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REVIEW ARTICLE

Dental Stem Cells and Tooth Banking for Regenerative Medicine

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Stem cell (SC) therapy has a promising future for tissue regenerative medicine. However, because SC technology is still in its infancy, interdisciplinary cooperation is needed to achieve successful clinical applications. Dental SCs have drawn attention in recent years because of their accessibility, plasticity, and high proliferative ability. Several types of dental SCs have been identified, including dental pulp SCs from adult human dental pulp, SCs from human primary exfoliated deciduous teeth, periodontal ligament SCs, and dental follicle SCs from human third molars. Similar to mesenchymal SCs, these dental SCs can undergo self-renewal and have multipotent differentiation ability, but do not have the ethical issues associated with other sources of SCs. Therefore, appropriate preservation procedures for dental SCs and teeth are now needed. Here, we discuss the opportunities for tooth-banking (as it is now clinically feasible and commercially available), the advantages and limitations of current cryopreservation techniques for dental SCs/teeth or tissues, and the current status of tooth banks.

1. Introduction

Stem cells (SCs) are undifferentiated cells capable of self-renewal and differentiation into multiple functional cell types. These cells are widely used in injury repair and tissue regeneration.[1,2] Adult SCs have been isolated from a variety of tissues including bone marrow, brain, liver, lungs, breast, skin, skeletal muscles, hair follicles and teeth.[3,4] Dental-derived SCs have been isolated and identified as the cell sources for tooth repair and regeneration. These cells are named according to their anatomical locations, and are characterized by their SC markers, colony-forming ability, and dental regenerative function.

Current research indicates that dental SCs may have the potential to regenerate bone, the periodontal ligament (PDL), and possibly teeth. Thus, appropriate cryopreservation of these dental cells, tissues and teeth are imperative to realize the opportunities of these SCs for medical applications, particularly for autotransplantation.[5] However, the optimal methods for tissue cryopreservation remain largely unknown. Masato et al described long-term tooth cryopreservation using a programmed freezer with a magnetic field, the so-called Cell Alive System (CAS).[6] Using the CAS method, the PDL showed good cell viability and differentiation capability after cryopreservation.[6] In support of this, further experiments by Temmerman et al demonstrated

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the successful cryopreservation of human pulpal tissues, when the cryoprotectant encompassed the entire pulp.[7] Thus, by using appropriate cryopreservation processes, tooth storage and banking will provide significant contributions to clinical autotransplantation.[6,7]

Remarkable progress has recently been made in SC biology and tissue engineering. Tooth tissue engineering involves the use of *in vitro* expanded cells in combination with supporting biocompatible materials in an appropriate environment. Traditionally, tooth-like structures produced from biodegradable polymer scaffolds were seeded with dissociated tooth germ, usually from postnatal pigs or cultured rat tooth bud cells, and then grown in the omentum of immunocompromised mice.[8] Recent advances have uncovered the potential for tooth tissue regeneration using SCs and a scaffold/extracellular matrix.

2. Dental-derived SCs

Dental SCs have been found in several tissues and can be divided into dental mesenchymal SCs (MSCs) and dental epithelial SCs (Figure 1 and Table 1).[4,9–23] MSCs from human dental tissues include dental pulp SCs (DPSCs) in human permanent teeth,[4,9,10] SCs from human exfoliated deciduous teeth (SHEDs),[11] periodontal ligament SCs (PDLSCs),[24] and dental follicle SCs (DFSCs) from human third molars.[25,26] Dental epithelial SCs have also been found in continuously growing incisors in mice and in molars from various mammalian species.[20,21]

2.1. DPSCs and SHEDs

DPSCs are SCs derived from dental pulp (Figure 1). These cells are quiescent and reside in a specific perivascular microenvironment where they maintain their SC characteristics. DPSCs show a multipotential differentiation ability, which is similar to that of MSCs**[REFERENCE?]**. These DPSCs express MSC markers, including Stro-1 and CD146, and undergo colony forming *in vitro* and can regenerate the dentin/pulp complex *in vivo***[REFERENCE?]**.

SHEDs are multiple SCs found in the pulp tissue of human exfoliated deciduous teeth. They were originally identified as a population of extensively proliferative clonogenic cells, and can differentiate plastically into neuronal cells, adipocytes and odontoblasts. In addition, SHEDs show higher proliferation rates than DPSCs, and can form significant amounts of alveolar and orofacial bone for tissue regeneration.[4,11]

2.2. PDLSCs

Periodontal disease is prevalent in the adult Taiwanese population.[27] As periodontal tissues are able to regenerate after mild trauma, researchers in the early 1970s postulated that PDLSCs might play an important role in periodontal repair.[12] PDLSCs were first isolated by Seo et al, and were found to be capable of differentiating into cementoblast-like cells, adipocytes and collagen-forming cells.[13] Cell-surface markers of PDLSCs include Stro-1 and CD146/Muc18.[28] Moreover, PDLSCs have been used to generate a root/periodontal complex to support normal tooth function in a mini-pig animal model.[14] Isolation of PDLSCs from rats and sheep was also recently reported.[15,16]

2.3. DFSCs

Dental follicles comprise the neural crest, which is derived from ectomesenchymal tissue surrounding the developing tooth germ.[17] Human dental follicles can be isolated after wisdom tooth extraction, and they play an important role in tooth eruption by regulating osteoclastogenesis and osteogenesis.[17,29–31] After tooth eruption, the dental follicle differentiates into cells of the periodontium, including alveolar osteoblasts, the PDL, fibroblasts and cementoblasts.[32] The pluripotency of DFSCs has also been demonstrated. For example, the neuronal-differentiation ability of DFSCs was documented using the neural progenitor cell markers Notch-1 and Nestin.[33] Meanwhile, the adipocyte differentiation capability of DFSCs was demonstrated by cultivating dental follicle cells with an adipogenesis medium.[18] These

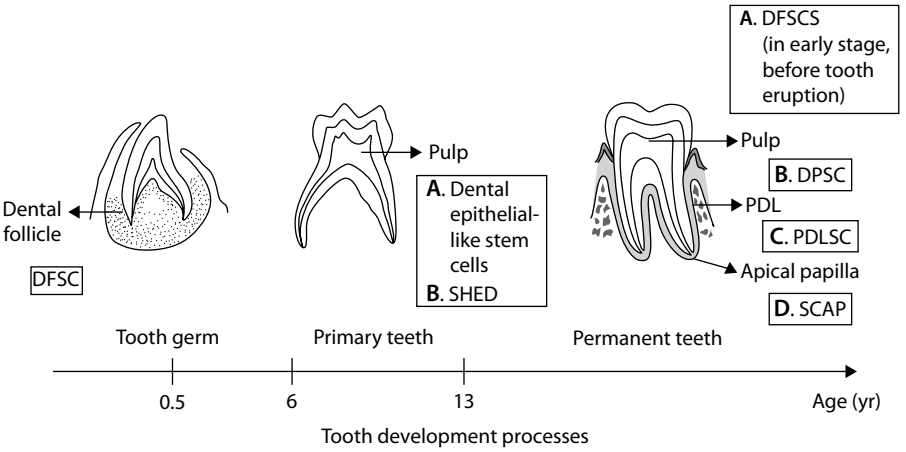

Figure 1 Tooth developmental stages and the derivation of dental-derived stem cells. DFSCs = dental follicle stem cells; SHED = stem cells from human primary exfoliated deciduous teeth; DPSCs = dental pulp stem cells; PDLSCs = periodontal ligament stem cells; SCAP = stem cells from apical papilla.

Table 1 Dental stem cells

Tooth type	Dental germ	Primary teeth		Permanent teeth				
						Wisdom teeth		
Tooth eruption stage	0–6 mo	6–13 yr		>6 yr		16–24 yr		
Dental tissue	Follicle	Pulp	Pulp	Pulp	Periodontal ligament	Pulp	Follicle	Apical papilla
Stem cell type	DFSC	Dental epithelial stem cell-like cells	SHED	DPSC	PDLSC	Dental epithelial stem cell-like cells	DFSC	SCAP
Multipotentiality								
Osteogenic	ND	ND	+	+	+	ND		
Odontogenic	ND	ND				ND	+	+
Cementogenic	ND	ND			+	ND	+	+
Dentinogenic	ND	ND	+	+		ND		
Adipogenic	ND	ND	+	+	+	ND	+	+
Chondrogenic	ND	ND	+	+	+	ND	+	+
Myogenic	ND	ND	+	+	+	ND	+	+
Neurogenic	ND	ND	+	+	+	ND	ND	ND
Reference		20, 21, 22	9, 11	4, 9, 10	12–16		17–19	23

DFSC = dental follicle stem cells; SHED = stem cells from human exfoliated deciduous teeth; DPSC = dental pulp stem cells in human permanent teeth; PDLSC = periodontal ligament stem cells; SCAP = stem cells from apical papilla; ND = not determined in humans.

observations suggest the presence of pluripotent SCs in human dental follicles. In addition to human wisdom teeth, SCs have been isolated from mouse or bovine dental follicles.[19,34]

2.4. Dental epithelial SCs

Tooth enamel, the most mineralized tissue of the body, is first formed in the crown stage of dental development.[35] Before the tooth erupts into the mouth, the ameloblasts are broken down. Consequently, human enamel, unlike continuously growing mouse incisors and some mammalian molars, is unable to regenerate itself.[36–38] Dental epithelial SCs in the mouse cervical loop form a unique structure, the apical bud.[39] The apical bud is a condensed SC compartment responsible for replenishing the growing dentition when it interacts with mesenchymal cells.[20,21,40]

3. Tooth-banking: A Preliminary Step for Future Tissue Regeneration

Extracted teeth are traditionally thought to be medical waste. Historically, the therapeutic potential of dental SCs was not well understood, and there were no appropriate storage methods for potential donor teeth or SCs. Many types of dental SCs have now been identified from human teeth and surrounding tissues. Unlike embryonic SCs, which involve the destruction of human embryos, dental SCs are accessible and available and, most importantly, there are few if any ethical considerations.

The potential roles of dental-derived SCs in regenerative medicine are summarized in Table 1.[4,9–23] With advances in tissue engineering, dental SCs have shown their potential in regenerating odontoblasts,[41] dentin/pulp-like structure, and dentin.[42] Furthermore, dental SCs can differentiate into adipocytes[10] and neurons,[43] and promote the proliferation and differentiation of endogenous neural cells.[44] It is also possible that myocardial infarction[45] and liver dysfunction[46] could be treated with dental SCs in the near future. Thus, the therapeutic capability and clinical benefits of dental SCs are not limited to dental use but can also be used for regenerative medicine (Table 2).[23,45,47–53]

Because of the opportunity to preserve dental SCs for medical applications, the term "tooth bank" was first raised in 1966.[54] Several attempts to preserve dental SCs have also been reported by other groups (Table 3).[6,47,54–56] However, the absence of appropriate preservation methods for teeth and/or dental SCs remains a significant limitation.

With the rapid development of advanced cryopreservation technology, the first commercial tooth bank was established as a venture company at National Hiroshima University in Japan in 2004.[6] By systematic organization, an increasing number of teeth have been cryopreserved for future generative medicine.[57]

3.1. Tooth cryopreservation and tooth banking

"Cryo" means cold in Greek, and cryopreservation is a process in which cells or whole tissues are preserved by cooling to subzero temperatures, typically –196°C.

Table 2 Benefits of dental stem cells

A.	Few ethical concerns
B.	Autograft
	1. Increasing the success rate of tooth auto-transplantation[47,48]
	2. Better proliferation and immunoregulation than bone marrow-derived MSCs[49]
C.	Dental stem cell-based tissue engineering
	1. Oral medicine Tooth regeneration[50] Pulp/dentin regeneration[23] Periodontal ligament regeneration[51]
	2. Other medical application Bone formation[52] Stroke therapy[53] Heart disease[45]
D.	General benefits
	1. Efficient and easy to access source of MSCs
	2. Potential for commercial banking

MSCs = mesenchymal stem cells.

In reproductive medicine, cryopreservation plays a very important role in cell and tissue preservation. For example, in 1949, Polge et al reported the successful cryopreservation of sperm using glycerol[58] and the first human artificial insemination with frozen sperm was reported in 1953.[59] Using glycerol as a cryoprotectant, Smith's group successfully preserved erythrocytes.[60] In 1963, Voronoi et al used a freeze-dry technique to preserve skin.[61] In a further advance, in 1985, mouse embryos were preserved with ice-free cryopreservation at −196°C.[62] In 1999, Kuleshova et al successfully used a vitrification method to preserve human oocytes.[63] Cell and tissue banking is now well developed.[64] However, the possibility of organ cryopreservation, including teeth, is still under investigation.

The formation of ice nucleation and the growth of a crystal structure are the main limitations of traditional cell/tissue preservation because these processes cause cell death. Accordingly, vitrification offers a better method of preserving tissues. Vitrification converts a material into a glasslike amorphous solid without crystalline structure. For this reason, it can protect cells and tissues from dying during cryopreservation. There are three critical factors that affect vitrification.[65] (1) Cooling and thawing rates: two cooling rates are reported when freezing. One is the conventional slow freezing method; the other is vitrification with an ultra high freezing rate. For the thawing rate, a rapid warming method is recommended.[66] As heat is transferred from the external environment inwards when thawing, a slow warming rate causes the outside part to defrost first, but the central part is still cold enough to freeze the outside part again. This is likely to damage the cell. Therefore, rapid cooling and thawing rates are highly recommended. (2) Concentration of the cryoprotectant: the chemical toxicity and potential for osmotic injury by the cryoprotectant are very important for cell viability.[67] However, vitrification requires a high concentration of the cryoprotectant, which is a concern when preserving functional cells and tissues. (3) Sample size and carrier systems: reducing the sample and carrier sizes allow higher cooling and thawing rates.

Since the limitations of cryopreservation and autoreplantation will eventually result in root canal therapy, it is reasonable that those multipotent DPSCs should also be preserved before pulp extirpation. Along with a greater focus on studying dental pulp SCs, tooth banking should be considered as a major source of dental SCs for now.[68,69]

The CAS cryopreservation method using a programmed freezer with a slight magnetic field was established by Masato et al at Hiroshima University, Japan. It was originally designed to preserve dental PDLSCs for autotransplantation.[6] More recently, Price and Cserepfalvi[70] have used the CAS and claimed to have preserved pulp viability and successfully homotransplanted frozen teeth. However, different opinions **[describe what the different opinions were. You mean other researchers tried this technique but failed?]** were reported, which suggested that further endodontic treatment is still needed after autotransplantation.[71] Thus, the impacts of CAS on the cryopreservation of teeth, dental pulp tissues, and DPSCs remain unclear.[72]

In addition to the activities at Hiroshima University, the sister school, Taipei Medical University (TMU) has recently completed a cooperative system and established a second tooth bank in 2008 (the TMU Tooth Bank).[47] After consecutive experimental studies using the CAS, the TMU Tooth Bank has successfully expanded from cryopreservation for autotransplantation to long-term preservation of dental SCs.[48] Now, patients who store teeth in the TMU Tooth Bank will have teeth for autotransplantation and also for SC isolation from thawed dental pulp tissue.

4. Perspectives

In terms of regenerative dentistry, it is recommended that dentists repair an edentulous area of a patient by regenerating or replacing new teeth.[50] Extending the dental oriented applications to other areas of medicine is the main reason for the popularity of research on SC therapy in recent years.[73] However, the potential treatments still need to be supported by *in vitro* and *in vivo* research. Therefore, alternative approaches for regenerative dentistry to repair an edentulous area with the patients' own teeth, so-called autotransplantation, should be considered as a treatment priority (Table 2).[23,45,47–53] It is also important to include tooth banking for dental SC preservation as a preventive treatment plan.

Table 3 Tooth banking methods

Location	Indication	Cryopreservation method	Tooth-derived stem cell	Tooth preserved
United States[54]	Tissue culture Autotransplantation	Solution: Saline/antibiotic/glycerol Control temperature: −20°C for 25 min Dry ice and alcohol bath for 15 min to reach −80°C Storage temperature: −80°C	−	−
Denmark[55]	Autotransplantation Replantation	Solution: DMEM culture medium 10% human serum 10% DMSO Control temperature: 1.2°C/min to −40°C 6°C/min to −100°C Storage temperature: −196°C (LN)	−	+
Korea[56]	PDL cell viability	Solution: DMEM:F−12 = 3:1 10% FBS 10% DMSO Control temperature: −**[NO VALUE?]** Storage temperature: −196°C (LN)	−	−
Japan[6]	PDL cell viability	Solution: BAMBANKER 2 10% DMSO Control temperature: Programmable freezer (ABI Corp. Ltd. **[CITY, STATE, COUNTRY?]**) 75 mA electric current to generate a magnetic field −5°C for 15 min 0.5°C/min to −30°C Storage temperature: −150°C	−	+
Taiwan[47]	Autotransplantation DPSC isolation PDLSC isolation	Solution: BAMBANKER 2 10 % DMSO Control temperature: Programmable freezer (ABI Corp. Ltd.) 75 mA electric current to generate a magnetic field −5°C for 15 min 0.5°C/min to −30°C Storage temperature: −150°C	+	+

DMEM = Dulbecco's modified Eagle Medium; DMSO = dimethyl sulfoxide; LN = **[WHAT DOES "LN" STAND FOR?]**; PDL = periodontal ligament; FBS = **[FETAL BOVINE SERUM?]**; DPSC = dental pulp stem cells; PDLSC = periodontal ligament stem cells.

It is highly likely that tooth banking will be the future of the SC era.

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