Therapeutic Potential of Stem Cells From Human Exfoliated Deciduous Teeth(Shed)- A Review

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Abstract

Stem cells are groups of undifferentiated cells that have the ability to become any cell. Recent studies have shown that the stem cells present in the pulp of human teeth can also be used for the same purpose. Stem cell therapy is a safe procedure. The physician must follow proper cell administration techniques. Patients must also be screened for treatment candidacy as all people may not be a candidate for stem cells. Dental pulp stem cells are stem cells present in the dental pulp, which is the soft living tissue within teeth. They are pluripotent, as they can form embryoid body-like structures in vitro and teratoma-like structures that contained tissues derived from all three embryonic germ layers when injected in nude mice. Recent advancements prove that stem cells from exfoliated deciduous teeth prove more effective than the permanent ones. In a clinical trial stem cells extracted from children's baby teeth were used to regrow the living tissue in teeth damaged by injury. In the future they may be used to replace cells and tissues that have been damaged or lost due to disease. The review has covered various aspects to find if SHED is a potent product. Its viability and sustention as discussed by various authors has been discussed. Role of SHED in pulp tissue engineering, neural tissue engineering, their differentiation towards hormone secreting cells etc has been discussed. Hence the stem cells from human exfoliated deciduous teeth also present another opportunity to dentistry to contribute to the development of tissue engineering.

Keywords: *stem cells, deciduous teeth, exfoliated, SHED, Therapeutic potential*

Introduction

Stem cells are specialised human cells that have the ability to develop into any cells. They can repair damaged tissues.Stem cells are of various origins,they are derived from bone marrow, blood, umbilical cord. Dental stem cells derived from wisdom teeth and milk teeth have the ability to regenerate a whole tooth when they are obtained from pulp. Dental pulp is a highly vascularized connective tissue encapsulated in mineralized structure formed by enamel, dentin, and cementum. The dental pulp is a source of different populations of stem cells, such as dental pulp stem cells (DPSC) in permanent teeth and stem cells from human exfoliated deciduous teeth (SHED) [1]. A naturally occurring exfoliated deciduous tooth is similar in some ways to an umbilical cord, containing stem cells that may offer a unique stem-cell resource for potential clinical applications [2]. Possibility of cell-based therapies to treat disease, which is often referred to as regenerative or reparative medicine is becoming a promising field of research on stem cells[3]. SHEDs have high proliferation potency and are multipotent mesenchymal stem cells. Taking these advantageous properties together, SHEDs are one of the candidate cell types for tissue regeneration study [4]. Tooth loss is a common condition that has been encountered in clinics, it affects mastication and also a series of physiological and psychological problems. The

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tooth root is complex , it consists of hard and soft tissues including the cementum, dentine and periodontium [5]. The potency and quality of adult stem cells in the body is inversely proportional to the age. The perspective of obtaining SHED from deciduous teeth is quite practical because everyone sheds deciduous teeth[6] . Previously our team had conducted numerous original studies [7-13] and surveys [14-21] over the past 5 years. Now we are focusing on applying the knowledge to write the review on new recent advancements in various fields. This review focuses on the therapeutic potential of SHED and its various applications in different tissues.

BASIC CHARACTERISTICS OF STEM CELLS

In recent advancements, stem cell therapy has become a very promising and advanced scientific research topic. The development of treatment methods has evoked great expectations. Stem cells have great potential to become one of the most important aspects of medicine. In addition to the fact that they play a large role in developing restorative medicine, their study reveals much information about the complex events that happen during human development[22]. SHED are heterogeneous populations of cells that have been isolated from exfoliated deciduous teeth [4].

STEM CELLS IN DENTISTRY

Stem cells have the ability to repair and regenerate dental tissues like dentine, teeth, bone, cartilages, skin, adipose tissues, and glands. The research of stem cells in scientific and therapeutic potential in scientific and therapeutic potential in oro-facial diseases is yet not reached its pinnacle but future is flamboyant in regenerative dentistry. Scientists have reported for the first time that baby /milk teeth, the temporary teeth that children begin losing around their sixth birthday, contain a rich supply of stem cells in their dental pulp. The scientists say this unexpected discovery could have important implications because the stem cells remain alive inside the tooth for a short time after it falls out of a child's mouth, suggesting the cells could be readily harvested for research [3].

4. VIABILITY OF STEM CELLS:

The blood supply to the pulpal tissues enters through the apical area of the tooth. Hence, when a tooth is extracted, the pulp should appear red in color, indicating that the pulp received blood flow up until the time of removal, which is indicative of cell viability.During an extraction, if the pulp is gray in color, it is likely that blood flow to the pulp has been compromised, and thus, the stem cells are likely necrotic and are no longer viable for recovery. Tooth that are very loose, either through trauma or disease (e.g. Class III or IV mobility), often have a severed blood supply, and are not candidates for stem cell recovery. That is why the recovery of stem cells from deciduous teeth is preferred after an extraction versus the tooth that is "hanging on by a thread" with mobility. Pulpal stem cells should not be harvested from teeth with apical abscesses, tumors or cysts [23] .

SUSTENTION OF STEM CELLS

After an extraction of either a temporary, permanent tooth or another appropriate surgical procedure, the dentist places the tooth/ tissue sample in a screw-top vial containing a hypotonic phosphate buffered saline solution, which provides nutrients and helps to prevent the tissue from drying out during transport. Keeping a tooth into this vial at room temperature induces hypothermia. The vial has to be carefully sealed and placed into the thermette, a temperature phase change carrier, after which the carrier is then placed into an insulated metal transport vessel. The thermette along with the insulated transport vessel maintains the sample in a hypothermic state during transportation. The viability of the stem cells depends on both time and temperature, and careful attention is required to ensure that the sample will remain viable. Time from harvesting to arrival at the processing storage facility should not exceed 40 hour [23,24] .

CHARACTERISTICS OF SHED

SHEDs are a different population of cells that are isolated from dental pulp tissues which remain in exfoliated deciduous teeth. Similar to the mesenchymal stem cells (MSCs), SHEDs exhibit fibroblast□like appearance, attaches on plastic tissue culture surface, express mesenchymal stem cell surface marker and have multipotential differentiation ability (4). SHEDs have higher multiplication rate when compared to dental pulp stem cells (DPSCs) and bone marrow derived mesenchymal stem cells (BMMSCs)[2,25] . This

could be due to the high expression of genes related to cell proliferation and extracellular matrix in SHEDs comparing with DPSCs [25]. MSCs can be isolated from many tissue types. Though, there is no specific marker to clearly identify these cells. According to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, the minimum criteria to identify MSCs are as follows [26]. First, the isolated MSCs should attach to plastic tissue culture plates [9]. Second, expression of MSCs to several specific surface markers, namely CD105, CD73 and CD90[9] . They should not express CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR[9]. Finally, MSC should be able to differentiate into osteoblasts, adipocytes and chondroblasts in vitro[26]. The following section describes general SHEDs' characteristics and addresses MSCs' characteristics of SHEDs according to these criteria.

ROLE OF SHED IN DENTAL PULP TISSUE ENGINEERING

Despite the introduction of new materials, medicines, and tools for the clinical management of dental pulp diseases, the principles of root canal treatment have not evolved significantly from the disinfection and obturation paradigm. This well-established approach presents high rates of success in the daily clinics, but it is very rarely based on the substitution of organic tissues with synthetic and, in many cases, inactive materials [27]. This often restricts the completion of root development in immature teeth [28] . Hence, the development of clinically approachable techniques that allow the regener- ation of a functional dental pulp that is capable of depositing organized and mineralized matrix is of great interest [27,29]. The shift towards regenerative endodontics leads to the rescue of tooth viability and further development of the root structure.The capacity of SHED to specialise into fully functional odontoblasts are capable of depositing a mineralized structure comparable to dentin invivo was observed later. Similar to the previous study, SHED were seeded within a scaffold cast in a tooth slice and implanted subcutaneously into the dorsum of mice. 32 days after implantation, a dental pulp- like tissue was centripetally formed in the pulp chamber of the tooth slice. The tissue formed with SHED had a positive expression for the markers of odontoblastic differentiation such as dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP-1). During the experiment, mice had been injected with the periodical injections of tetracycline hydrochloride to reveal the deposition of mineralized matrix. Remarkably, well-defined fluorescent lines originated from the chelation of calcium ions in the newly deposited dentin offered the evidence that SHED can differentiate into fully functional odontoblasts invivo [30].

SHED FOR NEURAL TISSUE ENGINEERING

Neurodegenerative disorders are characterized by the loss of neurons, leading to functional disabilities. Although some of them, such as Parkinson and Alzheimer's disease, mainly affect the older people, they are not part of the natural aging process. Parkinson disease affects 9.7 to 13.8 per 100,000 population, while Alzheimer's disease has become a major public health concern as the world's population ages [30,31]. In addition to these diseases, there are approximately five million people living with traumatic brain disability in the United States alone [32]. Other injuries such as stroke, peripheral nerve injury, and spinal cord injury also is a huge burden to society. Due to the limited regenerative capacity of the nervous system, stem cell-based therapies have emerged as treatment options. The neural crest-cell origin of the dental pulp makes SHED an interesting cell model for neuron tissue regeneration research [33]. The neural developmental potential of SHED invivo was demonstrated by Miura and colleagues by injecting SHED into the dentate gyrus of the hippocampus of mice. The cells survived in the environment which was provided and it continued to express neural markers such as NFM for more than ten days [2]. The potential of SHED to undergo neurogenic differentiation invivo opened avenues for the use of these cells as an alternative model to treat different neuron-related conditions like focal cerebral ischemia, spinal cord injuries, Alzheimer's disease, and others. The enhanced regeneration observed with SHED not only applies to lesions in the central nervous system, but also was observed in peripheral nerve injuries. It was possible to promote the regeneration of sciatic nerve defects in rats by treating the lesions with SHED conditioned media. The axon density and number of regenerated myelinated fibers observed in the group treated with SHED conditioned media were similar to the autograft used as a control [25] .These promising results pertaining to the neuron differentiation potential of SHED in several in vitro settings and animal models

increase the interest of using these cells as an alternative to treat different neuronal diseases and injuries [1] .

SHED DIFFERENTIATION TOWARDS HORMONE SECRETORY CELLS

In addition to their therapeutic use in dental pulp and neuroregeneration, SHED also has the potential to be used in the treatment of liver diseases and diabetes. Organ transplantation could be the choice of treatment for patients suffering from fatal liver conditions such as cirrhosis and hepatocellular carcinoma. However, the lack of donors encourages the development of therapeutic alternatives [1] . Under proper stimulation, SHED expresses a set of hepatic markers such as hepatic nuclear factor-4 alpha, alpha-fetoprotein, and insulin-like growth factor-1. Remarkably, 90% of the hepatocytes that were obtained were positive for the expression of albumin. In addition, there were significant increases in the concentration of urea in the median amount of cytoplasmic glycogen storage within the cells after differentiation [34] .

SHED IN ADIPOGENIC DIFFERENTIATION

A total of 1×10 5 DFCs and SHEDs were seeded into each well of a 6-well plate. When the cells reached 70% convergence, the culture medium was replaced with a complete α-MEM which was supplemented with 10% FBS, 2 mm insulin (Sigma, USA), 0.5 mm isobutylmethyl- xanthine (IBMX; Sigma, USA), and 10 nM dexamethasone (Sigma, USA)(35). α-MEM supplemented with 10% FBS served as the negative control. The medium was refreshed every 2 days. After 15 days of culture, the cells were washed three times with PBS after fixation with 4% paraformaldehyde for 15 min and then incubated with a 0.3% Oil Red O (Sigma, USA) solution for 15 min. After washing thrice with PBS, cells were periodically observed and were photographed under a phase-contrast inverted microscope (Olympus, Japan). The expression of adipogenic genes PPARγ2, LPL and adiponectin was analyzed using real-time PCR[6].

SHED IN OSTEOGENIC DIFFERENTIATION

A total of 1×10 5 DFCs and SHEDs were seeded into each well of separate 6-well plates. Upon reaching a level of 70% convergence, cells were cultured in osteogenic medium containing 10% FBS, 10 mM L-glycerophosphate (Sigma, USA), 100 μM dexamethasone (Sigma, USA), and 50 μg/ml ascorbic acid (Sigma, USA) for 21 days. α-MEM which was supplemented with 10% FBS served as the negative control. The medium was refreshed every 2 days. After 3 weeks, cells were washed twice with PBS after fixation with 4% paraformaldehyde for 15 min and then incubated with a 0.1% alizarin red solution (Sigma, USA) in Tris-HCl (pH 8.3) at 37°C for 30 min. After washing twice with PBS, cells were observed and photographed under a phase-contrast inverted microscope (Olympus, Japan). The expression of the osteogenic genes BSP, ALP and OCN was analyzed using real-time PCR. Relative expression levels were calculated using the $2-\Delta\Delta CT$ method and normalized to the reference GAPDH gene [6].

FUTURE SCOPE

Teeth derived from autologous cells, such as dental pulp derived stem cells and SHED, which can be deposited in cell banks, can be produced remains to be established. Immune responses to the cells used are unknown but these have to be understood before any clinical trials can be performed. With more extensive research it may be possible, in future, to regenerate the structures of the teeth .

Conclusion

As the stem cells from human exfoliated deciduous teeth can be obtained from naturally "disposable" tissues without significant morbidity to host and with limited ethical concern, they present another opportunity for dentistry to contribute to the development of tissue engineering. Several studies offer evidence that SHED can differentiate into odontoblasts, neurons, hepatocytes, endothelial cells, alpha cells, and others. The wide variety of cell types creates a plethora of opportunities for the use of SHED in tissue regeneration processes. There is still the need to deepen the understanding of the mechanisms underlying the differenti- ation processes before SHED-based therapies can become a clinical reality .

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Ethical Clearance: As it is a review article so it is

not required.

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