REVIEW ARTICLE

Role of tissue engineering in dental pulp regeneration

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ABSTRACT

Stem cells constitute the source of differentiated cells for the generation of tissues during development, and for regeneration of tissues that are diseased or injured postnatally. In recent years, stem cell research has grown exponentially owing to the recognition that stem cell-based therapies have the potential to improve the life of patients with conditions that span from Alzheimer's disease to cardiac ischemia to bone or tooth loss. Growing evidence demonstrates that stem cells are primarily found in niches and that certain tissues contain more stem cells than others. Among these tissues, the dental pulp is considered a rich source of mesenchymal stem cells that are suitable for tissue engineering applications. It is known that dental pulp stem cells have the potential to differentiate into several cell types, including odontoblasts, neural progenitors, osteoblasts, chondrocytes, and adipocytes. The dental pulp stem cells are highly proliferative. Collectively, the multipotency, high proliferation rates, and accessibility make the dental pulp an attractive source of mesenchymal stem cells for tissue regeneration. This review discusses fundamental concepts of stem cell biology and tissue engineering within the context of regenerative dentistry.

Key words
Apexogenesis, dental pulp stem cells, pulp/dentin tissue regeneration, apexification, pulp revascularization

INTRODUCTION

The creation and delivery of new tissues to replace diseased, missing or traumatized pulp is referred to as regenerative endodontics, which provides an innovative and novel range of biologically-based treatments for endodontic disease.[1] Treatment of young permanent tooth with a necrotic root canal system and an incompletely developed root is fraught with difficulty.[2] Apexification enables a calcified barrier to form at the root apex by placing a biocompatible material against the periapical tissues via the root canal. Calcium hydroxide and mineral trioxide aggregate (MTA) have been materials of choice for apexification procedure, but neither of them is ideal.[3] Numerous clinical case reports have suggested that many teeth that traditionally would receive apexification may be treated for apexogenesis.[4] Thus there is continuedneed to develop biologically based treatment regimens that offer the potential for continued hard tissue formation of the young permanent tooth with a necrotic root canal system and incompletely developed root.[2] The discovery of stem cells in deciduous teeth elucidates the intriguing possibility of using dental pulp stem cells for tissue engineering.[5]

It has been noted that there is a population of putative post natal stem cells in human dental pulp.The most striking feature of Dental pulp stem cells (DPSCs) is their ability to regenerate a dentin-pulp-like complex that is composed of mineralized matrix with tubules lined with odontoblasts, and fibrous tissue containing blood vessels in an arrangement similar to dentin-pulp stem complex found in normal human teeth.[6]

The post-natal pulp contains several niches of potential progenitor/stem cells, which may have importance in mediating reparative dentine formation. Indeed, progenitor/stem cell niches are continually being identified in all connective tissues of the body, where they play a fundamental role in wound repair processes. This subset of undifferentiated cells can represent as little as 1% of the total cell population. However, they produce multiple highly differentiated progeny in response to specific extracellular signals.[7] Central to the niche is the 'true' adult or 'mother' stem cell which displays an infrequent, yet almost unlimited self-renewal. At mitosis, these cells give rise to a renewed mother stem cell and a daughter transit amplifying progenitor cell.

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These daughter progenitor cells possess a more limited capacity for self-renewal, but are highly proliferative. They also appear to control multi-potentiality, and are capable of following along several cell lineages to ultimately produce terminally differentiated cells such as osteoblasts, odontoblasts, and, adipocytes, chondrocytes, and neural cells.[7]

STEM CELLS

A stem cell is defined as a cell that can continuously produce unaltered daughters and, furthermore, has the ability to generate cells with different and more restricted properties. Stem cells can divide either symmetrically (allowing the increase of stem cell number) or asymmetrically. Asymmetric divisions keep the number of stem cells unaltered and are responsible for the generation of cells with different properties. These cells can either multiply progenitors or transit amplifying cells.[8]

Types of stem cells

The stem cells were first suggested by Danchakoff and by Sabin and Maximow.[9]

Stem cells can be classified according to:

- Potential for differentiation into totipotent, pluripotent, multipotent, and unipotent cells
- The tissue of origin for embryonic /adult stem cells
- Their capacity for tissue re-population *in vivo* in short, medium or long time regeneration.

Different types of stem cells are described in Figures 1 and 2.

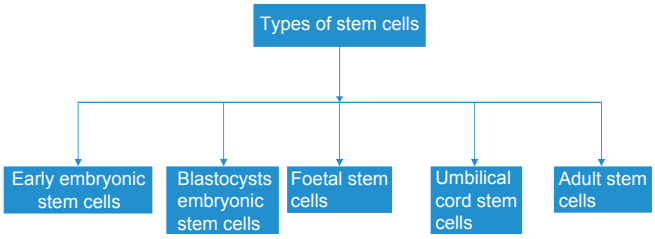

Figure 1: Types of stem cells

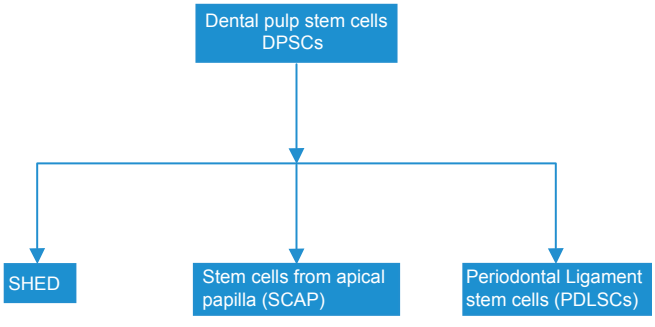

Figure 2: Types of dental pulp stem cells

Dental pulp stem cells

Dental pulp entrapped within 'sealed niches' of the pulp chamber, is an extremely rich site for stem cell collection. These stem cells are called DPSCs when found in adults and stem cells from human exfoliateddeciduous (SHEDs).[5,10]

SHED

The use of SHED for tissue engineering might be more advantageous that that of stem cells from adult human teeth; they were reported to have a higher proliferation rate than stem cells from permanent teeth.[5] They are ideally suited for young patients at the mixed dentition stage who have suffered pulp necrosis in immature permanent teeth as a consequence of trauma.[11] The main task of these cells seems to be formation of mineralized tissue which can used to enhance orofacial bone regeneration.[12]

Stem cells from apical papilla

SCAP were recently discovered by Sonoyama.[13] SCAP exhibits a higher proliferative rate and appears more effective than PDLSC for tooth formation.[9] Compared with DPSC, SCAP have a great numbers of STRO-1 positive cells, faster proliferation, a greater number of population doublings and increased capacity for *in vivo* dentine regeneration.[14]

Periodontal ligament stem cells

Presences of multipotent post natal stem cells in human PDL were first described by Seo.[15] They can be isolated from Cryo-preserved periodontal ligaments while maintaining their stem cell characteristics, including the expression of MSC surface cell markers, single-colony-strain generation, multipotential differentiation and cementum/periodontal-ligament-like tissue regeneration, thus providing a ready source of MSCs.[16]

Apexification and new trends of regenerative endodontics

Apexification is a procedure to promote the formation of an apical barrier to close the open apex of an immature tooth with a nonvital pulp such that the filling material can be contained within the root canal space.[17] Maturogenesis is a more appropriate term than Apexification, because not only the apex but the entire root is allowed to mature as in non-traumatized tooth.[18] The use of term "Revascularization" was adopted by Iwayato describe the clinical healing of periapical abscesses and continued root formation in immature teeth with nonvital pulps.[19]

The clinical decision whether to perform apexogenesis or apexification for immature teeth appears to be clear cut with teeth deemed to have vital pulp tissue are subjected to apexogenesis and teeth deemed to have non vital pulp tissue receives Apexification.[20] Calcium hydroxide and

mineral trioxide aggregate (MTA) have been materials of choice for Apexification procedures, but neither materials is ideal. A number of shortcomings can be summarized as for Ca $(OH)_2$ apexification:

1. Long time span for entire treatment;
2. Multiple visits with heavy demands on patient and carers and inevitable clinical costs;
3. Increased risk of tooth fracture using $Ca(OH)_2$ as a long term root canal dressing.[21]

However MTA has provided more favorable results and improved patient compliance. Pulpal tissue preservation, uniform calcified bridge formation, rate of bridge formation and providing excellent seal make MTA a new choice for Pulpal maturogenesis.[22] From a physiological point of view calcified material benefits vs. regeneration in Apexification is ongoing point of debate as calcific metamorphosis is a degenerative disease.[23] The advantages of pulp revascularization lie in the possibility of further root development and reinforcement of dentinal walls by deposition of hard tissue, thus strengthening the root against fracture.[24]

Two types of pulp regeneration can be considered based on the clinical situations:

1. Partial pulp regeneration
2. *De novo* synthesis of pulp.[20]

Ostbypostulated the tissue reorganization in the canal space filled with blood clot. It was observed that tissue formed in the canal was not pulp but granulation or fibrous tissue and in some cases the ingrowths of Cementum and bone growth.It has been experimentally shown that apical part of pulp may remain vital and, after reimplantation, may proliferate coronally, replacing the necrotized portion of pulp.[25] Successful regeneration depends on race between the new tissue and bacteria populating the pulp space is strengthened by the fact that the incidence of revascularization is enhanced if the apex shows radiographic opening of more than 1.1 mm and the tooth is replanted within 45 min (thus increasing chances of revascularization by 18%).[26]

Pulp tissue engineering and shift of treatment protocol

Before isolation of DPSCs, pulp regeneration was tested using Modern tissue engineering concepts by growing pulp cells onto synthetic polymer scaffolds of polyglycolic acid (PGA) and *in vitro* and *in vivo* analysis performed.[27] These approaches are basically a proof of principle to test whether cultured pulp cells can grow well and produce matrix on PGA, and whether the engineered pulp can be vascularized.[28]

While vascularization is a universal issue for an engineered tissue, it is of special concern for pulp tissue engineering b'coz of the lack of a collateral source of blood supply. Use of angiogenic factors inducing factors such as vascular endothelial growth factors (VEGF) could enhance and accelerate pulp angiogenesis.[28] Regeneration of tissue into the apex of an immature permanent tooth may come from stem cells already residing in vital pulp tissue, the apical papilla, PDL or alveolar bone.[29] Stem cells have been identified in greater number within the PDL of diseased teeth where the inflammatory process actively recruited immature cells.[30]

Mooney first described technique to engineer new pulp-like tissue from cultured human pulpal fibroblast. Regeneration of pulp or periodontal tissues relies on the provision of appropriate biodegradable scaffolds which are capable of containing or being seeded with growth factors and bioactive signaling molecules, supporting cell organization and growth of vascular supply.[31] Yet no matrix has been proved ideal; collagen and polymer scaffolds are able to support *in vitro* survival of DPSC and PDLSC.[32] Cordeiro seeded SHED and endothelial cells onto biodegradable scaffolds within human tooth slices then implanted them into immunocompromised mice. It was observed that cells differentiated into odontoblast like and endothelial like m *in vivo* resulting in tissue closely resembling dental pulp with a viable blood supply.[33]

Management of immature teeth: A new perspective

Clinical reports show that after conservative treatment severely infected immature teeth with periradicular periodontitis and abscess can undergo healing and apexogenesis and maturogenosis. Regenerative endodontics promotes a shift in protocol for treating endodontically involved immature permanent teeth by conserving any dental stem cells that might remain in the disinfected viable tissues to allow tissue regeneration and repair to achieve apexogenesis/maturogenisis.

Even though there exists no evidence based guidelines regarding cases that can be treated with conservative approach recent case reports have shown that the traditional approach of apexification may compromise certain cases that have the potential to undergo apexogenisis. Several reports have documented this observation. These case reports have generated interest in understanding the observation that important type of cells and tissues must have survived after disinfection thereby allowing the root to undergo maturation. Example of HERS and SCAP in the apical papilla. SCAP have been proposed to the cell source of root odontoblasts.[13]

CONCLUSION AND PROSPECTS

Although Calcium hydroxide and MTA are time tested materials for apexification. But literature is replete with the finding that prognosis is sometimes questionable for teeth that inherit a thin and weak root after successful apexification, which subsequently makes these teeth susceptible to fracture. Therefore recent advances in

regenerative dentistry may help clinicians to choose a conservative approach for generation of an apical barrier. The discovery of dental pulp stem cells provides a new protocol for treating immature teeth with open apices. As a number of clinical case reports challenge the traditional approach for managing immature teeth by apexification. Of late, there has been increasing interest in choosing a conservative approach first, while apexification is performed in cases of failure.

At the same time it is of utmost importance to provide more evidence based research and conclusions in tissue engineering and regenerative dentistry in order to strive to give patients the best possible treatment.

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