

## Review Article

# Current and Future Perspectives for Dentin-pulp Tissue Engineering - An update

Swati Srivastava<sup>1\*</sup>, Lubna Alotheem<sup>2</sup>, Haneen Alotheem<sup>3</sup>, Einas Alahmad<sup>3</sup>, Shahad Alogayyel<sup>3</sup>

<sup>1</sup>Assistant Professor, Department of Conservative Dental Sciences, College of Dentistry, Qassim University, Buraydah, Kingdom of Saudi Arabia

<sup>2</sup>Qassim Private College, Buraydah, Kingdom of Saudi Arabia

<sup>3</sup>Qassim University, Buraydah, Kingdom of Saudi Arabia

\*Corresponding author email: [swatisrivastava412@gmail.com](mailto:swatisrivastava412@gmail.com)

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## Abstract

The ball has been set rolling with the isolation of dental pulp stem cells in 2000 for tissue engineering to generate dental pulp. The rationale lies in the possibility of further root development and reinforcement of dentinal walls by deposition of hard tissues which are usually a clinical problem of interest for dentists. In such scenarios, mechanical preparation of the root canal may further weaken the thin dentinal walls and increase the risk of tooth fracture. On the other hand, regenerative endodontic procedures (REPs) could be a better solution. Many exciting areas of endodontic research are emerging and contributing to an increasing momentum of activity in this discipline including regenerative procedures in permanent teeth. This review is an attempt to update on the current trends.

## Key words

Apical papilla, Bioengineering, Cell homing, Dental triad, Regenerative endodontics.

## Introduction

Regenerative endodontics provides hope of converting the non-vital tooth into vital once

again. It focuses on substituting traumatized and pathological pulp with functional pulp tissue. AAE defines regenerative endodontics as

“biologically-based procedures designed to physiologically replace damaged tooth structures, including dentin and root structures, as well as cells of the pulp-dentin complex.”

The foundation of regenerative endodontic procedures (REPs) was established by Nygaard-Ostby who observed that the formation of a blood clot was not only desirable, but also sought after as a means of ensuring adequate healing [1]. Despite its shortcomings, this pioneer study laid the foundation for the subsequent studies in the field of regenerative endodontics. In 2004, Banchs and Trope [2] published a case report describing a new treatment procedure for the management of the open apex called “revascularization.” Unlike traditional apexification or the use of apical barriers, revascularization procedures allow for increase in both the length of the root and root wall thickness. Hargreaves et al. recommended the interplay of the three major components for pulp regeneration formed by stem cells, bioactive molecules and bioactive scaffolds. During REP therapies, this triad aims to reconstruct these microenvironments.

A dental stem cell is a self-renewable cell type in the tooth involved in the maintenance of adult or developing dental tissues. These dental stem cells and their daughter cells grow and differentiate dependent on growth factors or bioactive molecules released by the dental stem cell niches. Bioactive molecules are signaling molecules such as growth factors or chemical cues that control a variety of cellular responses through specific binding of transmembrane or intracellular receptors in a target cell. The bone morphogenic protein (BMP) superfamily of proteins such as BMP-2, BMP-4, BMP-7, and transforming growth factor beta 1(TGF-  $\beta$ 1) are found to modulate cellular functions. There exists a rich cocktail of bioactive molecules in dentin and pulp matrices capable of directing all of the signaling events involved in dentin-pulp regeneration [3].

Bioactive Scaffolds should be biodegradable and able to facilitate cellular infiltration, vascularization, cell differentiation, and eventually its replacement by the appropriate tissues. A variety of biomaterials include tissue extracts such as blood clot, platelet- rich plasma (PRP), and platelet-rich fibrin (PRF), pure platelet-rich fibrin, leukocyte platelet-rich fibrin; ceramics such as calcium hydroxyapatite, tricalcium phosphate, and mineral trioxide aggregate; synthetic polymers such as polylactic and polylactic-co-glycolic acids; biopolymers such as collagen, hyaluronan and chitosan; and self- assembling peptide hydrogel. Most of the biomaterials that have been proposed lack approval from the Food and Drug Administration (FDA) which makes them non-suitable for clinical use [4]. Chrepa, et al. demonstrated in vitro an FDA-approved Hyaluronic acid hydrogel -Restylane, an injectable scaffold which promoted SCAP survival, mineralization, and differentiation into an odontoblastic phenotype. As it is available in injectable form, it may represent a suitable scaffold for immediate clinical use in endodontic regenerative procedures [5].

#### **A New Bioengineering Dental Triad**

The above discussed traditional triad fails to accomplish healthy functional tissue. These non-satisfactory results suggest that some important factors are still missing in current REPs. Hence, a new dental bioengineering approach has been suggested which aims the importance of dentin niche and the influence of trophic factors [6].

**Dentin Niche / Niche engineering** - It is a conducive environment in which tissue regeneration occurs. Both direct pulp capping and pulpotomy, the main vital pulp therapy procedures, are based on the ability of DPSCs of the remaining vital dental pulp to accomplish repair. The presence of dental stem cell niches in remaining vital pulp would represent an advantage and would be crucial to obtain good results in REPs, even in cases with small fragments of residual vital pulp [7].

**Trophic factors/ Survival signals** - Dentin matrix contains a reservoir of growth factors and other bioactive molecules sequestered or “fossilized” within the mineralized matrix. These factors regulate cell proliferation and differentiation during tooth development and get entrapped in the dentin matrix where they remain functional during life [8]. Dentin-entrapped factors are potentially controlled by TFs regulating “positional memory” during development. Their exact concentration could be highly heterogeneous. General REP’s treatments that release them from dentin would be benefited by these factors.

### **Current developments and challenges in Regenerative endodontics**

#### **Vasculature Engineering**

Angiogenesis is important not only for nutrient supply during regeneration of the tissue but also, potentially, for stem cell recruitment. During the last decade, there have been major advances in the understanding the process of neoangiogenesis which can be categorized into 3 main approaches [9]:

**1.Incorporation of growth factors** - Angiogenic factor–incorporated scaffolds with sustained release Vascular endothelial growth factor (VEGF) is a signal protein which is the most potent angiogenic and vasculogenic factor, promoting endothelial cell proliferation, migration, and survival. Yadlapati, et al. [10] found that VEGF-loaded biodegradable fiber may be considered a viable option to stimulate angiogenesis and new tissue formation during endodontic regeneration procedures.

**2.Coculture of progenitor/target cells with endothelial cells** - A number of studies have shown that the coculture of stem/progenitor cells with endothelial/endothelial progenitor cells significantly enhances angiogenesis acting synergistically [11, 12]. Findings indicate the coculture of DPSCs and endothelial cells as a promising source for regenerative endodontics.

**3.Microfabrication of vasculature or decellularized matrices** -This approach aims to

create a blueprint for microcirculation in a biodegradable scaffold so that endothelial cells attach to microchannels and proliferate, giving rise to a functional vasculature [13, 14]. Although this method has been investigated in relation to engineering complex tissues such as the liver and brain, it is yet to be attempted in dental tissue engineering. This approach could prevent one of the most common issues in pulp regeneration strategies, which is the lack of perfusion in the periphery of newly implanted pulp tissue hindering full-length pulp regeneration.

#### **Pulp tissue graft**

Minced pulp tissue has been used as a source of pulpal MSCs for tissue regeneration. Minced pulp tissue transplantation yields migrating cells that retain odontogenic and osteogenic differentiation potential. Here, the explant culture method into pulp regeneration approach bypasses the need for in vitro cell culture. This is the basis of the proposed protocol for “**pulp tissue grafting**” approach to regenerate pulp-dentin complex in teeth that require endodontic intervention without the need for in vitro culture of pulpal stem cells [15].

#### **Low-intensity pulsed ultrasound (LIPUS) treatment**

Mesenchymal stem cells (MSCs) from dental tissues may respond to low-intensity pulsed ultrasound (LIPUS) treatment, potentially providing a therapeutic approach to promoting dental tissue regeneration. The mechanism has not yet been fully elucidated but is attributed to its non-thermal biomechanical affects. In particular, through acoustic microstreaming and physical radiation, LIPUS may affect the cell membrane and cytoskeleton to trigger downstream signaling processes. Therefore, the relatively easy and inexpensive process of application may provide an ideal therapy in the dental clinic for the regeneration of dental tissues [16, 17].

#### **Infection and apical papilla**

Apical papilla undergoes liquefaction necrosis in

tandem with the dental pulp because they are interconnected. However, there is growing evidence that the apical papilla and its rich resident stem cell population is capable of surviving prolonged endodontic infection and apical periodontitis and that it represents a rich source of undifferentiated mesenchymal stem cells in REPs [18]. This can probably explain why MSCs such as SCAP can survive during apical periodontitis. The biological reason for this apparent resilient survival may be explained by the relatively low density of blood vessels in the apical papilla in comparison to the adjacent dental pulp.

### **Disinfection vs Regeneration**

Most REPs include minimal-to-no mechanical debridement. Hence, they rely heavily on the chemical debridement and intracanal. The irrigants used should be selected on the basis of their bactericidal/bacteriostatic properties and their ability to promote survival and proliferation of stem cells [19]. Thus, an important concept of regenerative endodontics is to achieve disinfection while creating conditions conducive to stem cell survival, proliferation, and differentiation. It has been demonstrated that 6% NaOCl denatures growth factors embedded in dentin. However, EDTA solubilizes these growth factors from dentin, thereby increasing their bioavailability [20]. Importantly, the growth factors embedded in the dentin matrix such as TGF- $\beta$  and dentin sialoprotein (DSP) are known to be potent stimulators of stem cell proliferation and differentiation.

### **Potential role of NETs (Neutrophil Extracellular Traps)**

Recently, a novel bacterial killing mechanism termed neutrophil extracellular traps (NETs) has been described that uses reactive oxygen species signaling and results in cellular DNA extrusion causing microbial entrapment and death [21]. NETs and their components, such as histones, may provide novel prognostic markers for pulp pathologies. Indeed, the determination of their levels within the infected pulp, could be exploited to target the application of novel

disease management strategies. They have potential role within pulpal infections and how these structures may influence the pulp's vitality and regenerative responses is an area of further research.

### **Cell-homing Approach - Regenerative Endodontics for Adult Patients**

Tremendous effort has been made to revitalize disinfected immature permanent teeth in children and adolescents with diagnoses including pulp necrosis or apical periodontitis. But can the concept of regenerative endodontics be extended to revitalize mature permanent teeth with diagnoses including irreversible pulpitis and/or pulp necrosis in adults is the question.

The concept of cell homing in dental pulp and dentin regeneration was first proposed in 2010 [22]. In tissue regeneration, cell homing includes two separate cellular processes: cell recruitment and differentiation. Recruitment is directional cell migration to tissue injury or defects. Differentiation indicates the process of transformation of stem/progenitor cells into increasingly mature and matrix synthesis cells. In dental pulp and dentin regeneration, stem/progenitor cells differentiate into odontoblasts, pulp fibroblasts, and other niche cells [23]. Cell homing shows a potential of dental pulp regeneration in mature permanent teeth in adults, as shown by He, et al. [24]. Sometimes, the cell homing approach for tissue regeneration is also referred to as the cell-free approach, which indicates that no cells are transplanted. The term "Cell homing" is preferred because resident cells are recruited into endodontically prepared root canals, and, hence, it is not exactly "cell free." Cell homing is a complementary and/or alternative approach to cell transplantation for dental pulp and/or dentin regeneration.

The development of biology-based approaches to regenerate or repair dental pulp is possible today because of recent advances in tissue engineering and biomaterials. However, because of several severe problems afflicted with this approach, it

might not be feasible for a clinical setting in the near future. Galler, et al. [25] suggested a modification of the classical tissue engineering paradigm, where resident cells are attracted by endogenous, dentin derived growth factors that further induce cell proliferation and differentiation and a bioactive scaffold material laden with these growth factors that serves as a template for tissue formation.

Regeneration of dental pulp by cell homing rather than cell delivery may accelerate clinical translation. Cell homing offers a feasible strategy for dental offices, and the described envisioned treatment protocol has the potential to become part of the therapeutic spectrum in endodontics in the near future.

#### **What the future holds? Immediate versus Delayed Induction:**

Currently, there are no clinical trials reporting on immediate induction for regenerative endodontics despite the need of randomized clinical trials in this field. Randomized clinical trials comparing immediate induction versus delayed induction protocols shows clinical success rate of 71% for delayed induction and a 33% success rate for immediate induction [26]. The association between success rate and etiology are possible predictor factors. At the time pulp necrosis is diagnosed, the stage of root development might be a relevant factor for periapical and apical healing. Unfortunately, there is no information of the stage of root development at the time of induction for most studies. Determination of the stage of root formation and etiology are possible critical factors for any therapeutic decision.

#### **Conclusion**

The field of regenerative endodontics is evolving. Present concepts of regeneration of dental tissues can revolutionize the dental health provision. However, there is still considerable debate as to what represents clinical success with such procedures. Primarily the source and potency of pulpal mesenchymal stem cells are of

paramount importance and could be limiting factors in making this technology available for routine endodontic therapies for patients. Regenerative procedures could eventually promote tooth longevity in our aging population. Further clinical studies could make significant progress toward achievement of good patient-based, clinician-based and scientist based outcomes.

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