

Regenerative Endodontics; Pulp Reincarnation

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Abstract : Regenerative endodontics is the creation and delivery of tissues to replace diseased, missing, and traumatized pulp. Current regenerative procedures successfully produce root development but still fail to re-establish real pulp tissue and give unpredictable results. There are several drawbacks that need to be addressed to improve the quality and efficiency of the treatment. This review provides an overview of regenerative endodontics and its goals, and describes possible techniques that will allow regenerative endodontics to become a reality.

Keywords: Regeneration, Stem Cells, Scaffolds, Growth Factors, Tissue Engineering

I. Introduction

From a biologic perspective, the goal of endodontics is to prevent or treat apical periodontitis. An optimal way to accomplish this goal is to either maintain pulpal health in cases of pulpal inflammation or to regenerate healthy pulpal tissue in cases of pulpal necrosis. Thus, there is considerable interest in research and clinical studies aimed towards regeneration of a functional and healthy pulp-dentin complex [1,2]. 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. Current regenerative procedures successfully produce root development but still fail to re-establish real pulp tissue and give unpredictable results.

Regenerative endodontics has been defined as, “biologically based procedures designed to replace damaged structures such as dentin, root structures, and cells of the pulp-dentin complex” [1].

This review provides an overview of regenerative endodontics and its goals, and describes possible techniques that will allow regenerative endodontics to become a reality. Although the challenges of introducing endodontic tissue engineering therapies are substantial, the potential benefits to patients and the profession are equally ground breaking.

II. Historical Development of the Field

Pioneering work supporting the concept of regenerating dental tissues was reported more than 50 years ago when Dr.B.W. Hermann described the application of calcium hydroxide (Ca[OH]₂) for vital pulp therapy[3], and Professor NygaardØstby evaluated a revascularization method for reestablishing a pulp-dentin complex in permanent teeth with pulpal necrosis[4].

The foundation of tooth regeneration was laid when stomatologist G. L. Feldman (1932) proposed that through biological-aseptic principle of tooth therapy, regeneration of pulp might be achieved and used dentine fillings for stimulating pulp regeneration[5]. In 1957, Gavrilov demonstrated regeneration of dentin and cementum of tooth root in dogs[5]. In 2001, Iwaya *et al.* described a procedure termed revascularization that resulted in thickening of the root canal walls and continued root development. In 2004 [6], Banchs and Trope proposed a clinical protocol for revascularization of infected immature teeth[7]. Regenerative dental procedures are emerging as a vital, evolving field of dental care.

III. Major Components Of Regenerative Endodontics

I. Stem Cells

Dental pulp can be viewed as a core of innervated and vascularized loose connective tissue surrounded by a layer of odontoblasts. The major cell type of this core region is the fibroblast. Together with blood vessels, lymphatics, and neurons, this core tissue is embedded in an extracellular matrix consisting of collagen and other fiber types. Pulpal mesenchymal stem cells are thought to be localized in the perivascular region and the cell-rich zone of Hohl adjacent to the odontoblastic layer; both have been proposed to serve as cell sources for replacement odontoblasts[8][9]. Thus, several cell types must be developed in order to form this core of loose connective tissue.

At least five different types of postnatal mesenchymal stem cells have been reported to differentiate into odontoblast-like cells, including dental pulp stem cells (DPSC), stem cells of human exfoliated deciduous teeth (SHED), stem cells of the apical papilla (SCAP), dental follicle progenitor cells (DFPC), and bone marrow-derived mesenchymal stem cells (BMMSC).

II. Growth Factors/Morphogens

Several growth factors have been evaluated for their ability to trigger the differentiation of selected mesenchymal stem cell populations into odontoblast-like cells. Growth factors act as signals to induce cellular proliferation and/or differentiation. Examples of key growth factors in regenerative dentistry include bone morphogenetic protein, transforming growth factor-beta, fibroblastic growth factor, platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF). Growth factors found dentin are also being investigated for their potential applications[10].

The major drawback in growth factors is that a different set of growth factors is required to induce stem cells from different sources to achieve specific differentiation. Along with this safety, quantity and time of delivery of the growth factors pose a significant challenge.

III. Scaffolds

An important component of tissue engineering is a physical scaffold. Tissues are organized as three-dimensional structures, and appropriate scaffolding is necessary to (1) provide a spatially correct position of cell location and (2) regulate differentiation, proliferation, or metabolism. It is known that extracellular matrix molecules control the differentiation of stem cells[11][12], and an appropriate scaffold might selectively bind and localize cells, contain growth factors, and undergo biodegradation over time. Thus, a scaffold is far more than a simple lattice to contain cells.

Scaffolds can be classified as either natural or synthetic. Examples of natural scaffolds include collagen, glycosaminoglycans, demineralized or native dentin matrix, and fibrin. From the perspective of focusing on practical clinical applications, platelet-rich plasma (PRP) appears to satisfy several of these criteria. PRP is autologous, fairly easy to prepare in a dental setting, rich in growth factors, degrades over time, and forms a three-dimensional fibrin matrix. In addition, these properties may play a role in the reported outcomes of classical revascularization methods applied to patients

The second major category of scaffolds is based upon synthetic materials. Examples include polylactic acid (PLA), polyglycolic acid (PGA), polylactic-coglycolic acid (PLGA), polyepsilon caprolactone, hydroxyapatite/tricalcium phosphate, bioceramics.

This is a critical domain of research for the development of regenerative endodontics as a predictable clinical procedure.

IV. An Overview Of Potential Technologies For Regenerative Endodontics

- i.** Root canal revascularization via blood clotting.
- ii.** Postnatal stem cell therapy.
- iii.** Pulp implantation.
- iv.** Scaffold implantation.
- v.** Injectable scaffold delivery.
- vi.** Three-dimensional cell printing.
- vii.** Gene delivery.

I. Root Canal Revascularization via Blood Clotting

Several case reports have documented revascularization of necrotic root canal systems by disinfection followed by establishing bleeding into the canal system via overinstrumentation [6][7][13]. An important aspect of these cases is the use of intracanal irrigants (NaOCl and chlorhexidine) with placement of antibiotics (e.g. a mixture of ciprofloxacin, metronidazole, and minocycline paste) for several weeks. This particular combination of antibiotics effectively disinfects root canal systems[14][15][16] and increases revascularization of avulsed and necrotic teeth, suggesting that this is a critical step in revascularization. The selection of various irrigants and medicaments is worthy of additional research, because these materials may confer several important effects for regeneration in addition to their antimicrobial properties. For example, tetracycline enhances the growth of host cells on dentin, not by an antimicrobial action, but via exposure of embedded collagen fibers or growth factors [17]. However, it is not yet known if minocycline shares this effect and whether these additional properties might contribute to successful revascularization.

There are several advantages to a revascularization approach. First, this approach is technically simple and can be completed using currently available instruments and medicaments without expensive biotechnology. Second, the regeneration of tissue in root canal systems by a patient's own blood cells avoids the possibility of immune rejection and pathogen transmission from replacing the pulp with a tissue engineered construct.

II. Postnatal Stem Cell Therapy

The simplest method to administer cells of appropriate regenerative potential is to inject postnatal stem cells into disinfected root canal systems after the apex is opened. Postnatal stem cells can be derived from multiple tissues, including skin, buccal mucosa, fat, and bone[18]. A major research obstacle is identification of a postnatal stem cell source capable of differentiating into the diverse cell population found in adult pulp (e.g., fibroblasts, endothelial cells, odontoblasts).

III. Pulp Implantation

The majority of in vitro cell cultures grow as a single monolayer attached to the base of culture flasks. However, some stem cells do not survive unless they are grown on top of a layer of feeder cells[19]. In all of these cases, the stem cells are grown in two dimensions. In theory, to take two-dimensional cell cultures and make them three dimensional, the pulp cells can be grown on biodegradable membrane filters. Many filters will be required to be rolled together to form a three dimensional pulp tissue, which can be implanted into disinfected root canal systems.

IV. Scaffold Implantation

To create a more practical endodontic tissue engineering therapy, pulp stem cells must be organized into a three-dimensional structure that can support cell organization and vascularization. This can be accomplished using a porous polymer scaffold seeded with pulp stem cells[20]. A scaffold should contain growth factors to aid stem cell proliferation and differentiation, leading to improved and faster tissue development. Growth factors were described in the previous section. The scaffold may also contain nutrients promoting cell survival and growth, and possibly antibiotics to prevent any bacterial in-growth in the canal systems. In pulp-exposed teeth, dentin chips have been found to stimulate reparative dentin bridge formation. Dentin chips may provide a matrix for pulp stem cell attachment and also be a reservoir of growth factors[21]. The natural reparative activity of pulp stem cells in response to dentin chips provides some support for the use of scaffolds to regenerate the pulp dentin complex.

V. Injectable Scaffold Delivery

Rigid tissue engineered scaffold structures provide excellent support for cells used in bone and other body areas where the engineered tissue is required to provide physical support[22]. However, in root canal systems a tissue engineered pulp is not required to provide structural support of the tooth. This will allow tissue engineered pulp tissue to be administered in a soft three-dimensional scaffold matrix, such as a polymer hydrogel. Hydrogels are injectable scaffolds that can be delivered by syringe. Hydrogels have the potential to be noninvasive and easy to deliver into root canal systems. In theory, the hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure

VI. Three-Dimensional Cell Printing

The final approach for creating replacement pulp tissue may be to create it using a three-dimensional cell printing technique. In theory, an ink-jet-like device is used to dispense layers of cells suspended in a hydrogel to recreate the structure of the tooth pulp tissue. The three-dimensional cell printing technique can be used to precisely position cells [23], and this method has the potential to create tissue constructs that mimic the natural tooth pulp tissue structure.

VII. Gene Therapy

A recent review has discussed the use of gene delivery in regenerative endodontics[20]. One use of gene delivery in endodontics would be to deliver mineralizing genes into pulp tissue to promote tissue mineralization. However, a literature search indicates there has been little or no research in this field, except for the work of Rutherford [24]. He transfected ferret pulps with cDNA-transfected mouse BMP-7 that failed to produce a reparative response, suggesting that further research is needed to optimize the potential of pulp gene therapy.

V. ADA Codes for Pulpal Regeneration Procedures

The 2011-2012 American Dental Association (ADA) Current Dental Terminology recognized pulp regeneration as an endodontic procedure and gave it code (D3354).

1. First Phase of Treatment (D3351):

Consists of debridement and antibacterial medication

2. Interim Phase (D3352):

Consist of interim medication replacement

3. Final Phase (D3354):

Completion of regenerative treatment in an immature permanent tooth with a necrotic pulp. It does not include final restoration.

VI. Conclusion

It is evident that recent rapid advances have opened the door to exciting new opportunities in the quest for healing immature teeth with pulpal necrosis. Extension of these advances to the treatment of mature teeth with pulpal necrosis would provide significant therapeutic benefits.

The future development of regenerative endodontic procedures will require a comprehensive research program directed at each of these components and their application to our patients.

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