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Application of Stem Cells in Pediatric Dentistry

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Abstract

A stem cell is a master of all cells in the body, which can divide and regrow with regeneration and vascularization. The deciduous teeth, which are not infected and vital with pulp stem cells, help in the formation of adult stem cells. The extracted stem cells from human exfoliated deciduous teeth (SHED) and immature dental pulp stem cells can be harvested as stem cells that form dental tissue regeneration SHED, osteogenic potential 40%, and neurogenic potential either by in vivo or in vitro method. The article here focuses mainly on the stem cell classification of dental stem cells, their characterization, therapeutic application, application in the deciduous tooth procedure, banking of SHED, and recent innovative methods for banking SHED. This review was done to highlight the integral role of pedodontists in educating patients about stem cell banking services and must become a part of such services.

Keywords**:** *Human exfoliated deciduous teeth, Immature dental pulp stem cells, Pedodontist, Pediatric dentistry, Stem cells.*

Introduction

Stem cells are unspecialized cells of the human body that can develop into various types of specialized cells, such as the blood, muscle, or brain cells [1, 2]. Having the unique ability to self-renew and recreate functional tissues, these thrive as a potent resource for developing cells and tissues for treating various diseases [2].

These cells have a dominant role in regenerative medicine and have proven to help regenerate human cells, tissues, and organs, thereby helping to evoke their normal functionality after any disease or injury. There have been immense advances in the field of stem cell therapy with novel approaches to apexogenesis, which help young patients affected by periapical periodontitis or pulpitis [1, 2].

The human deciduous teeth differ predominantly from permanent teeth concerning developmental processes, functions, and tissue structure. The adult stem cells in the human exfoliated deciduous teeth (SHED) [3, 4] are available substantially. Reckoning out the availability of various deciduous teeth stem cell (DTSC) populations that include the presence of stem cells in the human exfoliated deciduous teeth (SHED) and the immature dental pulp stem cells (IDPSCs) has opened up a plethora of opportunities for harvesting the young stem cells [3, 4]. Renowned researchers worldwide are chiefly focusing on making use of stem cell therapy using SHED to treat various diseases. Even though there is ample research that's yet to be done, we have hard-core evidence that undeniably conveys the fact that primary human teeth exist as the foremost choice for therapeutic stem cell therapy in comparison to

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wisdom teeth as well as orthodontically extracted teeth [3]. The ultimate prerequisite for successful stem cell therapy includes the need for pedodontists to possess ample knowledge regarding the stem cells procured from deciduous teeth. The present article reviews targets essentially to dig into the characteristics, storage process, and applications of stem cells on the commercial front and delves into their therapeutic prospects in the future.

Classification

It is possible to classify dental stem cells as shown below [5] (Figure 1).

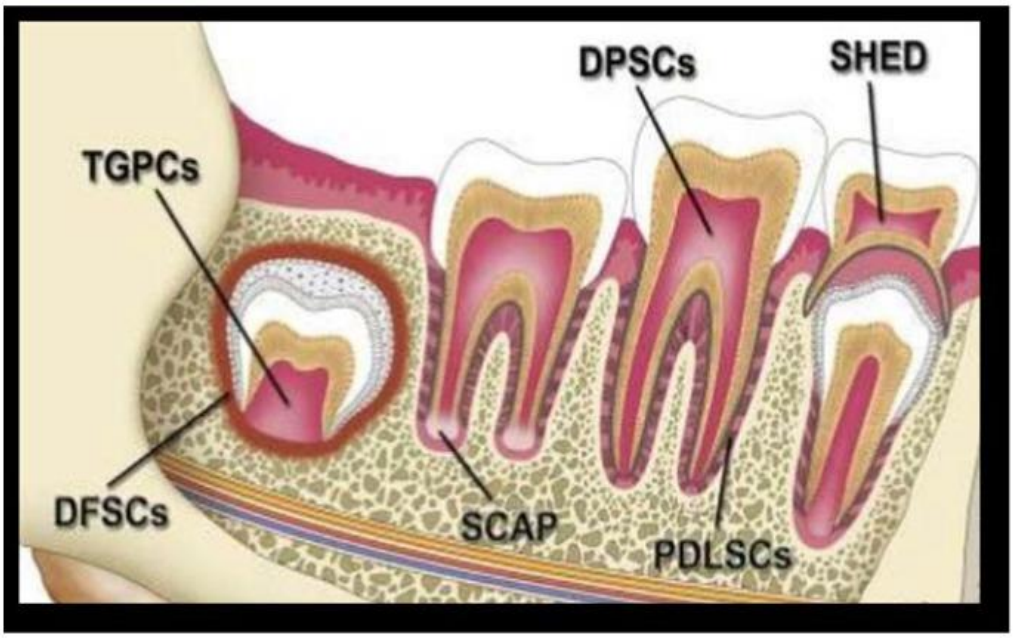

Figure 1. Classification

DPSC: Dental Pulp Stem Cells, Pulp procured from teeth that are extracted for orthodontic purposes, and also the pulp extracted from third molars. PDLSCs: Periodontal Ligament-derived Stem Cells. GMSCs: Gingiva-derived Mesenchymal Stem Cells. SHED: Stem cells from Human Exfoliated Deciduous teeth. IDPSCs: Immature Dental Pulp Stem Cells from deciduous teeth. DFSCs: Dental Follicle Stem Cells. TGPCs: Tooth Germ Progenitor Cells. SCAP: Stem Cells from the Apical Papilla [5,6]

Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

SHED is nothing but stem cells procured from exfoliated deciduous teeth. Dr. Songtao Shi holds pride of discovering SHED way back in 2003, and this has laid the paths for harvesting stem cells in plenty of useful ways. On a comparative note, SHED holds a better proliferation rate and increased cell population against permanent teeth (SHED > DPSCs > BMSCs) [3, 7]. The general trend followed is extracting SHED from those teeth that are "disposable" and available readily from young patients. There is no need for counter therapy to deal with problems such as immune rejection issues or so, as the samples fall into the category of autologous transplant. Also, collecting the samples is a simple, quick, and effortless technique that can be done easily.[8] As opposed to cord blood collection, which is an expensive affair, SHED banking is comparatively economical. SHED do not suffer from any principal ethical constraints [9] as compared to embryonic stem cells and might even be taken as a second chance for patients as it's highly feasible to extract them during any regular dental checkup in kids concerning bank cord. Moreover, SHED opens the possibility of being used for other members of the family, including parents and grandparents as well

apart from minimizing the transition of unknown genetic elements spread across the human population [6], yet another feather added to its cap.

Characterization

In Vitro: Multi Lineage Differentiation Employment of Special Culture Media

In-vitro studies include probing into the multi-lineage differentiation of SHED into osteocytes, adipocytes, and neuron-like cells that generally express early and late neuronal markers [6, 10]. The insulin and C-peptide secretion paves the way for the differentiation into islets of the pancreas.

In Vivo: Dental Tissue Regeneration SHED

Here, it is placed in immune-compromised mice and yielded human-specific odontoblast-like cells with a dentin-like architecture that expressed dentin sialophospho protein [3].

Using human tooth slices as scaffolds displayed similar results but with higher microvessel density while implanting SHED into the subcutaneous tissue of immune-deficient mice. Another important observation included the differentiation of SHED into blood vessels that anastomosed with the host vasculature [11] Pulp-like tissues resulted in the differentiation effect of SHED only after their implantation into the root canals of mice for 28 days. Dentin, the yellow tissue forming the bulk of the teeth, was produced with the help of the newly formed pulp. But, SHED differentiation needs even more detailed research and probing to understand its injection effects on human oral tissues.

Oestrogenic Potential 40%

Monumental new bone formation potential was observed when injecting SHED colonies into immune-compromised mice [12]. In vivo SHED after transplantation was capable enough to turn recipient murine cells into osteoblasts, and this was not the case with DPSCs. Correction of the calvarial defects in mice markedly displays the osteoinductive nature of SHED and its advantages [5].

Neurogenic Potential

To understand the neural development potential of SHED, the dentate gyrus of the hippocampus of immune-compromised mice was injected with SHED, which showed exemplary neural markers, including neurofilament M (NFM). There have been a couple of studies that show the impact of SHED on neuronal and glial cell markers, both of which could pose an impact on the neural crest cell origin of the dental pulp [12].

Differentiation into Hormone Secreting Cells

Hepatic recovery was observed in mice when SHED was implanted into the liver. Normal glucose levels were attained due to the differentiation of SHED into Islets of pancreas [5].

Therapeutic Applications

Dental Tissue Engineering

Implanting mice with SHED into functional odontoblasts expressing dentin sialoprotein resulted in tubular dentin. Cells present in the walls of the blood vessels within the tooth scaffolds/slice presented with positive B-galactosidase staining, which closely resembled the non-stained (host) blood vessels. The foremost benefit involved with SHED, as confirmed by this investigation, is its regenerative property of pulp-like tissue in vivo, whose characteristics portray a close resemblance to the natural tooth. All these, without a doubt, point to the fact that SHED can be used for engineering dental tissue medicine [8, 11]. In the present-day world, SHED plays a significant role in treating bone fractures, bone marrow transplants for cancer, immunity-related conditions such as lupus erythematosus, and spinal fusion surgery. There are numerous types of research delving into the

therapeutic nature of stem cells, and a couple of them have the approval of the U.S. FDA as well [13]. Induced pluripotent cells (IDPSCs) resulting from the SHED (stem cells of deciduous teeth) displayed better reprogramming efficiency just like embryonic cells and hence, could prove to be extremely useful for treating pediatric disorders.

Medicine

Induced pluripotent cells formed from stem cells of deciduous teeth showed higher efficiency of reprogramming similar to embryonic cells and can be applied for treating paediatric disorders [5].

Application of Stem Cells in Deciduous Tooth

Restoration in Dental Caries

One of the most common dental diseases affecting primary teeth is dental carries, and when the infection goes into the pulp chamber, the necrosis of the pulp canal occurs, and the infected pulp needs to be removed. Rather than choosing conventional treatments, tissue engineering approaches using SHED has the potential to replace irreversibly inflamed or necrotic pulps with healthy and functionally competent tissue that is capable of forming new dentin. Directed recruitment of these cells might be achieved through the local application of enriched cell populations by harvesting cells from autologous or non –autologous shed deciduous primary teeth [14].

Apexogenesis and Apexification

This is a specialized technique involving the regeneration of the tissue into the apex of an immature permanent tooth. For this purpose, stem cells and growth factors seeded on scaffolds are essential. Extracting stem cells isn't a problem as these cells are already present in the apical papilla, vital pulp tissue, alveolar bone, or PDL [15]. These cells find their use in the areas next to the blood vessels, peripheral nerve endings, and in the perivascular region. The SCAP, when stimulated, forms new dentin deposits and the rest of the apex with support from Hertwig's Epithelial Root Sheath (HERS), thereby being a significant part of the apical development and regeneration process.

The Regenerative Endodontics Committee of the American Association of Endodontists endeavoured various approaches to achieve revascularization. Presently, making use of a tri-antibiotic compound involving metronidazole, minocycline, and ciprofloxacin is favoured over calcium hydroxide treatment. Regeneration and pulp canal filling can be achieved using neighbouring tissues in the presence of total pulp necrosis and an aseptic micro-atmosphere. Researchers witnessed the formation of cementum tissues, both, at the apex and the pulp canal, soon after removing and replacing pulp tissue in rhesus monkeys [16].

Investigations

Cultured human pulpal fibroblasts helped in producing new pulp-like tissue using an in-vitro technique making use of biodegradable scaffolds seeded with growth factors and bioactive signaling molecules—these helped in cell organization and growth of a vascular supply. As indicated in the report, polymer scaffolds thrive as a cornerstone for the durability of DPSC and PDLSC contrary to the nature of calcium phosphate [5].

Using immune-compromised mice as specimens for study, SHED and endothelial cells were implanted into biodegradable scaffolds within human tooth slices. The implanted cells were kept under constant observation to understand whether they differentiated into odontoblast-like cells and endothelial-like cells in vitro, and the tissues were also followed up to confirm whether they stimulated dental pulp having viable blood supply [5]. To explore the benefits of autologous DPSC, first molars extracted from a canine pulpless animal model were used to

check whether pulp regeneration was possible. Observations proved that DPSCs were competent enough to generate pulp-like tissues having blood vessels and dentin-like tissues with thickening of the root canal walls [5]. A three-case report showcased the healing ability of large periapical lesions in immature permanent teeth affected by apical periodontitis. DPSCs with PLGA-PEG as a scaffold were also noted [17]. The human apical papilla expressed abilities to retain its stem cells' vitality and showed increased osteogenic and angiogenesis potential under such circumstances.

Banking

Steps Involved

Step 1: Collecting the Tooth

The tooth extracted by the dentist is carefully scrutinized to visually confirm the availability of healthy pulpal tissue. Once this is done, the collected sample is transferred to a container containing up to four teeth. To preserve the teeth and prevent dehydration during shipment, the container also has a sterile saline solution for this purpose. Soon after keeping the tooth inside, the container is tightly sealed and kept in a thermette (temperature change phase carrier) which is then shifted to an insulated metal box [5]. Such an elaborate process is necessary to keep the sample in a hypothermic phase. A stem cell's viability is entirely based on the time and temperature maintained; hence, it becomes indispensable to take necessary care. The maximum time allocated for a stem cell to reach the processing storage facility from when the cells are collected should not exceed 40 hours.

Role of a Clinician/Pedodontist

Choosing the Right Tooth

Indications

Always choose primary incisors and canines that have no pathological history and have at least one-third of the root remaining intact. Studies clearly show that the stem cells extracted from dental pulp display characteristics based on root resorption. The research team could not isolate any stem cell failing to show root resorption potential from the dental pulp. Only those pulp from the teeth exhibiting higher levels of root resorption had the potential to generate SHED [6]. Hence, such observations help us conclude that SHED can be isolated during the normal eruption phase.

Extracted third molars and permanent teeth, besides the primary teeth, were removed for orthodontic purposes. The exfoliated tooth must exhibit red coloration of the pulp (pulp vital)

Contraindications

1. When the resorb time takes long, it results in the obliteration of pulp in the primary tooth.
2. Major tooth decay resulting in compromised pulp [18].
3. Cysts, apical abscesses, or tumors present in the teeth.
4. Trauma or periodontal conditions that lead to class III or IV mobility of the teeth [19].
5. Compromised pulp that has a grey coloration.

Patient Education and Registration

Either the dentist is responsible for creating awareness regarding the availability of tooth banking to their patients or the stem cells from the teeth must be enrolled with the potentially available nearest stem cell banking services in the vicinity. Only after this step, the dentist schedules an appointment for tooth extraction, and the organization works in proximity with the dentist's clinic to take care of all the required materials and instructions needed for the extraction and transfer. The company Store-A-Tooth provides a comprehensive tooth collection kit that satisfies all required protocols (Figure 2) [5].

Figure 2. Tooth Collection Kit

Step 2

Immediately following the isolation of the stem cell, successive steps take place at the storage firm. Firstly, Dulbecco's phosphate buffered saline (PBSA), devoid of calcium and magnesium ions, is used to clean the surface of the tooth not once or twice but three times. Povidone iodine is used to disinfect the tooth, after which it is once again washed with PBSA. From the pulp chamber, pulp tissue rich in stem cells is isolated from the tooth's center and washed out with salt water. If there is any contamination observed, the tissue goes to a sterile petri dish, where it's washed thrice using PBSA.

Collagenase Type I and dispase come in handy for tissue digestion which occurs for an hour approximately at a temperature of 37°C. To achieve single-cell suspensions, the isolated cells pass through a 70 µm filter and are then cultured using a Mesenchymal Stem Cell (MSC) medium. It takes around 24 hours to secure the visibility of isolated colonies, and only at this stage do the donors get a confirmation regarding the cell's health and viable nature [5]. Stem cells are stored predominantly using one of the two methods, namely (a) Cryopreservation and (b) Magnetic freezing.

Cryopreservation

Sub-zero degrees maintained in this method help in preserving the cells or the entire tissue by cooling them to such temperatures. Cells are maintained below a temperature of <−150°C with the help of liquid nitrogen vapor. Cryopreservation works are based on the ideology of pausing any biological activity associated with the cells at these temperatures for a definite period, with the vitality remaining unquestioned. The cells are later defrosted whenever required. Right until the time when the donor requires the cells for therapy, the requirement for cell culture setting for frozen pulp remains dormant. Cells extracted near the end of log phase growth are ideal for cryopreservation. The optimal cell count for successful recovery is 1–2 × 106 cells in 1.5 ml of freezing medium [5, 20].

Magnetic Freezing

Also referred to as a live cell system (CAS), magnetic freezing is an alternative technique proposed initially by Hiroshima University. A weak magnetic field is applied to any tissue that lowers the freezing point by up to 6-7°C. After uniform chilling of the body, the magnetic field can be turned off [5].

Recent Innovations

There are new innovative methods of banking in SHED [16]. To overcome the "replicative senescence" phenomenon, the reversible arrested proliferation phase of SHED, and the recent innovation methods of banking SHED come into the picture. The reasons for such a phenomenon include stress occurring because of cultures such as hyperoxia and elevated temperature. The extent of cellular senescence is directly dependent on the length and rate of loss of telomere during cell division. Maintaining the telomere length is based on the Telomerase Reverse Transcriptase (TERT). The lentiviral transduction in combination with a puromycin selection marker, proved of use in restoring the ectopic expression of TERT among SHED. The research results were favorable for TERT-SHED's vigorous proliferation capacity and proved that TERT immortalized SHED was a concrete source for stem-cell therapy [5, 6].

Conclusion

Dental stem cells would play a pivotal role in enhancing the overall health of the human race in the future. The research results until now support using SHED as it is one of the better and more advantageous resources, even though there are certain areas that need further probing. It is desired that the number of clinicians, pedodontists, and dentists in middle-income and high-income countries who enlighten patients about banking services and become a part of such services increase potentially in numbers, given the simple nature and convenience of extracting stem cells from the tooth. The bitter truth remains that the scenario existing in low-income countries must be enhanced, but even until then, it never harms to keep spreading awareness regarding the tremendous potential of dental stem cells. To make this possible, it would be of great help if dental stem cells became a part of the curriculum in academics.

Conflict of Interest

There is no conflict of interest.

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