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Preserving stem cells from extracted deciduous teeth

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Abstract

Stem cells from human exfoliated deciduous teeth (SHED) are a promising source of dental stem cells due to their high proliferation rate, multipotency, and ease of collection. This review highlights the classification and properties of dental stem cells, including SHED and their differentiation potential into odontogenic, osteogenic, neurogenic, and adipogenic cell types, as well as the conditions for the preservation of dental stem cells. SHED can be isolated from exfoliated deciduous teeth, particularly between ages 5 and 9, with the best results from teeth with healthy pulp. The cryopreservation process involves collecting, disinfecting, and expanding the stem cells, which are then stored for future regenerative medicine applications. Dental stem cell banking is becoming increasingly popular due to the non-invasive nature of the process and its cost-effectiveness compared to other sources of mesenchymal stem cells. However, challenges such as infection, lack of viable tissue, and a need for standardized manufacturing practices remain. Additionally, long-term studies are needed to confirm the efficacy of current cryopreservation techniques for future clinical use. The review concludes by emphasizing the potential of SHED in tissue regeneration and the importance of further research to optimize stem cell banking protocols and procedures.

Keywords: Cryopreservation, dental pulp, dental stem cells, regenerative medicine, SHED, stem cell banking

1. Introduction

Stem cells have gained immense attention in regenerative medicine due to their ability to differentiate into various specialized cell types, thus offering potential treatments for numerous degenerative diseases and tissue injuries. Among the different sources of stem cells, dental stem cells (DSCs) represent a unique and promising category, particularly for use in tissue engineering and regenerative therapies. These stem cells, derived from dental tissues such as dental pulp, periodontal ligaments, and apical papilla, offer numerous advantages due to their accessibility, multipotency, and relatively non-invasive collection procedures.

The concept of dental stem cell banking has also gained popularity as stem cells from human exfoliated deciduous teeth (SHED) and other dental stem cells offer a viable and easily accessible option for future regenerative medicine applications. Unlike other sources of mesenchymal stem cells (MSCs), such as bone marrow or adipose tissue, the collection of dental stem cells does not require invasive procedures, making it an attractive alternative. Cryopreservation of dental stem cells, particularly from deciduous teeth, has the potential to provide biological insurance for future therapies. However, challenges remain in optimizing cryopreservation techniques, ensuring long-term viability, and standardizing protocols in accordance with current good manufacturing practices.

This review aims to highlight the classification, characteristics and preserving conditions of various dental stem cells with a particular focus on SHED. Additionally, the isolation and cryopreservation methods of these cells will be discussed, along with the current challenges and future directions for their use in clinical applications.

2. Dental stem cells (DSCS): Classification and properties

Stem cells are unspecialized cells that have the ability to differentiate into specific cell types. Up to now, seven types of human dental stem or progenitor cells have been identified and characterized: dental pulp stem cells (DPSCs) (1); stem cells from human exfoliated deciduous teeth (SHED) (2); periodontal ligament stem cells (PDLSCs) (3); dental follicle progenitor cells (DFPCs) (4); and stem cells from apical papilla (SCAP) (5). DPSCs, SHED, and SCAP are commonly classified as pulp-related dental stem cells, while PDLSCs and DFPCs are grouped as stem cells associated with the periodontium (6). Additional dental-related stem cells have been discovered more recently, including gingival mesenchymal stem cells (GMSCs) (7), and human natal dental pulp stem cells (NDP-SCs) (8).

Additionally, some markers, such as bone matrix protein and bone sialoprotein, are expressed at low levels in bone marrow stem cells (BMSCs) and absent in DPSCs (1). DPSCs also express markers associated with neuronal and glial cells, which could be attributed to their neural crest cell origin (9).

3. Stem cells from human exfoliated deciduous teeth (Shed)

In 2003, Miura and his team were the first to discover SHED from the remaining pulp in the crowns of deciduous incisors in children aged 7–8 years (2). They isolated these clonogenic and proliferative cells using a method that closely resembled the technique Gronthos and colleagues used to isolate DPSCs (1). When cultured in a neuronal differentiation medium, SHED formed sphere-like clusters, where highly proliferative cells grouped together, either attaching to the culture dish or floating in the medium (2). By dissociating these clusters, the cells were able to grow individually as fibroblast-like cells, indicating a high capacity for proliferation similar to that seen in neural stem cells. In fact, SHED display a faster proliferation rate and higher cell population doublings compared to BMSCs (2).

Under suitable culture conditions, SHED have shown the capacity to differentiate not only into odontogenic, osteogenic, neurogenic, and adipogenic lineages (2), but also into myogenic and chondrogenic cell types (10-12). These findings suggest that deciduous teeth could serve as an excellent source of stem cells for repairing damaged dental tissues, promoting bone regeneration, and potentially treating neurodegenerative disorders.

SHED can be isolated using methods similar to those employed for DPSCs. These stem cells present notable advantages compared to other postnatal dental stem cells, as they come from a non-invasive, easily accessible, naturally discarded source with minimal ethical or legal issues (13). Like DPSCs, SHED express early MSC surface markers STRO-1 and CD146. STRO-1 and CD146 positive cells have been observed around the blood vessels in the residual pulp, suggesting that SHED may originate from a perivascular niche (2). The categories and differentiation potential of stem cells derived from dental tissues are detailed in Table 1.

SHEDs express typical MSC markers such as CD105, CD146, STRO-1, and CD29, while being negative for CD31 and CD34. They also express embryonic stem cell markers Oct4 and Nanog, neural stem cell marker nestin, and stage-specific embryonic antigens SSEA-3 and SSEA-4 (2). Their ability to cross lineage boundaries enhances the potential application of SHED in therapies targeting various tissues. SHED can differentiate into adipogenic, chondrogenic, neuronal, and endothelial cells (2, 14, 15). Additionally, SHED can differentiate into osteoblasts and express receptors for TGF-ß, FGF, and VEGF (15, 16).

Table 1. Categories and differentiation potential of dental-tissue derived mesenchymal stem cells (70).

	Source	In vitro multipotency	In vivo ectopic tissue formation
*DPSC	Pulp adult teeth	Osteogenic, dentinogenic, adipogenic, chondrogenic, neurogenic, myogenic, cardiomyogenic, hepatocyte-like cell, melocyte	Dentin-pulp-like complex
			Bone-like tissues
			Adipose
			Muscle
**SHEDs	Pulp deciduous teeth	Osteogenic, dentinogenic, adipogenic, chondrogenic, neurogenic, myogenic, bone induction, endothelial cell	Pulp-like tissues Bone-like tissues

* DPSC: Dental pulp stem cells, * SHEDs: Stem cells from human exfoliated deciduous teeth.

The quantity of stem cells found in exfoliated deciduous teeth diminishes as the dental pulp recedes and is gradually replaced by gum tissue. As a result, deciduous teeth that fall out naturally often contain little to no pulp and, thus, a minimal or nonexistent SHED population. To ensure a sufficient number of stem cells can be harvested, it is recommended to focus on teeth with at least one-third of their original root length remaining after the onset of root resorption. For multi-rooted teeth, it is preferable to select teeth where the furcation area is still intact.

The optimal age range for extracting deciduous teeth to harvest dental pulp stem cells is between 5 and 9 years old. However, it's important to note that the dental pulp from teeth affected by cavities is unsuitable for research purposes.

4. Isolation and cryopreservation of stem cells from dental tissues

4.1 Dental Stem Cell Banking

Dental stem cells obtained from extracted teeth are particularly appealing because the process of isolating these multipotent cells is relatively straightforward, bypassing the need for invasive procedures typically associated with other common MSC sources, like bone marrow or adipose tissue. A highly promising source of dental stem cells can be found in deciduous teeth, which are naturally lost during development. As human deciduous teeth are shed and replaced by permanent teeth, the stem cells in the pulp chamber can be collected and stored, offering a convenient and painless option for regenerative treatments. SHED cells, which proliferate rapidly and have multipotent capabilities, are often preferred over other bankable dental stem cells obtained from wisdom teeth or teeth extracted for orthodontic purposes (22). The procedure for isolating and banking cells from teeth is also more cost-effective in comparison to MSCs derived from cord blood, a widely-used banking source (23). Moreover, studies have shown that cryopreserved dental MSCs maintain both their post-thaw viability and differentiation potential even after short or long-term storage (24, 25).

4.2 Cryopreservation Procedure

One of the major challenges in the cryopreservation of dental tissue is the time delay between harvesting and storing the tissue (26). Once a tooth is lost or extracted, the vital pulp begins to deteriorate, resulting in irreversible damage to the cellular components. Research has shown that to preserve the viability of DPSCs, the process of cell isolation and cryopreservation should take place within 120 hours of the tooth being extracted (27). To limit tissue degradation, teeth are typically placed in sterile hypotonic phosphate-buffered saline to prevent dehydration. This step can be carried out by the individual, their parents or guardians (in the case of SHED), or a dental professional. The tooth is then sealed in a vial and placed in a thermette (a temperature-stabilizing carrier) before being transported to the storage facility in an insulated metal container, which is often supplied by the tooth bank (23). This method ensures the sample remains in a hypothermic state throughout the process, which is known as sustentation. It is crucial that, at the time of extraction, the central pulp appears red, signaling sufficient blood flow at the time of collection. Teeth with a central pulp that appears grey should be discarded, as this is a sign of pulp necrosis. The ideal deciduous teeth for collection and storage are canines and incisors, as they often contain healthy pulp and are naturally becoming loose. Deciduous canines and incisors with no signs of disease and with at least one-third of their root intact are suitable for banking (28). In contrast, deciduous molars are generally not recommended for SHED banking, as their slower resorption process can negatively affect the pulp chamber and the viability of the stem cells (29).

4.3 Cell Isolation and Expansion

Most tooth banks isolate and expand the cells before freezing them. However, a thorough disinfection process must be completed before isolating the dental pulp.

Sterilization is typically done by washing the cells with PBS and povidone-iodine (23). Additionally, commercially available bactericidal solutions, such as chlorhexidine, may be used to minimize microbial presence and further reduce the risk of cross-contamination (27). Once the tooth has been cleaned and disinfected, DPSCs are isolated using protocols initially developed by Gronthos et al. following these specific steps (30):

1. The pulp tissue is manually extracted from the tooth using sterile forceps or a dental excavator. Alternatively, the pulp can be removed by irrigating the pulp chamber with sterile salt water or saline solution.
2. The extracted pulp is placed in a sterile petri dish and washed multiple times with PBS to further minimize the risk of bacterial contamination.
3. The pulp tissue is then digested using either a Collagenase type-I (3 mg/mL) and Dispase II (4 mg/mL) solution or a 0.5% Trypsin/EDTA solution for 1 hour at 37 °C.
4. The digested pulp is filtered through a 70 µm cell strainer to obtain a single-cell suspension.
5. The cell suspension is cultured at 37 °C with 5% CO2 in a growth medium composed of Alpha-modified Eagle's medium (α-MEM) supplemented with 20% fetal bovine serum (FBS), 2 mM L-glutamine, 100 µM L-ascorbate-2-phosphate, 50 U/mL penicillin, and 50 µg/mL streptomycin.
6. At this stage, there is the option to differentiate the expanded MSCs into specific cell lines, such as odontogenic (31), adipogenic (32), or neural (33), by modifying the culture medium.

To characterize or enhance the homogeneity of MSC cultures, techniques such as fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting can be utilized to select for Stro-1+ or CD146+ cells. Previous research has shown that DPSCs can be effectively identified based on these cell surface markers (34). However, it is important to recognize that purifying MSC populations might not always be advantageous, as it can potentially impair their differentiation capabilities and therapeutic effectiveness (32).

5. Dental stem cells-based pulp tissue engineering

Biological-based therapies for dental pulp can be divided into cell-free and cell-based strategies. The cell-free approach focuses on the chemoattraction of the body's own cells into the root canal (35, 36). In contrast, the cell-based approach involves transplanting responsive stem cells along with an appropriate scaffold into the root canal to stimulate tissue regeneration and formation (37-40).

Root canal revascularization through blood clot formation relies on inducing bleeding into the canal system by over-instrumenting the apex (41). This method presents an appealing option for direct clinical use, as it removes the necessity for cell retrieval, culturing, and transplantation that are required in cell-based strategies (17, 37, 41, 42).

The residual pulp from SHED has the ability to regenerate tissue similar to dental pulp, capable of depositing mineralized tissue (2, 14-16). This regenerative potential was first demonstrated in 2003 in a landmark study by Miura et al., where SHED combined with HA/TCP were able to differentiate into odontoblasts within 8 weeks after implantation in immunocompromised mice. However, unlike DPSCs, these cells did not initially regenerate a fully developed dentin-pulp complex in vivo (1, 2). This capability was reported in subsequent studies (14, 15, 43).

Although SCAP have the ability to form odontoblast-like cells and produce dentin in vivo, they are most likely the source of primary odontoblasts responsible for root dentin formation. This restricts their isolation to the period before root development is complete (44). Therefore, third molars are an ideal source of SCAP, as their root formation typically completes between the ages of 18 and 25. In contrast, SHED can be collected from naturally exfoliating post-natal tissue, providing a window of around 11 years to harvest these stem cells, primarily from deciduous central incisors and canines.

It is important to note that although dental stem cell types share similar properties, each type has distinct characteristics. For instance, a key feature of DPSCs is their potential for odontoblastic differentiation, making them well-suited for dentin regeneration strategies and ideal for seeding onto dentin. In endodontic therapy, promising results have been observed from the autogenous transplantation of BMP2-treated pellet cultures of pulp progenitor/stem cells onto amputated pulp, which stimulated reparative dentin formation in a dog model (45). SHED, in comparison, exhibit a faster proliferation rate and greater population doublings than DPSCs and BMMSCs. Unlike DPSCs, however, SHED cannot regenerate a complete dentin-pulp-like complex in vivo. Nevertheless, they retain the ability to differentiate into osteoblasts, promote bone formation, and generate dentin and dental pulp (2, 18, 46). Notably, DPSCs do not exhibit bone-forming potential following in vivo transplantation (47). Like DPSCs and SHED, SCAP expanded ex vivo can also undergo odontogenic differentiation in vitro.

In this study, SHED were seeded onto poly-L-lactic acid scaffolds, which were cast into 1 mm thick tooth slices and transplanted into the subcutaneous space of immunocompromised mice. After 4 weeks, the pulp chamber space showed the formation of a well-vascularized pulp-like tissue (18).

While these findings present a promising outlook for regenerative endodontics, the use of tooth substrates only a few millimeters thick does not fully capture the

challenges of regenerating pulp tissue within incomplete roots (14).

A key aspect of this study is that the cells were delivered into the root canals using injectable scaffolds. Since injection is a common procedure among dentists, the learning curve for clinicians to adopt this technology in practice can be significantly shortened.

6. Neuronal properties of dental stem cells

Over the last decade, researchers have shown increasing interest in stem cells for understanding disease pathogenesis and aiding the development of new therapeutic approaches (48). In model organisms, both endogenous and exogenous neural stem cells (NSCs) have been studied for their ability to regenerate damaged nervous tissue (49). Given the scarcity and limited accessibility of adult NSCs in humans, using exogenous stem cells with neural potential has emerged as a viable option for stem cell therapy. While embryonic stem cells (ESCs) and BMSCs have been explored for neuronal treatment, stem cell populations derived from cranial neural crest cells may offer a greater potential for neuronal differentiation and regeneration (50).

SHED have demonstrated potential for neural differentiation and differ from DPSCs in several ways, including a faster proliferation rate, more frequent cell-population doublings, the formation of sphere-like cell clusters, osteoinductive capacity in vivo, and their inability to regenerate a dentin-pulp-like complex (2). As a result, SHED may represent a population of multipotent stem cells that are potentially more primitive than other postnatal stromal stem-cell populations previously studied. Under non-neuronal inductive conditions, SHED express a variety of neural markers such as nestin, ßIII-tubulin, glutamic acid decarboxylase (GAD), NeuN, GFAP, NFM, and 2′,3′-cyclic nucleotide-3′-phosphodiesterase (CNPase). After four weeks in neural induction culture, SHED lose their fibroblastic morphology, develop multicytoplasmic processes, and show increased expression of neuronal markers like ßIII-tubulin, GAD, and NeuN, while the expression of nestin, GFAP, NFM, and CNPase remains unchanged (2). These results were supported by a study by Govindasamy et al., which compared the neural differentiation potential of SHED and DPSCs (51). The study found that SHED had a higher proliferation rate than DPSCs under non-neuronal inductive conditions, and SHED also exhibited higher expression of pluripotent markers such as OCT4, SOX2, NANOG, and REX1 compared to DPSCs. Conversely, DPSCs showed higher expression of neuroectodermal markers like PAX6, GBX2, and nestin, suggesting that SHED are more primitive or pluripotent cells than DPSCs. OCT4, SOX2, and NANOG, which are overexpressed in SHED, are key transcription factors responsible for maintaining pluripotency in early embryos and ESCs (52). However, when SHED and DPSCs were cultured in non-coated dishes containing neuronal induction factors FGF and EGF for 15 days, both cell types were able to form distinct neurospheres, with the cells aggregating into floating spheres. Upon attachment to coated dishes, the neurospheres derived from both SHED and DPSCs spontaneously displayed outgrowth and dendrite-like structures, expressing neuronal markers such as ßIII-tubulin, NF, and GFAP. While both SHED and DPSCs originate from the same source, DPSCs demonstrated higher neurosphere formation and neuronal marker expression compared to SHED. Given that nestin is essential for neurosphere formation (53), the high levels of nestin in undifferentiated DPSCs may enable them to differentiate into neuronal cells more efficiently than SHED. Nevertheless, SHED can also be induced to form neuronal cells in the presence of the appropriate microenvironment (51).

The neural developmental potential of SHED was specifically explored by injecting them into the dentate gyrus of the hippocampus in immunocompromised mice. Histological analysis revealed that SHED survived for 10 days within the brain microenvironment of the mice and continued to express neural markers like NFM (2).

Similarly, SHED demonstrated therapeutic advantages in recovery following spinal cord injury (SCI) (54). SHED was found to prevent SCI-induced apoptosis in neurons, astrocytes, and oligodendrocytes, aiding in the preservation of neural fibers and myelin sheaths. Additionally, SHED promoted the regeneration of severed axons by directly inhibiting various axon growth inhibitory signals through paracrine mechanisms. Moreover, SHED contributed to the replacement of lost or damaged oligodendrocytes after SCI by specifically differentiating them into mature oligodendrocytes under the severe conditions of SCI (54).

SHED has also been suggested as a potential source of stem cells for treating Parkinson's disease, as they have been shown to differentiate into dopaminergic neurons (55). In a study by Wang and colleagues, SHED were first induced to form neurospheres in a serum-free medium enriched with EGF and basic FGF. These SHED-derived spheres were then directed to differentiate into dopaminergic neurons using a combination of sonic hedgehog (SHH), FGF8, GDNF, and forskolin. Under these conditions, SHED-derived spheres generated several ßIII-tubulin- and MAP2-positive neurons, with some expressing tyrosine hydroxylase (TH), the enzyme involved in dopamine synthesis. To enhance cell survival, Wang and his team transplanted SHED-derived spheres, rather than fully differentiated neurons, into the striatum of 6-hydroxydopamine-treated rats, as mature neurons are known to have low survival rates (56). This intrastriatal transplantation improved motor deficits in the Parkinsonian rats and increased dopamine levels in the striatum. It is likely that this induction system activated transcription factors within SHED-derived spheres, regulating TH expression. Once transplanted into the striatum, these cells responded to environmental cues, further differentiating into their committed lineage (55).

7. DSC For ocular regeneration

The corneal epithelium undergoes continuous self-renewal, where terminal superficial cells are naturally shed and replaced by a group of stem cells located in the limbus, the area between the cornea and sclera, known as limbal epithelial stem cells (57). Besides their role in routine epithelial renewal, these stem cells possess the ability to regenerate the entire corneal epithelium after injury.

SHED have been extensively studied due to their easy isolation from autologous tissues and their ability to differentiate into various cell types (2). Recent research has demonstrated the potential of SHED to regenerate corneal epithelial cells. Gene expression profiles of epithelial and stem cells in SHED were found to be more similar to limbal epithelial stem cells than to differentiated corneal epithelial cells, particularly for genes like ABCG2, K12, and K3 (58). In an in vivo LSCD animal model, SHED successfully restored corneal epithelial tissue. LSCD was induced in New Zealand white rabbits through chemical burns, and a month after injury, a keratectomy was performed to remove the pannus. SHED cell sheets were then transplanted onto the exposed rabbit stroma, protected by an acellular human amniotic membrane that was sutured to the episclera. Without treatment, the chemical damage led to corneal vascularization, conjunctivalization, and opacity. Histological examination of untreated eyes revealed a lack of stratified epithelium and the presence of disorganized, neovascularized conjunctival tissue (58, 59). In contrast, eyes treated with SHED sheets showed clearer corneas with reduced neovascularization. Histological analysis confirmed the regeneration of corneal epithelium in treated eyes, displaying cuboidal basal cells, intermediate flattened cells, and polygonal surface cells, similar to normal corneal epithelial tissue (57-59). These findings suggest SHED's potential in tissue engineering and regenerative therapies for corneal epithelial repair.

8. DSC-differentiated hepatocytes for treatment of liver diseases

Several studies have shown that DSCs, such as tooth germ progenitor cells (60), DPSCs (61-63), tooth-pulp stem cells CD117+ (63) and SHED (64) or SHED-CD117+ (65) are capable of differentiating into functional hepatocytes in vitro.

Ishkitiev et al. demonstrated that DSCs derived from the pulp of fully developed wisdom teeth or deciduous teeth could be differentiated into hepatic-like cells (66). Specifically, they induced the differentiation of wisdom tooth pulp cells and deciduous tooth pulp cells into hepatocyte-like cells using a combination of HGF, Dex, OSM, and ITS-x. These differentiated DSCs expressed hepatocyte-specific proteins and exhibited hepatic functions, including glycogen storage and urea production (66).

Recently, Okada et al. compared the hepatic differentiation capabilities of SHEDs and BM-MSCs in vitro, with and without H2S treatment (67). Their study found that, in the absence of H2S, SHEDs and BM-MSCs CD117+ expressed similar levels of stem cell transcription factors. However, upon differentiation, SHEDs exhibited significantly higher levels of hepatic markers such as ALB, αFP, and CPS-1 compared to BM-MSCs. In contrast, no significant differences were noted between H2S-treated SHEDs and BM-MSCs regarding the expression of ALB, αFP, and CPS-1, as well as glycogen production and urea concentration after hepatic maturation. These findings indicate that SHEDs, especially when combined with H2S, may be a promising option for clinical liver disease treatments.

To emphasize the clinical potential of DSCs in treating hepatic diseases, Ishkitiev et al. demonstrated that human hepatically differentiated CD117+ SHED (hdSHED) could successfully engraft and function in the livers of rat models with hepatic conditions (65). The presence of human-specific markers like albumin and αFP in the rats' serum confirmed that the transplanted hdSHED were incorporated into the liver structures of rats with acute liver injury. This research supports the idea that differentiating DSCs into hepatocyte-like cells and subsequently transplanting them could be an effective approach for treating liver diseases in humans.

In previous studies, DSCs were pre-differentiated in vitro before being transplanted into animal models (60, 65, 68). Recently, Yamaza et al. demonstrated that naïve SHEDs, which had not been differentiated in vitro, were able to enhance hepatic function and directly convert into hepatocytes in CCl4-treated mice (64). Additionally, these in vivo SHEDs contributed to hepatic recovery through tissue replacement and exhibited anti-fibrotic and anti-inflammatory effects.

9. Conclusion

Dental stem cell banking is gaining traction as it offers individuals a form of 'biological insurance' to aid in the regeneration of tissues lost due to injury or disease. To achieve this, research and development are necessary to identify the best methods for long-term cryopreservation. Future research will focus on creating cryopreservation media that ensure the safe and effective transfer of cellular therapies within clinical settings, aiming to eliminate the use of serum and harmful cryoprotectants without compromising cell viability and function. Standardized protocols and procedures that comply with current good manufacturing practice (cGMP) will also need to be established. Additionally, practical factors such as the cost and scalability of cell banking procedures must be considered to enable the widespread application of cell therapies. A critical issue that remains unaddressed is the impact of long-term cryopreservation (>10 years) on cell

viability and phenotype. Addressing this concern through long-term studies is essential to validate the effectiveness of current cryopreservation methods for future clinical use.

DPSCs are among the most accessible stem cells, as they can be collected directly by dental practitioners. In contrast, SHED are a valuable source of dental stem cells and can be obtained from discarded tissue of extracted or exfoliated deciduous teeth. These stem cells can be commercially banked as whole pulp tissue, and SHED are subsequently isolated and expanded in vitro using enzymatic digestion of the pulp tissue followed by fluorescence-activated cell sorting (23). Despite the relative ease of obtaining SHED (23), their routine collection and clinical use are currently limited by issues such as infection, insufficient viable pulp tissue, and the absence of standardized good manufacturing practices (2, 69).

Deciduous teeth naturally fall out, making them a potentially valuable and easily accessible source of stem cells. Additionally, dental tissues are relatively simple to manipulate and store in stem-cell banks. The ability to preserve DSCs for future differentiation into hepatocytes, which could then be used for transplantation without the risk of immunologic reactions, holds great promise for advancing cell therapy for liver diseases. Historically, liver diseases were often deemed irreversible, but advances in cellular and molecular biology have shown that hepatic cellular recovery is feasible. Therefore, the potential of using DSCs for liver disease treatment is very promising. However, additional research is needed to ensure the long-term safety of DSC-based transplantation, and clinical trials are essential to confirm their effectiveness in restoring liver function in patients with liver diseases.

Biological therapies for dental pulp encompass both cell-free and cell-based methods. Cell-free techniques rely on attracting host cells into the root canal, while cell-based approaches involve using stem cells with scaffolds for tissue regeneration. Although revascularization through blood clotting streamlines the procedure and avoids handling cells, SHED present a promising alternative. Initially, SHED faced challenges in regenerating a complete dentin-pulp complex, but they have demonstrated potential in forming dental pulp-like tissue and differentiating into bone-forming cells. Research involving SHED in scaffolds has led to the development of vascularized pulp-like tissue in animal models, indicating potential for practical applications in regenerative endodontics. The use of injectable scaffolds, a common dental practice, may facilitate easier clinical implementation.

Recent research has underscored the promise of stem cells in advancing disease research and creating new therapies. Exogenous neural stem cells (NSCs) have been examined for their potential to repair nervous system injuries. Given the limitations associated with the availability of adult NSCs, alternative sources such as SHED are being explored. SHED are recognized for their enhanced proliferation and neural differentiation capabilities compared to DPSCs (70).

SHED are capable of forming neurospheres and expressing a range of neuronal markers. They have demonstrated efficacy in treating spinal cord injuries by promoting neural regeneration and reducing cell death. Moreover, SHED show promise for Parkinson's disease treatment, with evidence indicating their ability to differentiate into dopaminergic neurons and improve symptoms in animal models. Ongoing research is crucial to further their clinical application and optimize therapeutic results.

The corneal epithelium, which relies on limbal epithelial stem cells for maintenance, naturally regenerates through the shedding of superficial cells and repopulation from these stem cells. SHED have proven to be a valuable resource because of their straightforward isolation and multipotent differentiation abilities. Recent research underscores SHED's potential for regenerating corneal epithelium, with gene expression profiles that closely resemble those of limbal stem cells. In an animal model of limbal stem cell deficiency (LSCD), SHED successfully restored the corneal epithelium, resulting in clearer corneas and reduced neovascularization compared to untreated controls. These results indicate that SHED could be a promising option for corneal tissue engineering and regeneration.

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