, the same blocks were submitted to conventional histological analyses to confirm the diagnosis, which was performed by experienced pathologists.
It was possible to identify a significant amount of the cells, which showed appropriate nuclear positive immunostaining for pluripotent markers especially in the sites of epithelial tissue invasion. Such phenomenon was observed in the samples obtained from both: men and women.
Our data indicate ECC as a possible source of pluripotent stem cells as well as demonstrate a role of these cells in tumor formation.

Clinical Research

210 - THE INFLUENCE OF LEFT VENTRICULAR DYSFUNCTION AND CARDIOVASCULAR RISK FACTORS IN ENDOTHELIAL, HEMATOPOIETIC AND MESENCHYMAL STEM CELL COMPARTMENTS FROM BONE MARROW

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The left ventricle dysfunction is a complication of cardiovascular diseases, and cell therapy using the bone marrow mononuclear fraction in cardiac patients has induced improvement in ejection fraction and symptomatology. The bone marrow fraction represents a heterogeneous cell population, containing among others cell types, the mesenchymal stem cells, endothelial progenitor cells and hematopoietic stem cells. Cardiac patients have an impairment of these compartments.
The aim of this study was to correlate the left ventricle dysfunction and cardiovascular risk factors with variations in frequency and functional capacity of mesenchymal, endothelial and hematopoietic stem compartments, looking for a way to identify patients who would have greater success using autologous cell therapy.
This study included 36 patients referred for coronary artery bypass grafting or aortic valve replacement. The mononuclear fraction was isolated from sternum bone marrow and the analysis of the compartment was made through clonogenic assays and flow cytometry.
Patients with left ventricle dysfunction, diabetes and intermediate-risk in EuroScore and SyntaxScore presented impairment in endothelial compartment. The use of aspirin and β-blockers was associated with a higher frequency of cells in the hematopoietic and endothelial compartments, respectively.
Left ventricle dysfunction, diabetes mellitus and unfavorable clinical scores were associated with deficiencies in the stem cells compartments, and cell therapy using autologous bone marrow cells for these patients may not be the best therapy of choice.

211 - MORPHOLOGY AND MORPHOMETRY OF FELINE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN CULTURE

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Mesenchymal stem cells (MSC) are increasingly being proposed as a therapeutic option for treatment of a variety of different diseases in human and veterinary medicine. Stem cells have been isolated from feline bone marrow, however, very few data exist about the morphology of these cells and no data were found about the morphometry of feline bone marrow – derived MSCs (BM-MSCs).
The objectives of this study were the isolation, growth evaluation, differentiation potential and characterization of feline BM-MSCs by their morphological and morphometric characteristics.
The study protocol was approved by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Federal University of the State of Paraná, Southern Brazil (CEUA-SCA/UFPR number 044/2008).
Fourteen housecats (ages ranging from nine months to 10 years, seven male and seven female) were used for the study. In vitro differentiation assays were conducted to confirm the multipotency of feline MSC, as assessed by their ability to differentiate into three cell lineages (osteoblasts, chondrocytes, and adipocytes). To evaluate morphological and morphometric characteristics the cells are maintained in culture. Cells were observed with light microscope, with association of dyes, and they were measured at 24, 48, 72 and 120 h of culture (P1 and P3). The non-parametric ANOVA test for independent samples was performed and the means were compared by Tukey's test.
On average, the number of mononuclear cells obtained was 12.29 (± 6.05x106) cells/mL of bone marrow. The mean number of MSCs colonies after cultivation of 470 and 752 cells/cm2 was 15.5±7.30 and 10,25±6,25 in P1, respectively. In P2, the mean number was 8.58±6.16 and 16.26±9.25, to each concentration. Morphologically, BM-MSCs were long and fusiforms, and squamous with abundant cytoplasm. In the morphometric study of the cells, it was observed a significant increase in average length of cells during the first passage. The cell lengths were 106.97±38.16 µm and 177.91±71.61 µm, respectively, at first and third passages (24 h). The cell widths were 30.79±16.75 µm and 40.18±20.46 µm, respectively, at first and third passages (24 h). The nucleus length of the feline BM-MSCs at P1 increased from 16.28 µm (24h) to 21.29 µm (120h). However, at P3, the nucleus length was 26.35 µm (24h) and 25.22 µm (120h).
This study has demonstrated the isolation of adult feline BM-MSC, proliferation potential and differentiation into three lineages. Cultures of feline MSCs cultures undergo changes as they are expanded, as observed in cultures of human MSCs. This information could be important for future application and use of feline BM-MSCs.

212 - DECREASED NUMBER OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS (EPCs) IS ASSOCIATED TO THE HYPERTENSION SEVERITY LEVELS IN PREMENOPAUSAL WOMEN

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Endothelial progenitor cells (EPCs) are involved in neovasculogenesis and maintenance of vascular homeostasis and their impairment may have a role in the pathogenesis of hypertension.
This study aimed to analyze the expression profile of circulating EPCs and different cardiovascular risk factors in hypertensive women aged 30 to 50 years compared with the same age healthy normotensive women.
A case-control study was conducted enrolling 45 women volunteers, aged from 30- 50 years (41 ± 6) in the Ambulatory of the Cardiologic Emergency Hospital of Pernambuco (PROCAPE). EPCs numbers were determined by flow cytometry in peripheral blood as the CD45-/CD34+/KDR+ cells. The women were classified as healthy normotensive controls (CT) with SBP (systolic blood pressure) 180 mmHg and DBP>110 mmHg (n=15). The group was interviewed regarding smoking habits, physical exercise and body mass index (BMI), and measured the level of blood pressure at quiescent. An analysis in records of test results cholesterol, high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c), triglycerides and fasting glucose in the month of collection of blood samples.
The results found a significant reduction in circulating EPCs numbers in MH (74%) and SH (88%) when compared to the CT and reduction of 67% in SH when compared to MH, an inverse relationship between the number of cells and the stage of hypertension. SH group showed an increase of 49% CD45+ cells demonstrating inflammation and a reduction of 61% CD45-/CD34+ cells. Regarding the biochemical serum was found: HDL-c [MH (52±7); SH (48±5)]; LDL-c [MH (130±8); SH (143±15)]; triglycerides [MH (138±19); SH (153±40)]; fasting glucose [MH (95±7); SH (121±39)] and BMI [MH (31±4); SH (29±3)]; revealing that 67% of women with severe hypertension had metabolic syndrome (MS).
The reduction of the EPCs may contribute to increased cardiovascular risk in this population. Development of hypertension and the parameters related to MS are directly correlated with a decrease of circulating EPCs. Therefore, the EPCs counts may be considered a suitable biological marker to follow up the evolution of the hypertensive state in women.

AUTHORS INDEX