

Stem Cell Regeneration: A Comprehensive Review

Dr. Smriti Kapur¹, Dr. Monika M Sehgal², Dr. Samar Grover³, Dr. Kazal Sohi³, Dr. Neha Rohilla³, Dr. Jaskiran Sidhu⁴

¹Reader, ²Professor, ³PG student, Department of Prosthodontics, JN Kapoor, D.A.V Centenary Dental College, Haryana, India

⁴BDS, Canada

ABSTRACT:

Background: Teeth are an ectodermal organ that is derived from sequential reciprocal interactions between oral epithelial cells and cranial neural crest–derived mesenchymal cells. Human tooth-associated stem cell populations include dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), and stem cells from human exfoliated deciduous teeth (SHED), have been isolated from dental pulp and periodontal ligament tissues. Although dental stem cells possess colony formation, proliferation and multipotent differentiation capacity to generate osteogenic, adipogenic and chondrogenic lineages in vitro similar to BMMSCs under certain conditions, they also displayed their own distinctive regenerative potential different from each other in vivo, suggesting that tissue specific stem cells might be the optimal choice for self-tissues repair and regeneration.

Key words: Stem cell, Regeneration

INTRODUCTION

The loss of a tooth is a minor deformity and a major pain. Although dental implants are available, the healing process can take months on end, and implants that fail to align with the ever-growing jawbone tend to fall out. Teeth are an ectodermal organ that is derived from sequential reciprocal interactions between oral epithelial cells and cranial neural crest–derived mesenchymal cells. Human tooth-associated stem cell populations include dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), and stem cells from human exfoliated deciduous teeth (SHED), have been isolated from dental pulp and periodontal ligament tissues. More importantly, DPSCs and PDLSCs are capable of forming a dentin–pulp complex and Cementum–periodontal ligament, respectively, when transplanted subcutaneously into immunocompromised mice, demonstrating their potential for regenerating human dental tissues *in vivo*. However, to achieve the goal of functional bio-tooth regeneration, a more thorough study of the intricate processes of tooth growth and development is necessary, in order to fully understand the processes of mutual induction between odontoblasts and ameloblasts and the complicated multiple structures of tooth composition.¹⁻³

Researchers have recognized that dental pulp contains *ex vivo*- expandable cells called dental pulp cells which include:

1. Alkaline phosphatase,
2. Type I collagen,
3. Bone sialoprotein,
4. Osteocalcin
5. Osteopontin,
6. Transforming growth factor β -(TGF- β),
7. Bone morphogenetic proteins (BMPs)

They also respond to the induction of BMP, fibroblast growth factor 2 (FGF- 2), matrix extracellular phosphoglycoprotein (MEPE), and TGF- β by undergoing osteogenic differentiation.⁴

DISCUSSION

Collectively, this experimental evidence suggests that dental pulp cells might be similar to osteoblast- like cells in terms of expressing bone markers and forming mineralized nodules when cultured under osteoinductive conditions. It was discovered that dental pulp cells might represent a distinct population of cells because of their unique ability to form specific crystalline structures in mineralized nodules, similar to physiological dentin but different from bone structures. Hence to justify it a Trial was carried out by selecting a population from human third molars provided evidence that dental pulp is extremely rich in stem cells, that are c-kit+/CD34+/STRO-1+/CD45 and capable of differentiation toward several stromal-derived differentiated cells and mainly osteoblasts and odontoblasts.⁵⁻⁹

Following the temporal expression pattern of specific markers when dental pulp cells are differentiating into osteoblasts, it is been shown that they express osteocalcin and fetal liver kinase 1 (flk-1), vascular endothelial growth factor receptor 2 (VEGF-R2) and 30% of them express specific antigens for endothelial cells (CD54, von-Willebrand 1 and 2, CD31 and angiotensin-converting enzyme). These stem cell populations synergically differentiated into osteoblasts and endotheliocytes, with flk-1 exerting a pivotal role in coupling osteoblast and endotheliocyte differentiation, suggesting that osteogenesis and angiogenesis mediated by human dental pulp stem cells are regulated by distinct mechanisms.⁶

This mechanism is probably specific to dental stem cells as the osteogenesis mediated by the dental cells cannot recruit hematopoietic marrow elements seen in the osteogenesis generated by bone marrow mesenchymal cells. But a substantial repair resulted from transplantation of SHED cells with hydroxyapatite/tricalcium phosphate into large calvarial defects in mice indicating that they are a suitable resource for Odontogenic regeneration.⁷⁻¹⁰

Looking at the ongoing clinical trials, it is too early to speculate whether all therapies based on stem cells will prove to be clinically effective. Stem cells from apical papilla (SCAPs), a type of dental stem cells essential for the developing dental pulp-dentin complex, alveolar bone and

tooth root, have been isolated from root tips of growing teeth, and are similar to DPSCs but with a markedly higher proliferative capacity and mineralization potential.¹¹⁻¹³

SCAPs express high level of STRO-1, CD-146, and negatively express CD34 and CD45. Studies showed that SCAPs had a greater capacity for dentin regeneration compared to DPSCs. Furthermore, SCAPs also exhibit a higher proliferation and better tooth regeneration capacity compared to PDLSCs.¹⁴

It has been reported that SCAPs and PDLSCs with a HA/TCP carrier can produce a functional biological tooth root in a swine model and finally resemble a functional tooth with an artificial crown. In addition, besides of healthy SCAPs, SCAPs derived from inflamed root tips also exhibit high proliferation and multipotency. Complex molecular mechanisms underlying SCAPs differentiation and proliferation have been investigated extensively. B-FGF has been reported to enhance stemness of SCAPs and differentiation capacity under certain conditions. Canonical WNT signaling also participate in osteo/odontoblastic differentiation of SCAPs. MicroRNAs play vital roles in regulateing odonto/osteogenic differentiation capacity of SCAPs.¹⁵

IMPACTED 3RD MOLARS

As stem cells from developing stage, SCAPs hold superior potential for regenerative medicine, and more mechanism study and clinical trial are expected in the future in order to make better use of them. DFCs and Tooth Regeneration The dental follicle, a loose ectomesenchyme originated connective tissue, surrounds tooth germ during tooth development and plays important roles in tooth eruption and tooth root development. Undifferentiated ectomesenchymal cells known as dental follicle stem cells or dental follicle cells (DFCs) can be obtained from impacted third molars or ectopic impacted teeth, and express high level of STRO-1, CD44, CD105, Nestin and Notch-1. DFCs are multipotent stem cells dental follicle cells are precursor cells of periodontal fibroblasts, osteoblasts and cementoblasts during the process of periodontal tissues development. It has been reported that DFCs hold the properties similar to MSCs, which were able to form a connective tissue-like structure with mineralized clusters after being induced in osteogenic differentiation medium. After transplantation of DFCs with treated dentin matrix scaffold, root-like tissues stained positive for markers of dental pulp and periodontal tissues were found in the alveolar fossa. Also data showed that rat DFCs formed a tooth root when seeded on scaffolds of a treated dentin matrix (TDM) and transplanted into alveolar fossa. Apart from generating periodontium alone, DFCs have also been observed to improve regenerative capacity of healthy PDLSCs and even rescue degeneration of inflamed PDLSCs, indicating that DFCs could assist PDLSCs to regenerate periodontal tissues via ameliorating local microenvironment. Additionally, human dental follicle tissue after cryopreservation has been proven to be a reliable resource for regenerative medicine. As DFCs support bone regeneration in defect models of the calvaria of immunocompromised rats, they are also a promising cell medication for bone regeneration.¹⁶⁻¹⁹

COLLECTION, ISOLATION, AND PRESERVATION OF STEM CELLS FROM**Step 1: Tooth collection (sustentation)**

Freshly-extracted deciduous/third molar is transferred into vial containing hypotonic phosphate buffered saline. Vial is then carefully sealed and placed into thermette, after which the carrier is placed into an insulated metal transport vessel. Thermette along with insulated transport vessel maintains the sample in a hypothermic state during transportation. This procedure is described as sustentation. The time from harvesting to arrival at processing storage facility should not exceed 40 h.²⁰

Step 2: Stem cell isolation

Tooth surface is cleaned by washing three times with Dulbecco's phosphate buffered saline without Ca²⁺ and Mg²⁺. Disinfection is done and again washed with PBSA. Pulp tissue is isolated from the pulp chamber and is placed in a sterile petri dish, washed at least three times with PBSA. The tissue digestion is done with collagenase Type I and dispase for 1 h at 37°C. Isolated cells are passed through a 70 µm filter to obtain single cell suspensions. Then the cells are cultured in a MSC medium. Usually isolated colonies are visible after 24 h.²¹

Step 3: Stem cell storage.

The approaches used for stem cell storage are: (a) Cryopreservation (b) magnetic freezing. Cryopreservation It is the process of preserving cells or whole tissues by cooling them to sub-zero temperatures. Cells harvested near end of log phase growth (approximately 80–90% confluent) are best for cryopreservation. Liquid nitrogen vapour is used to preserve cells at a temperature of <−150°C. In a vial 1.5 ml of freezing medium is optimum for 1–2 × 10⁶ cells.²²

Magnetic freezing

This technology is referred to as cells alive system (CAS), which works on principle of applying a weak magnetic field to water or cell tissue, which will lower the freezing point of that body by up to 6–7°C. Using CAS, Hiroshima, University (first proposed this technology) claims that it can increase the cell survival rate in teeth to 83%. CAS system is a lot cheaper than cryogenics and more reliable.²³

Criteria of tooth eligibility for stem cells from human exfoliated deciduous teeth banking

Primary incisors and canines with no pathology and at least one third of root left can be used for SHED banking. Primary molar roots are not recommended for sampling as they take longer time to resorb, which may result in an obliterated pulp chamber that contains no pulp, and thus, no stem cells. However in some cases where deciduous molars are removed early for orthodontic reasons, it may present an opportunity to use these teeth for stem cell banking.²⁴

Stem cells from apical papilla (SCAP)

MSCs residing in the apical papilla of permanent teeth with immature roots are known as SCAP. These were discovered by Sonoyama et al. SCAP are capable of forming odontoblast-like cells, producing dentin *in vivo*, and are likely cell source of primary odontoblasts for the formation of root dentin. SCAP supports apexogenesis, which can occur in infected immature permanent teeth with periradicular periodontitis or abscess. SCAP residing in the apical papilla survive such pulp necrosis because of their proximity to the periapical tissue vasculature. Hence even after endodontic disinfection, SCAP can generate primary odontoblasts, which complete root formation under the influence of the surviving epithelial root sheath of Hertwig.²⁵

Periodontal ligament stem cells (PDLSCs)

Previous authors have described the presence of multipotent postnatal stem cells in the human periodontal ligament (PDLSCs). When transplanted into rodents, PDLSCs had the capacity to generate a cementum/periodontal ligament-like structure and contributed to periodontal tissue repair. These cells can also be isolated from cryopreserved periodontal ligaments while retaining their stem cell characteristics, including single-colony strain generation, cementum/periodontal ligament-like tissue regeneration, expression of MSC surface markers, multipotential differentiation and hence providing a ready source of MSC. Using a mini pig model, autologous SCAP and PDLSCs were loaded onto hydroxyapatite/tricalcium phosphate and gel-foam scaffolds, and implanted into sockets in the lower jaw, where they formed a bio-root encircled with the periodontal ligament tissue and in a natural relationship with the surrounding bone. Another set of authors have suggested that PDLSCs had regenerative potential when seeded onto three dimensional biocompatible scaffold, thus encouraging their use in graft biomaterials for bone tissue engineering in regenerative dentistry, whereas some other authors have reported cementum and periodontal ligament-like tissue formation when PDLSCs are seeded on bioengineered dentin.²⁶

TOOTH STEM CELL BANKING

Although tooth banking is currently not very popular the trend is gaining acceptance mainly in the developed countries. BioEden (Austin, Texas, USA), has international laboratories in UK (serving Europe) and Thailand (serving South East Asia) with global expansion plans. Stem cell banking companies like Store –A- Tooth (Provia Laboratories, Littleton, Massachusetts, USA) and StemSave (Stemsave Inc, New York, USA) are also expanding their horizon internationally. In Japan, the first tooth bank was established in Hiroshima University and the company was named as “Three Brackets” (Suri Buraketto) in 2005. Nagoya University (Kyodo, Japan) also came up with a tooth bank in 2007. Taipei Medical University in collaboration with Hiroshima University opened the nation’s first tooth bank in September, 2008. The Norwegian Tooth Bank (a collaborative project between the Norwegian Institute of Public Health and the University of Bergen) set up in 2008 is collecting exfoliated primary teeth from 1,00,000 children in Norway. Not last but the least, Stemade introduced the concept of dental stem cells banking in India

recently by launching its operations in Mumbai and Delhi. Proteins known as growth factors are what cause stem cells to differentiate into whatever type of cell they are bound to become. Introducing different growth factors force the cells to develop the desired type of tissue. This involves a lot of technical care and is a highly regulated process, which slows down progress. Mooney's team claims they have come up with a new technique that could streamline the process, making it a viable clinical option much more quickly.²⁷

Human dental stem cells that have been isolated and characterized are:

1. DPSCs
2. SHED
3. Stem cells from apical papilla (SCAP)
4. Periodontal ligament stem cells (PDLSCs)²⁸

CONCLUSION

Although dental stem cells possess colony formation, proliferation and multipotent differentiation capacity to generate osteogenic, adipogenic and chondrogenic lineages in vitro similar to BMMSCs under certain conditions, they also displayed their own distinctive regenerative potential different from each other in vivo, suggesting that tissue specific stem cells might be the optimal choice for self-tissues repair and regeneration.

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