STEM CELLS

REGENERATIVE MEDICINE

Concise Review: Liver Regenerative Medicine: From Hepatocyte Transplantation to Bioartificial Livers and Bioengineered Grafts

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ABSTRACT

Donor organ shortage is the main limitation to liver transplantation as a treatment for end-stage liver disease and acute liver failure. Liver regenerative medicine may in the future offer an alternative form of therapy for these diseases, be it through cell transplantation, bioartificial liver (BAL) devices, or bioengineered whole organ liver transplantation. All three strategies have shown promising results in the past decade. However, before they are incorporated into widespread clinical practice, the ideal cell type for each treatment modality must be found, and an adequate amount of metabolically active, functional cells must be able to be produced. Research is ongoing in hepatocyte expansion techniques, use of xenogeneic cells, and differentiation of stem cell-derived hepatocyte-like cells (HLCs). HLCs are a few steps away from clinical application, but may be very useful in individualized drug development and toxicity testing, as well as disease modeling. Finally, safety concerns including tumorigenicity and xenozoonosis must also be addressed before cell transplantation, BAL devices, and bioengineered livers occupy their clinical niche. This review aims to highlight the most recent advances and provide an updated view of the current state of affairs in the field of liver regenerative medicine. STEM CELLS *2017;35:42–50*

SIGNIFICANCE STATEMENT

This review aims to highlight the most recent advances and provide an updated view of the current state of affairs in the field of liver regenerative medicine through a thorough and systematic review of the current literature, with a focus on: the use of iPS cells, hepatocyte and stem cell transplantation, gene therapies for inherited metabolic diseases, development of bioartificial liver systems, and liver tissue engineering, as well as potential applications and current challenges of stem cell-based strategies in the treatment of liver disease.

INTRODUCTION

Liver transplantation is to date the only proven treatment for end-stage liver disease (ESLD) and acute liver failure (ALF). Due to the shortage of transplantable organs, however, alternatives to liver transplantation have long been sought after. With the advent of regenerative medicine, these alternative forms of treatment are becoming distinct possibilities—be it through cell and stem cell therapy, bioartificial liver (BAL) devices, or organ bioengineering. The liver is particularly amenable to these forms of therapy due to its innate capacity for intense regeneration and self-repair.

The oldest form of cell therapy is cell transplantation, which has been tested on a myriad of different liver diseases with uneven results. Primary hepatocyte transplantation, however, shares many of the limitations of whole-organ liver transplantation: scarcity of donor livers from which high-quality primary hepatocytes can be isolated, and possibility of allogeneic rejection. For this reason, the focus of liver cell therapy has shifted slightly onto the therapeutic potential of stem cells. Stem cell transplantation is especially promising in inherited liver disease-where it may be able, in combination with gene therapy, to offer permanent correction of metabolic deficiencies possibly without the use of immunosuppressive drugs. Stem cell-derived hepatocyte-like cells (HLCs) also provide the opportunity for noninvasive metabolic profiling and drug toxicity testing. With individualized medicine on the

rise, these therapeutic strategies will soon gain visibility in the field of hepatology.

The next step in liver regenerative medicine was the creation of a BAL system capable of bridging a patient either to liver transplantation or to recovery of the native liver through endogenous regeneration. These devices, as opposed to artificial liver support systems, contain live, functioning hepatocytes, and so are able to perform synthetic functions as well as blood detoxification by way of albumin dialysis. This holds great promise for the treatment of ALF. Finally, the paradigm of regenerative medicine is considered by many to be tissue engineering. Considerable advances have been made in the past decade toward the construction of a bioengineered liver through the de- and recellularization of a three-dimensional (3D) liver scaffold.

The aim of this review is to highlight the most recent advances made in the field of regenerative medicine for the treatment of liver disease, and to address the role that these new technologies may play in the clinical setting over the next few years.

Use of Primary Hepatocytes Versus Stem Cells

Isolated primary hepatocytes were the first and most obvious candidate for use in cell therapy, but they have several limitations related to both the nature of the cell and the scarcity of its source. Hepatocytes are not easily cultured in vitro and are susceptible to freeze-thaw damage [1]. The most important restriction to their use, however, is the difficulty that isolation of a sufficient quantity of metabolically active, high quality cells presents [2]. Not only is there a universal shortage of donor livers on which cell harvests may be performed, but the fact that hepatocytes are typically harvested from livers not suitable for transplantation makes quantity and quality of cells obtained highly variable [3]. Alternatives to the use of primary human hepatocytes are porcine hepatocytes and stem cell-derived HLCs. Due to concerns of xenozoonosis and antibody-mediated hyperacute rejection, the use of porcine cells in trials applicable to clinical practice has typically been limited to BAL devices, where the BAL's membrane prevents direct contact between patient and porcine hepatocyte.

Research in the field of regenerative medicine has lately set its focus on the generation of HLCs [4], namely from induced pluripotent stem cells (iPSCs) [5]. Several groups have developed standardized, efficient protocols [6, 7] for the development and isolation of iPSC-derived HLCs through soluble factors [8–10]; direct reprogramming of fibroblasts to HLCs has also been achieved [11, 12], with these cells demonstrating drug metabolic function [13]. Cell reprogramming for the production of autologous hepatocytes potentially allows these therapies to bypass the scarcity of human donor livers, as well as avoid allogeneic rejection [14]. However, reprogrammed cells are not without their disadvantages. As of today, fully mature HLCs have not yet been produced: studies have shown that stem cell-derived HLCs are phenotypically and functionally more similar to fetal than adult human hepatocytes [15]. Historically, they have lower levels of albumin production, as well as cytochrome P450 and urea cycle activity than hepatocytes, and persistently high expression of alpha-fetoprotein [16], although iPSC-derived HLCs may express hepatocyte-specific markers, glycogen and lipid storage activity, albumin secretion, and CYP450 metabolic activity, and have been able after transplantation to improve the functional status of a CCl4-injured mouse liver [17]. Notwithstanding this, their metabolic profile and CYP activity is sufficient to provide human in vitro models for toxicity testing and drug studies [18], a use which will be discussed more in depth in the following section. The main safety concern these cells pose is their possible tumorigenesis [19]. This issue has been partly bypassed through the avoidance of viral vectors by direct delivery of reprogramming factors [20, 21], but although tumorigenicity and immunogenicity of iPSCs have been found to decrease with reprogramming methods that do not involve genomic integration [22], the altered expression of the basal reprogramming factors involved in their differentiation has also been reported to be associated with cancer [23–25].

The most immediate application of iPSC-derived HLCs will foreseeably take place in the field of pharmaceutical development and individualization. Drug development is a long and expensive process with up to 90% of developed drugs failing clinical trials or being withdrawn from the market due to unexpected toxicity [26]. This underwhelming efficiency is likely due to imperfect toxicity correlations in vivo between animal model physiology and human patients, as well as irrelevant positive toxicity-related responses from in vitro assays that rely on primary cell cultures or immortalized cell lines [27, 28]. Drug assays in iPSC-derived HLCs provide a powerful in vitro alternative to existing methods for drug toxicity testing and metabolic profiling. iPSCs constitute a noninvasive, personalized approach to pharmacodynamic and pharmaceutical testing, since they can be produced from a blood sample rather than a liver biopsy [29]. They allow for prediction of interindividual differences in hepatic metabolism and drug sensitivity mediated by genetic polymorphisms, which influence both drug efficacy and adverse reactions [30], especially idiosyncratic drug-induced liver injury [31]. Disease-specific iPSCs have also been created that allow for a disease-in-a-dish approach to modeling [32] that may be applicable to a variety of genetic diseases [33] and may offer the opportunity not only for drug screening, but for gene correction as well [34]. Although standardization is necessary before these cells can be applied in routine pharmacotoxicology [35], the ability to test for undesired outcomes in a relevant biological model during the early stages of drug development will enhance the efficiency and affordability of the novel drug approval process. Similarly, another future use for stem cell-derived HLCs may be disease modeling, both in vitro and in vivo after cell transplantation [36].

Stem cells have been used not only to produce HLCs, but also to create a favorable environment for HLCs or primary hepatocytes to grow. Coculture with mesenchymal stem cells (MSCs) provides primary human hepatocytes with direct structural and paracrine trophic support, resulting in improved viability and function [37]. Another strategy that may improve hepatocyte functionality is 3D culture [38]. Aggregation into organoid-like structures has been tested in primary hepatocytes and stem cell-derived HLCs with and without MSC coculture, yielding promising results both in terms of functionality and engraftment [39, 40]. iPSC culture as aggregates in 3D suspension offers the advantage of culture at high densities, allowing for large-scale cell production—monolayer tissue

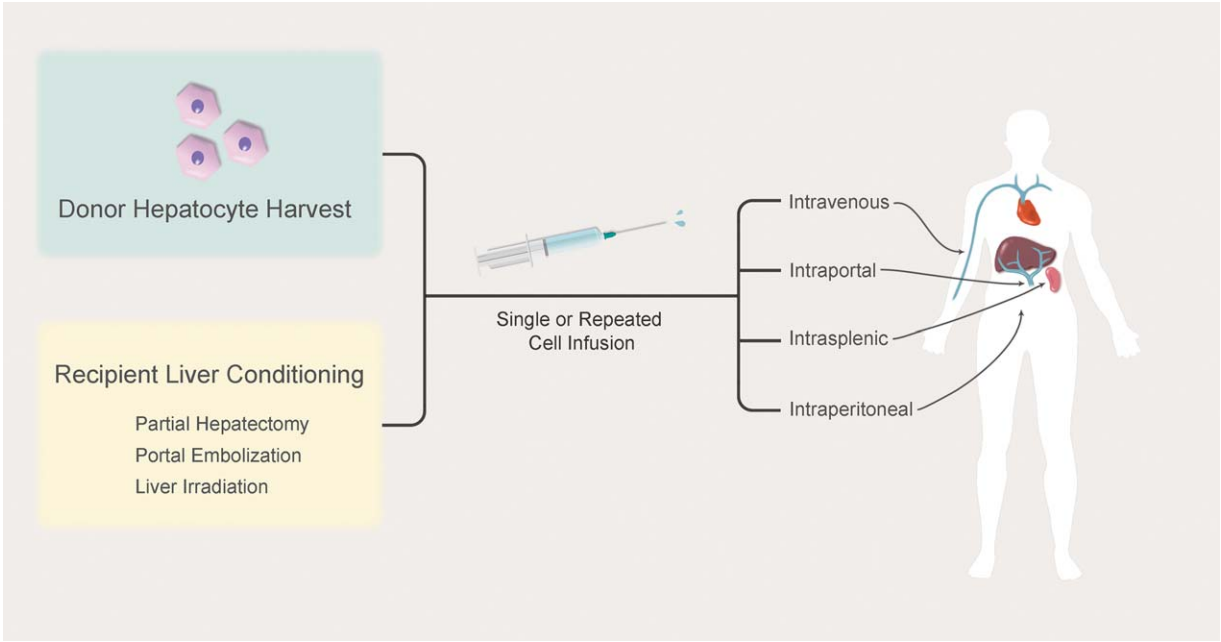

Figure 1. Infusion and engraftment strategies in cell transplantation. A hepatocyte harvest is performed on a donor liver, while the recipient liver is conditioned through partial hepatectomy, portal embolization, or liver irradiation for more efficient engraftment. The cells to be transplanted are then injected into a peripheral vein, portal vein, spleen, or intraperitoneally in the recipient through single or repeated cell infusion.

cultures would not be able to sustain the rapid cell expansion necessary for clinical application—, and at the same time increases functional maturation and longevity of HLCs [41]. In vitro expansion systems have also been developed that have proven successful in inducing iPSC proliferation [42], and the engraftment potential of iPSC progeny is actively being studied [43].

In conclusion, to date the ideal cell type for use in hepatic regenerative medicine remains the primary hepatocyte. Nevertheless, progress is rapidly being made in the direct reprogramming of somatic cells to HLCs and maturation of stem cell-derived HLCs, which may lead to the experimental introduction of these cells into the clinical setting in the near future.

Cell Transplantation

Cell transplantation has been tested in a number of patients with various forms of liver disease: most commonly metabolic diseases, but also acute and chronic liver disease. These clinical interventions have occurred as a result of the significant therapeutic benefit achieved in a number of preclinical models, mostly rodent. Cell transplantation has a number of key advantages. First, the procedure is less invasive than organ transplantation and multiple cell transplants can occur over time. Second, the native liver is left in place, allowing it the possibility of self-regeneration in the case of ALF. Third, with the promise of gene therapy and stem cell technology slowly coming to realization, the opportunity exists for an individualized, autologous approach to regenerative medicine.

Therapeutic benefit in a number of small animal models of metabolic liver diseases has been demonstrated using hepatocyte transplantation [44, 45]. The general concept behind these successes has been infusion of hepatocytes from highly inbred, syngeneic donors into recipients through intrasplenic or portal vein injection; the ultimate goal is to achieve a high enough engraftment so that a sufficient amount of the missing enzyme/protein is produced that a therapeutic effect is achieved—generally estimated to be 5%-10% for many diseases. While cell engraftment is initially low, estimated to be $<$1% of total liver mass [46], a number of strategies have been used to increase engraftment and/or proliferation of transplanted cells. Some diseases provide an inherent natural selective advantage for transplanted cells, as is the case in hereditary tyrosinemia type 1 [47] and alpha-1 antitrypsin deficiency [48]. Other methods have been tested in the case of diseases without a selective advantage for transplanted cells, including genetic modification of the donor cells [49, 50] and injury to the recipient liver [51, 52]. These methods have been successful in increasing donor cell expansion to sufficient quantities to produce a biological effect. While the safety of clinical hepatocyte transplantation has clearly been established, demonstration of therapeutic benefit has been modest and short-lived [53]. A major focus of ongoing clinical trials is improving engraftment and proliferation of donor hepatocytes using one or a combination of partial hepatectomy [54], portal embolization [55], liver irradiation [51], and repeated cell infusion [56] (Fig. 1).

The goal of cell transplantation in ALF is to provide time for (a) the native liver to regenerate or (b) liver transplantation to occur. Because of the differences in regeneration of rodent and human livers, it is difficult to interpret the positive results seen in ALF rodent models [57]. Notwithstanding this, significant improvements in survival have been demonstrated in various drug-induced rodent models of liver failure [58]. Clinically, a number of studies have occurred studying the impact of hepatocyte transplantation on various forms of ALF,

most commonly drug and viral-induced [53, 59]. However, as all of these studies were noncontrolled, it is not possible to draw definitive conclusions from them. Variations within these studies included: delivery method of cells (intravenous, intraperitoneal, intrasplenic, and portal vein infusion); dose of cells injected; and use of fresh or frozen cells. Therefore, it seems imperative that clinically-relevant models of ALF are utilized in future studies, and for these it would appear that large animal models will play a key role [60, 61].

In a number of small animal models of chronic liver failure, significant improvements were documented after hepatocyte transplantation [62, 63]. Clinically, a range of responses have been reported, and interpretation of results is difficult given the nature of the uncontrolled experiments [64]. Two general approaches have been attempted: allogeneic transplantation of donor hepatocytes from noncirrhotic liver donors; or autologous transplantation of hepatocytes isolated from a single lobe of the recipient's cirrhotic liver. Given the increased risk of portal hypertension after portal infusion of cells into a cirrhotic liver, intrasplenic injection has been the most common method of cell delivery [65]. More recently, preclinical data has indicated that extrahepatic lymph nodes may provide a superior niche for hepatocyte engraftment than the cirrhotic liver, in which cell engraftment and function is limited [66].

Successful hepatocyte transplantation in animal models of liver disease, primarily rodent, has not been replicated clinically [67]. While each of the three groups of diseases (metabolic, acute, and chronic) may have different etiologies, a few common hurdles will need to be overcome before significant and reproducible clinical success is achieved. The first major challenge is the shortage of high quality primary hepatocytes. As the shortage of donor organs is unlikely to improve in the future, alternative sources of high quality, engraftable cells will be needed. Ongoing research in this field includes the use of animal bioreactors to expand human hepatocytes [68], as well as identifying alternative sources of expandable cells. Recent data from several groups have identified populations of liver progenitor cells that allow expansion ex vivo [69]. While reproducible correction of mouse models has not been achieved, the data indicates that under the right conditions these progenitor cells can engraft and function in vivo in rodent models of metabolic disease and liver failure. The second major challenge is the immune response against transplanted allogeneic cells [54]. In the case of metabolic disease, this issue can be bypassed through the use of genetically corrected autologous hepatocytes, a procedure that has been reported only once for liver disorders [70]. Given the encouraging clinical success observed in the treatment of primary immunodeficiencies using ex vivo gene therapy with lentiviral vectors [71], it appears highly warranted that continued evaluation of ex vivo hepatocyte-directed gene therapy occurs. Although primary hepatocytes isolated from liver resection remain the optimal cell population for re-transplantation following gene therapy [72], the advent of nuclear reprogramming technology may allow for an alternative cell population in which to perform gene therapy and subsequent hepatocyte differentiation. While the utility of this approach to model "disease in a dish" is irrefutable, evidence to support the reprogrammed HLCs' ability to engraft and expand in vivo is still limited. However, encouraging results in mouse models of metabolic disease and liver failure have been reported [12, 73, 74].

Bioartificial Liver Systems

To date, liver transplantation remains the only definitive treatment for ALF [75]. However, there are several important limitations to liver transplantation, namely the nation-wide shortage of donor organs and the possibility of allogeneic rejection, together with the many long-term adverse effects of immunosuppressant medication. For this reason, alternative therapies are being sought out, with artificial and bioartificial liver support systems constituting one of the most promising solutions currently under development.

The ideal liver support system should detoxify waste molecules such as ammonia, provide synthetic function of albumin and coagulation factors, decrease inflammation, and promote cell regeneration. A BAL system incorporates hepatocytes into a purely mechanical, albumin dialysis-based artificial liver support device to achieve the aforementioned goals. Ideally, a BAL support system would use primary human hepatocytes. However, large amounts of high-quality human hepatocytes are not readily available. Therefore, a number of different cell lines currently being used. The two BAL systems that have undergone the most extensive human clinical trials are the Extracorporeal Liver Assist Device (ELAD) and the HepatAssist. Other systems include the Modular Extracorporeal Liver Support (MELS), the Amsterdam Medical Center Bioartificial Liver (AMC-BAL), and the Bioartificial Liver Support System (BLSS). To date, no BAL system has been showed to improve survival in ALF patients and none is FDA approved.

The ELAD uses HepG2/C3A hepatoblastoma cells loaded in four hollow-fiber cartridges holding 200 g of cells each. The VTI-208 trial, which is the largest BAL randomized controlled trial to date, assigned 96 patients to ELAD plus standard medical therapy (SMT) and 107 patients to SMT alone [76]. Results showed no statistically significant difference in overall survival at 28 and 91 days between both groups. However, stratified subgroup analysis showed that ELAD may have improved the outcome of patients under 50 years of age with Model for ESLD scores below 30. More randomized controlled trials are planned to confirm these results [77]. Concerns for use of the ELAD system include the theoretical risk of tumor cell migration into the patients' circulation, as well as the decreased hepatocytic functions of HepG2/C3A cells, especially in terms of ureagenesis and drug metabolism [78].

The HepatAssist liver support device uses 7 billion primary hepatocytes from healthy pig donors in a hollow-fiber bioreactor, and its largest clinical trial to date was published in 2004 [79]. Patients were randomized prospectively to BAL plus SMT (85 patients) or SMT alone (85 patients), with no statistically significant difference in 30-day survival. Porcine hepatocyte-based BAL systems pose concern for xenozoonosis, although no zoonotic infection has been observed in any clinical trial so far. The BLSS uses 100 g of primary porcine hepatocytes in a single hollow-fiber cartridge, as well. A Phase I trial performed in 2001 demonstrated safety in four patients [80], but further studies are required to demonstrate efficacy. The AMC-BAL incorporates 10 billion primary porcine hepatocytes into a nonwoven polyester matrix. A Phase I trial

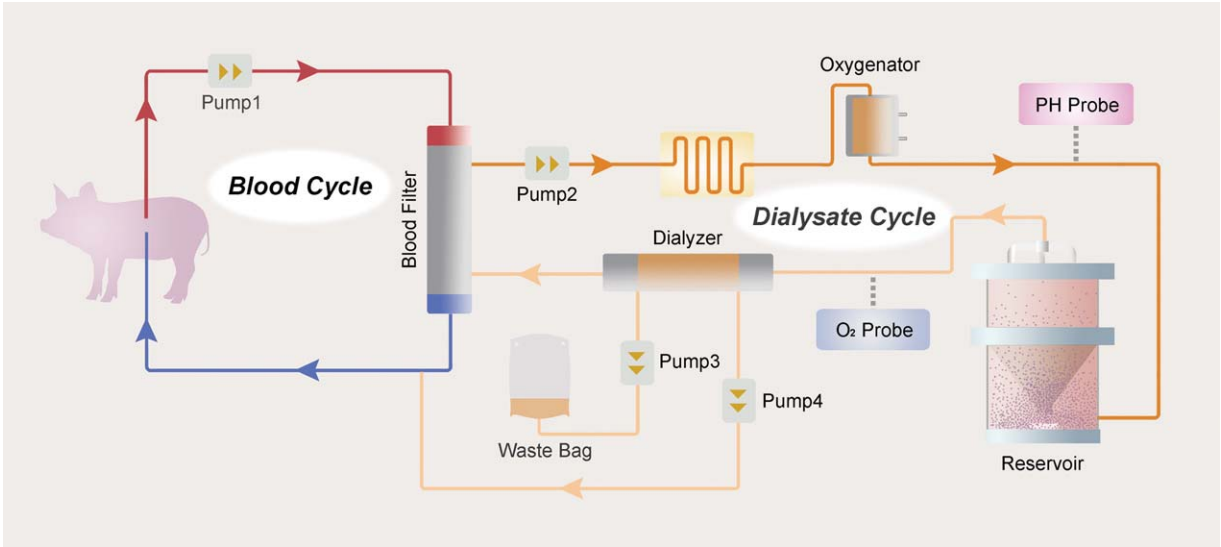

Figure 2. Schematic representation of the spheroid reservoir bioartificial liver device. The red and blue lines indicate the blood compartment, while the orange line indicates the acellular albumin dialysate compartment. The blood filter consists of a hollow fiber cartridge, and the spheroid reservoir, containing over 100 g of hepatocyte spheroids, functions as a suspension bioreactor with fluid entering below and exiting above.

successfully bridged 6 out of 7 ALF patients to liver transplant, with one patient recovering without transplant [81]. Due to legislative issues related to xenotransplantation, a human hepatoma cell line (HepaRG) is now being used as the hepatocyte source for this device: its efficacy and safety is currently being studied in rat models [82]. The MELS device contains polyether sulfone and hydrophobic multilaminated hollow fiber bundles interwoven inside a cell compartment that houses 18-44 billion primary porcine hepatocytes [83]. A Phase I trial performed in 2003 successfully bridged 8 out of 8 patients to liver transplant; all patients survived to 3-year follow-up.

Currently, primary porcine hepatocytes constitute the most realistic cell source to power a BAL device. However, the issue exists of primary porcine hepatocytes' loss of function and tendency to apoptose ex vivo. The second-generation porcine hepatocyte BAL system, Spheroid Reservoir Bioartificial Liver (SRBAL), resolved this issue by culturing the cells as 3D spherical aggregates instead of monolayers. The spheroid configuration allows for a higher number of hepatocytes per volume, and longer functional and survival times [84, 85]. A recent pivotal preclinical study showed a statistically significant survival benefit of SRBAL treatment in a drug-induced ALF pig model [60] (Fig. 2); human trials are planned but are not yet underway.

The first BAL system that made use of stem cell-derived HLCs was reported in 2016 [86]. This device contains 3 billion HLCs, termed hiHeps, induced from human fibroblasts [11, 12] in a multilayer radial-flow bioreactor. Its performance was tested in a drug-induced porcine ALF model, showing improvement in prothrombin time and ammonia levels, as well as a statistically significant improvement in survival. Other iPSC-based BAL devices had been previously described, but not tested [87]. To date, no stem cell-based BAL system has undergone human trial.

Going forward, a method for large-scale production of fully functional hepatocytes must be developed in order for BAL systems to be incorporated into clinical use. To this effect, a repopulation model was created in which a mouse model of hereditary tyrosinemia type 1 were used to expand transplanted human hepatocytes by virtue of the graft's selective advantage over the native mutant fumarylacetoacetate hydrolase (FAH)-deficient cells [68]. A porcine model of FAH deficiency has recently been developed that could potentially allow for large-scale production of high-quality and readily available human hepatocytes in an animal bioreactor [61]. The main issue with this technique is the possibility of immune rejection of the transplanted cells. Therefore, the next step may be to expand transplanted human hepatocytes through *in utero* transplantation before the fetus has developed a functional immune system [88], or through the creation of genetically-engineered immunodeficient FAH-negative pigs. Besides these re-population models, continuous advancements in the hepatocytic differentiation of iPSCs, embryonic stem cells (ESCs), and human fibroblasts may be able to create the metabolically active, functional HLCs needed for BAL systems in the future [11, 89].

Organ Bioengineering

Tissue engineered regenerated whole organs have the unique potential to overcome two major issues facing the field of transplantation: the shortage of donor organs as well as the need for ongoing chronic immunosuppression. Tissue engineering strategies have been to date been applied in building a biological substitute for a number of failing tissues including the heart [90], lungs [91], bladder, intestines, trachea, kidney, and liver [92].

A tissue engineered liver requires a scaffold on which to build a functional organ. A variety of scaffolds have been trialed, including biodegradable polymer matrices [93], two-dimensional hepatic tissue sheets [94], and decellularized xenogeneic liver matrices [95], with greatest success being

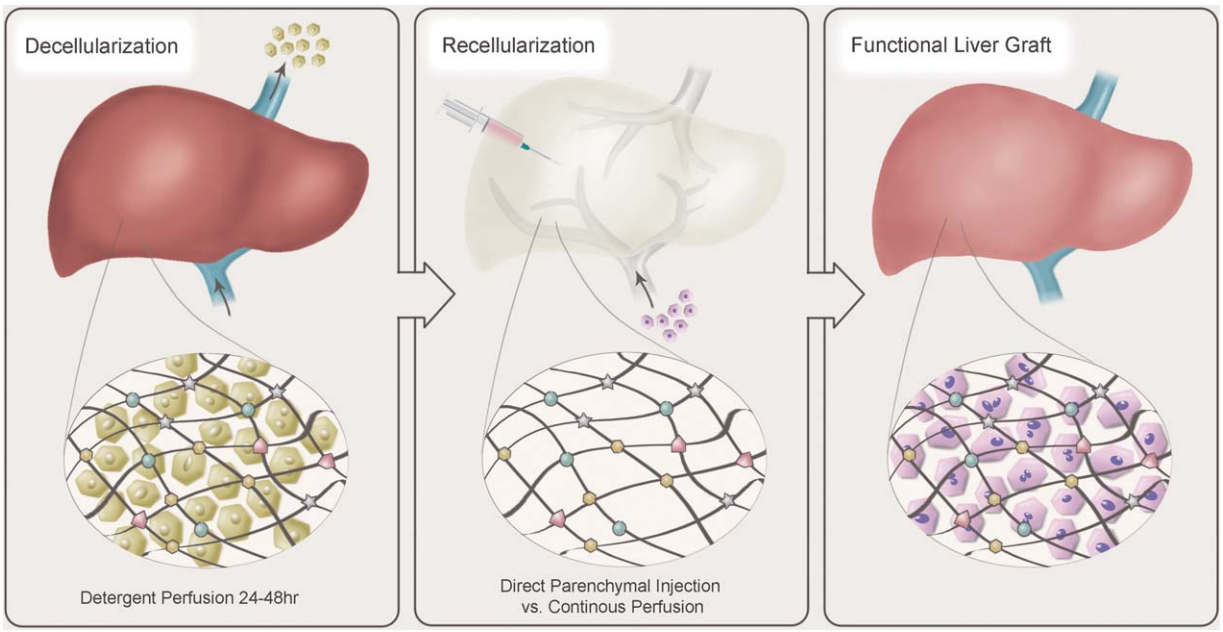

Figure 3. Decellularization and recellularization process for the creation of bioengineered livers. The liver is decellularized through detergent perfusion at physiologic pressures via the native vasculature for 24-48 hours. The resulting scaffold is then recellularized and reendothelialized with functional hepatocytes and endothelial cells either through direct parenchymal injections or through single or multistep continuous perfusion at physiologic pressures to produce a functional liver graft.

observed in xenogeneic (porcine or murine) liver scaffolds. In this case, the presence of an intact native extracellular matrix (ECM) is of central importance, as it not only provides a platform for cell ingrowth, but is also thought to mediate biochemical and molecular signaling [96].

In order to obtain a xenogeneic scaffold, complete decellularization of the native organ must be achieved. Detergent perfusion at physiologic pressures via the native vasculature for 24-48 hours has proven to be successful in attaining complete decellularization. Livers are first flushed with large volumes of isotonic saline solution, then perfused with detergents, which include sodium dodecyl sulphate, ethylene glycol tetraacetic acid, Triton X-100 1, 2, or 3%, and DNase [95]. Some protocols also utilize gamma irradiation to further reduce immunogenic properties [97]. The key to a successful decellularization is preservation of the ECM through maintenance of the collagen matrix together with destruction of the native organ DNA. In order to avoid immune reactions, a decellularized scaffold should have under 50 ng double stranded DNA per mg ECM [98]. Immunogenicity has been tested via implantation of naked scaffolds into allogeneic or xenogeneic hosts without evidence of increased immune response as measured by total white blood cell count, lymphocyte count, monocyte count, and lack of CD3+ T cell activation at the site of implantation [99].

Recellularization of the xenogeneic scaffold requires a large quantity of readily available, highly functional hepatocytes. Human survival is possible with 10%-30% (200-600 g) of residual hepatic parenchyma corresponding to 2.5-7.5 billion individual hepatocytes. These cells must be capable of proliferation and safe for transplantation. Adult human hepatocytes harvested from deceased donor grafts or partial hepatectomy are a potential cell source; however, adequate volumes of suitable cells are difficult to obtain. Furthermore, although the adult human liver is capable of significant regeneration following major hepatectomy, this process is poorly understood [100], and the adult hepatocyte demonstrates minimal in vitro proliferation proving thus far to be a poor candidate for organ regeneration. In contrast, human fetal liver cells demonstrate in vitro proliferation; however, they are not readily available and their hepatocytic functions remain relatively low [96]. Hepatoblastoma-derived cell lines (HepG2) offer unlimited expansion potential and have shown promise in clinical trials of BAL devices [101]. Unfortunately, the risk for uncontrolled metastatic spread prevents the use of these cells in an implantable liver. Xenogeneic cells sources such as porcine hepatocytes have also been trialed with some success in BAL devices but face major immunological barriers to clinical cell transplantation. The question of zoonotic viral transmission has been raised, but has not been realized in several clinical trials [102]. Autologous stem cells also show significant promise as a readily available and functional cell source: human iPSCs have been utilized to create an organ bud capable of liver specific protein production and drug metabolism [103]. Although this technology is promising, the human iPSCs produce albumin at a lower level than mature adult hepatocytes, raising concern for the applicability of this model within an adult liver. This whole-organ bioengineering approach with scaffolding has several important advantages over the in vitro creation of stem cell-derived transplantable organ buds, namely: difference between the ultrastructural organization of the bud and that of a normal liver, lack of an external bile tree, and inability to transplant such buds orthotopically [104], as well as size restriction [105]. Human bone marrow mesenchymal stem cells (hBMSCs) have also shown promise as a cell source for recellularization [106, 107], although they have not been studied in live matrices. A porcine model of fulminant hepatic failure rescued with hBMSCs, however,

demonstrated increased survival, suppression of cytokine storm, and reversal of liver failure within 7 days [108].

In order to achieve successful recellularization, both direct parenchymal injections as well as single or multistep continuous perfusion at physiologic pressures have been explored (Fig. 3), facilitated by the construction of a sterile organ chamber in which the scaffold is mounted and supported in tissue culture. A rat model was used to demonstrate proof of concept of whole liver decellularization and recellularization with mature rat hepatocytes [97]. Proliferation was confirmed via Ki67 antibody staining; albumin production and CYP1A1/2 activity confirmed ongoing metabolic function. Since hepatocytes demonstrate a significant increase in function with cell-cell and cell-matrix interactions, in vitro liver progenitor cell spheroids were produced which showed increased cell survival and differentiation when compared to single cell suspensions [109]. A rat hepatocyte spheroid tissue engineered liver was transplanted into a 90% rat hepatectomy model with an increase in overall survival from 16 to 72 hours; however, transplanted rats ultimately perished from small-for-size syndrome [110].

Regardless of the cell type selected, an intact vascular network is required to support the necessary cell mass. Therefore, a major challenge to hepatocyte recellularization has been the conservation of a functional vascular infrastructure. Rat anti-mouse CD31 was used to enhance reendothelialization of a liver scaffold with murine endothelial cells (MS1), and demonstrated in vivo patency at 24 hours in a porcine recipient [111]. Significant reendothelialization and in vivo vascular patency has also been demonstrated through the use of porcine umbilical vein endothelial cells at 72 hours following implantation in a porcine recipient [112].

Organ bioengineering represents a promising frontier for the creation of readily available and sustainable organs for transplantation. The decellularized xenogeneic scaffold with an intact ECM has proven an effective backbone on which to create a tissue engineered organ, and additional efforts aimed at selecting an ideal cell type for human application are ongoing. Still, further research into optimal cell seeding techniques and cell volumes required to sustain function is necessary.

Conclusion

A regenerative medicine approach to liver disease may in the future be a solution to the current shortage of donor livers available for transplantation. Cell transplantation has been tested in a number of pre-clinical and clinical models of various forms of liver disease, yielding promising results for the treatment of metabolic disorders in particular. Similarly, BAL systems may soon start playing a role in the treatment of ALF by either allowing for regeneration of the native liver or bridging the patient to liver transplantation. At the same time, major advances are also being made toward the creation of bioengineered, transplantable organs.

However, several important challenges must still be overcome before these therapeutic strategies are incorporated into clinical practice. First and foremost, the optimal cell type for each therapy must be determined, and be able to be obtained or produced in quantities sufficient for large-scale clinical application. Second, these cells must be able to be cultured efficiently in vitro, and in the case of cell transplantation and bioengineered livers engraft successfully in vivo. Third, each cell type must demonstrate safety in humans, with a special focus on concerns for xenozoonosis and tumorigenicity. Notwithstanding this, stem cell-derived HLCs are already being used in individualized medicine for the development and toxicity testing of new drugs.

Authors Contributions

C.T.N.: Conception and design, manuscript writing; R.D.H.: Manuscript writing; H.S.C. and S.A.M.: Manuscript writing; M.L.H.: Manuscript writing; Y.W.: Figure preparation; S.L.N.: Conception and design, final approval of manuscript.

Disclosure of Potential Conflict of Interest

The authors indicate no potential conflicts of interest.

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