Regenerative Stem Cells for Endodontic Tissue Engineering: Past, Present and Future

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Abstract

The ultimate aim of endodontic treatment focuses towards the complete preservation of physiologic, structural & mechanical integrity of native pulp – dentin complex. Regenerative endodontic procedure along with tissue engineering can be one of the novel approach to restore tooth structure. Over the last two decades, tissue engineering has promoted from science fiction to science. Tissue engineering & regeneration needs three basics ingredients i.e. morphogenetic signals including growth & differentiation factors, stem cells & a scaffold of extracellular matrix. As all tissues originate from stem cells, these stem cells are the first and utmost important key factor for endodontic regeneration. The present scientific review article is about discussing the basics of stem cells along with their current scenario and future perspectives in endodontic regeneration.

Key Words: *Dental pulp, Stem cells, Regenerative endodontic, Tissue engineering, Revascularization*

Introduction

Dental pulp is a unique tissue considering many perspectives. Even the mature pulp has embryonic connective tissue resemblance and therefore, is a rich source of stem cells. After tooth development, the dental pulp has ability to form dentin throughout life. The teeth affected by caries, dental wear lesions or trauma are generally treated by restorations or conventional dental pulp therapy such as pulp capping or root canal treatment. But to preserve the function & vitality of pulp, dental researches are focusing on strategies for vital pulp therapy that might provide a promising alternative instead of removing the whole pulp.

Millions of teeth are being saved every year by root canal treatment with the clinical success rate of more than 90%. However, many teeth are non-restorable because of several factors like apical root resorption and fracture, immature roots, or grossly decayed coronal structures.[1,2,3]

One novel attempt to preserve the tooth structure is based on regenerative endodontic procedures by application of tissue engineering.[4] Langer and Vacanti[5] has defined tissue engineering as an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function. The key elements of tissue engineering are stem cells, growth factors, and a scaffold of extracellular matrix. Stem cells have ability to self-renew and produce different cell types, thus promoting new regimes to regenerate missing tissues and treat diseases. Furthermore, oral tissues are assumed to be not only a source but also a therapeutic target for stem cells, as stem cells and tissue engineering therapies in dentistry help increasing the clinical interest day by day.[6]

History of Stem Cells Research[7,8]

The history of stem cell research began in the early 1960s when James Till and Ernest McCulloch and their colleagues at the University of Toronto came to know about the reservoirs of cells in mice with the properties of stem cells i.e. the capability to self-renew and to differentiate into specialized cells. This innovation helped the groundwork for the development of the first embryonic stem cell lines.

1961: Canadian James Till and Ernest McCulloch discovered the existence of stem cells in the bone marrow in mice.

1968: The postnatal stem cell therapy was initiated, when the first allogenic bone marrow transplant was successfully performed in the treatment of severe combined immunodeficiency (SCID).

1978: Stem cells were identified in human cord blood.

1981: First Embryonic stem cells were isolated from mouse blastocysts.

1992: Discovered stem cells in the adult human brain.
1995: First embryonic stem cell line developed from a non-human primate
1996: First mammal cloned from an adult (somatic) cell. Dolly the sheep was born at Roslin Institute, Scotland.
1998: The first human embryonic stem cell line was developed by Dr. J. Thomson at University of Wisconsin-Madison.
2006: First artificial liver cells generated from umbilical cord blood stem cells.
2007: First successful development of primate stem cell.
2008: First human embryonic stem cells generated without destruction of embryo and stem cells successfully used to regenerate knee cartilage in a human.
2009: Patient specific induced pleuripotent stem cells were developed.
2010: First & second trial of embryonic stem cells in human

- World's first human phase I clinical trial of stem cell therapy (cells derived from foetal stem cells) for disabled stroke patients.

2011: Reprogramming of bone marrow cells back into all-purpose stem cells of embryonic origin was done successfully.
2012: Conversion of cells found in urine, into pluripotent stem cells by Chinese scientists from the Guangzhou Institutes of Biomedicine and Health that can help to generate neurons and brain cells.

Origin of Stem Cells[6,9,10]

Stem cells may be originated from various sources and several researches are undergoing to promote the therapeutic use of stem cells from all sources. The stem cells can be categorised as:

- **Embryonic stem cells**: they are extracted from embryos and are considered to be most potential as they may generate any specialised cell in the human body.
- **Adult stem cells**: these are found in adult tissues such as the bone marrow, brain and blood but have limited potential compared to embryonic stem cells.
- **Cord blood stem cells**: this source of stem cells is cord blood and may have enormous potential in treating disease.

Classification of Stem Cells[6,11,12]

Totipotent Stem Cells: These are considered to be most versatile type of stem cell. When a sperm cell and an egg cell unite, they form a one-celled fertilized egg. Totipotent actually terms that it has the potential to give rise to any and all human cells, such as brain, liver, blood or heart cells. It can even generate an entire functional organism.
Pluripotent Stem Cells: These cells are like totipotent stem cells in that they can give rise to all tissue types. Unlike totipotent stem cells, however, they cannot give rise to an entire organism.
Multipotent Stem Cells: These are less plastic and more differentiated stem cells. They generate a limited range of cells within a tissue type.
Unipotent Stem Cells: Can differentiate into only one type of cells.

Types of Dental Stem Cells[13-17]

Till date, four types of human dental stem cells have been isolated and characterized:

1. Dental pulp stem cells (DPSCs),
2. Stem cells from exfoliated deciduous teeth (SHED),
3. Stem cells from apical papilla (SCAP), and
4. Periodontal ligament stem cells (PDLSCs)

- Among these, all cells are from permanent teeth **except SHED**.
- These dental stem cells are thought to be mesenchymal stem cells (MSCs) and can have different levels of abilities to become specific tissue forming cells.
- DPSCs and SHED are from the pulp and SCAP is from the pulp precursor tissue, the apical papilla.

Stem Cells Therapy in Endodontics

Apexogenesisor Apexification:[18,19]

- Pulp necrosis may be due to irreversible pulpal injury and is commonly because of endodontic infection. In younger patients with immature roots, where the possibility of retaining some vital pulp tissue and allowing continued root formation, a conservative approach is needed.
- When infection involves the whole root canal system, endodontic treatment need the removal of remaining pulpal tissue to the developing root apex level, i.e. at its loose contact with the apical papilla.
- In an immature permanent tooth, the regeneration of tissue into the apex may

derive from stem cells already present in vital pulp tissue, SCAP, PDL or alveolar bone. Also, stem cells and growth factors seeded on scaffolds may promote regeneration of tissues in-vitro or in-vivo.

- Stem cells are used to promote pulpal regeneration so as to treat immature permanent teeth in a conservative approach. Short-term regenerative endodontic procedures can lead to complete root formation causing an increase in root length and thickness.

Pulp Revascularization:[20,21]

- Immature tooth with pulpal necrosis, due to caries or trauma could stop further root development, resulting in thin root canal walls and blunderbuss apices.
- The absence of an apical stop makes root canal treatment difficult because of the inability to obtain a seal with conventional obturation techniques. Also, the thin root canal walls may become more susceptible to fracture.
- Pulpal tissue regeneration of an infected immature tooth might take place if favourable environment is present with absence of intra-pulpal infection and scaffold conductive to tissue ingrowth.
- Under this environment, the pulpal space might have repopulation of mesenchymal cells arising from dental papilla or apical periodontium.[21,22]

Whole Tooth Regeneration:[20]

- Tooth-like tissues have been regenerated by seeding of different cell types on biodegradable scaffolds. A common technique is to harvest cells, grow and differentiate cells in vitro, seed cells onto scaffolds, and implant them in vivo. In some cases, the scaffolds are re-implanted into an extracted tooth socket or the jaw.
- **Ikeda *et al*. (2009)**[24] successfully reported a complete functioning tooth replacement in an adult mouse through the transplantation of bioengineered tooth germ into alveolar bone in the lost tooth region. This technology was promoted as a basic model for future organ replacement techniques.

Previous Studies on Stem Cells Supporting Dental Regeneration

During repair process following pulpectomy in immature dogs' teeth, **Vojinovic´ & Vojinovic´ (1993)** confirmed periodontal cell migration into the apical pulp and found inflammation stimulated cellular recruitment.

For the first time, an in vitro study by **Mooney et al. (1996),** explained a methodology to engineer new pulp-like tissues from cultured human pulpal fibroblasts. Pulpal or periodontal tissues regeneration depends on suitable biodegradable scaffolds which may have or can be seeded with growth factors and signalling molecules, helping cell organization and growth of a vasculature.

Some periodontal studies by **King et al. (1997), King & Hughes (2001),** proved that cells may proliferate and migrate from adjacent normal PDL into the injured or damaged area. This theory helped supporting the role of stem cells present within PDL, and alveolar bone marrow that might be able to get stimulation at a distance and may migrate towards the immature root apices.

Histologically, **Thibodeau et al. (2007),** have confirmed the vital tissue within the root canal space following 'revascularization procedures', but the origin of this tissue remains unexplained.

Several related case studies by **Banchs & Trope (2004), Chueh & Huang (2006), Jung et al (2008),** showed that immature permanent teeth have a rich cellular and vascular supply and so, DPSC and SCAP may overcome disinfection, as these reports supported immature teeth with pulpal necrosis undergoing apexogenesis.

In-vitro study by **Mullane et al. (2008),** showed the revascularization following pulpal severing using tooth slices implanted into mice. Also, angiogenic growth factors inclusion remarkably stimulated the vascular sprouting, supporting the role of the environment on favourable healing.

Cordeiro et al. (2008) incorporated SHED and endothelial cells onto biodegradable scaffolds within human tooth slices then implanted them into immune-compromised mice. It was noticed that cells differentiated into odontoblast-like and endothelial-like cells in-vivo and generated the tissue which closely mimicked dental pulp with a viable vascular supply.

Gomez Flores et al. (2008) discovered a novel attempt for in-vivo periodontal regeneration using a multilayer human PDL cell sheet

technique which promoted the formation of immature cementum-like tissue and PDL. In case of open root apex, a similar scaffold structure adjacent to a vascular supply may favour apexification by thickening and closing the root apical end with hard tissue.

Objectives of Stem Cells Therapy in Endodontics[9,25]

The objectives of most researches on stem cells therapy are directed towards achieving the following:

1. Regeneration of pulp dentin complex
2. Regeneration of damaged coronal dentin
3. Regeneration of resorbed root, cervical or apical dentin and repair perforations
4. Whole tooth regeneration

Dental or Tooth Regeneration, Why??[20,26]

Since the humans only have two sets of teeth: the deciduous and permanent teeth, it has been desired for tooth regeneration as the ultimate dental treatment. Although predictable clinical outcomes have been achieved through titanium dental implants, but they do not function as identical as natural teeth because they lack an intervening PDL after osseo-integration. Hence, the natural functions of PDL are completely missing.

The ultimate goal of tooth regeneration is to have completely functioning bioengineered teeth that can replace lost natural teeth. Whereas, the regeneration of the tooth root seems to be more realistic and clinically approachable, especially for prosthodontists, because the regenerated tooth root can help an abutment tooth to allow fixed-prosthetic procedures, such as crown and bridge treatments. According to **Sonoyama et al. (2006)**[15], a root/periodontal complex constructed using PDL stem cells (PDLSCs), SCAP and a suitable scaffold, helped supporting an artificial crown to provide normal tooth function in a swine model. In addition, the cell sheet technology using DFCs in combination with a dentin matrix-based scaffold has resulted successfully to tooth root reconstruction.

Therefore, the new stem-cell-based methodology for the tooth root regeneration and its associated periodontal tissue may provide clinical applications for the treatment of damaged or lost teeth.

Hurdles for Regenerative Procedures

One of the major problem in the clinical application of tooth regeneration technology is the identification of a suitable and favourable autologous stem cell source in humans[20] but there are several hurdles with in-vitro regenerative procedures:

1. There is need of cell lines to be grown and expanded before being implanted into the root canal, leading to protracted clinical treatment times.
2. The implanted cells further need promising adherence to the disinfected root canal walls so that it may dictate a change in the clinician's way of currently debriding and disinfecting root canals.
3. Also, the lacks of crucial vascular supply in the implanted tissue, and technical difficulty of replanting the three-dimensional regenerated pulp without harming the cells, are some other limitations.

Current Trends and Future Perspectives of Stem Cells Regeneration

The whole tooth regeneration is supposed to be one of the biggest achievements in the field of dentistry. Tooth engineering to generate tooth structures in-vivo has already been established using many different types of stem cells from mice, rats and pigs. Also, Ikeda et al. (2009)[24] successfully elaborated a technique of complete functioning tooth replacement in a mouse using transplantation into the alveolar bone of bioengineered tooth germ reconstructed from epithelial and mesenchymal progenitor/stem cells in a collagen gel.

The bioengineered tooth, which was erupted and occluded, had the exact tooth structure, mineralized tissues for mastication, and response to noxious stimulations like mechanical stress and pain in cooperation with other oral and maxillofacial tissues. This study represented a substantial advancement and focused on the potential for bioengineered organ replacement in future regenerative treatments.

Based on same cell source used for the bioengineered tooth, the in-vivo reconstitution of a murine 'Bioengineered tooth unit' was recently developed. Interestingly, the unit composed not only a mature tooth and periodontal ligament but also alveolar bone. The unit promoted a fully functional tooth with vertical bone regeneration

when the unit was transplanted into a vertical alveolar bone defect in a mouse model. These findings aroused a new concept in tooth regeneration therapy: the transplantation of a bioengineered tooth has ultimate power for not only whole tooth regeneration but also as a treatment in clinical cases where tooth loss is associated with a serious alveolar bone defect.

As described earlier, one of the major hurdles in the clinical application of tooth regeneration technology is the identification of an appropriate autologous stem cell source in humans. In this regard, pulpal stem cells may be a suitable cell source because they can be differentiated to dental epithelial and mesenchymal cells and can be extracted from the patients' own somatic cells.

The viability of dental stem cells in frozen tissue might promote the possibility of banking exfoliated deciduous teeth, supernumerary teeth, third molars or teeth extracted for orthodontic reasons for later use in regenerative treatments.

Initiation of one's own cells tissue banking may solve the immunological and ethical issues involved with the use of allogenic cells. In particular, the banking of SHED or stem cells from immature third molar teeth would be advantageous with the condition of having high proliferative nature of these cells and the high incidence of traumatic dental injuries in the early permanent dentition.

Regarding future perspective, the use of gene therapy to regenerate dental tissue by the local delivery of cells that have been genetically altered to deliver physiological specific growth factors may be a possibility. Research is in its infant stages in terms of identifying exact novel genes and finding suitable vehicle for controlled cell-specific safe delivery. Including the ethical constraints for use of gene therapy, the in-vivo clinical applications for dental tissue regeneration are still a miles way off.

Conclusion

Still in the current perspective, the field of tooth tissue engineering is definitely one where questions are more than the answers. Regenerating the dental structures seems to be a complex proposition. Combination of high quality research works and effective collaborations between basic scientists and clinicians may be a definite way to direct this field toward its ultimate goal of regenerating individual tooth structure or the entire tooth, so that it could overcome the consequences of tooth related deformities.

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