Review Article

Regenerative Endodontics: An Overview

C. P. Sreedev, K. Karthick, Sebeena Mathew, Iswarya Raju

Department of Conservative Dentistry and Endodontics, KSR Institute of Dental Science and Research, Tiruchengode, Tamil Nadu, India

Abstract

The advancement of science and technology has huge positive impacts on the present day world. It has contributed immensely to every aspect of our lives, including the medical and dental care. The treatment concepts that were once perceived to be imaginative are today considered achievable. Even though millions of teeth are saved by root canal therapy every year, still, a significant number is rendered unrestorable and doomed to extraction. Thus, an alternative restorative therapy, which may substitute natural teeth, other than root canal therapy is needed. One biological approach to restore tooth structure is a regenerative endodontic procedure, where tissue engineering principles are applied. The field of tissue engineering has literally exploded during the past decade. The first is revascularization, where a new pulp tissue is expected to grow into the root canals from the remaining tissues exist apically in the root canal. The second includes tissue engineering where the replacement of the diseased pulp with a healthy tissue that is able to revitalize the tooth and restore dentin formation process.

Keywords: *Growth factors, revascularization, scaffolds, stem cells, tooth regeneration*

Introduction

The knowledge generated through basic science research, in the fields of stem cell biology, biomaterials (scaffolds), and morphogenetic signalling molecules, coupled with the recent advances in clinical research in the field of medicine, has resulted into an era, where tissue engineering-based therapies are no more a far-fetched dream, but indeed a reality.[1]

In 1996, Hoshino *et al*. reported that complete disinfection in the root canal space can be achieved using a combination of three antibiotics (ciprofloxacin, metronidazole, and minocycline).[2]

One of the first revascularization research efforts was made by Iwaya. An immature tooth with apical periodontitis and sinus tract was treated with irrigation and disinfection, using two antimicrobial agents: metronidazole and ciprofloxacin. At a subsequent appointment, the antibiotic paste was removed, and bleeding was induced into the canal. The canal was sealed with mineral trioxide aggregate (MTA), and after the MTA has set, a bonded restoration was placed. Revascularization procedure allows for an increase in both root wall thickness and the length of the root when compared to traditional apexification or use of apical barriers. Furthermore, the treatment can be completed in fewer visits than traditional apexification.[3]

In 2004, Banchs and Trope published a case report describing a new treatment procedure for the management of the open apex called revascularization. The protocol differs from other apexification techniques, in that, disinfection of the canal was performed with both sodium hypochlorite (NaOCl) and chlorhexidine.[4] These two scientists can be credited for sparking interest in regenerative endodontics.

Nakashima described three essential components of tissue engineering: stem/progenitor cells, morphogenetic signals, and three-dimensional (3D) scaffolds. All these three components play essential roles in the restoration of previously damaged structures. By addressing the

Address for correspondence: Dr. C. P. Sreedev, Department of Conservative Dentistry and Endodontics, KSR Institute of Dental Science and Research, Tiruchengode - 637 215, Tamil Nadu, India.
E-mail: sreedevcp@gmail.com

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three elements of tissue engineering in future clinical guidelines, it is hoped to provide a microenvironment that is conducive to regenerate pulp-like tissue in patients.[5]

Oda *et al*. reported successfully reprograming mesenchymal stromal cells, derived from the pulps of young human third molars by retroviral transduction of the transcription factors Oc3/4, Sox2, and Klf4. The resultant cells had high-induced pluripotent stem cells clonal efficiency, suggesting a potential role for dental pulp stromal cells in regenerative medicine.[6]

Goals and Objectives of Regenerative Endodontics

The main goal of root canal treatment is the prevention or treatment of apical periodontitis. For the treatment of immature permanent teeth, the goal is to restore the original physiologic structures and functions of the pulp-dentin complex. However, treating necrotic immature teeth has always been a clinical challenge for several reasons. It is difficult to achieve an appropriate apical seal with an open apex, using conventional root canal treatment. In addition, the discontinued development of dentinal walls after pulp necrosis can cause thin dentinal walls that make the tooth more prone to fracture.[7]

The primary goal is the elimination of symptoms and the evidence of bony healing. A secondary goal (which is desirable but perhaps not essential) includes increased root wall thickness and/or increased root length. A tertiary goal (which, if achieved, indicates a high level of success) is a positive response to vitality testing. Histologic confirmation of dental pulp with an intact odontoblastic layer and restoration of a functional pulp is the pinnacle of regenerative treatment goals.[5,8]

The interdisciplinary field of tissue engineering requires complex interactions between stem cells/progenitor cells, morphogenetic signaling molecules, and the scaffold or a matrix. Replicating cells of the triad of tissue engineering are the stem cells. The term stem cell was proposed for scientific use by Russian histologist Alexander Maksimov in 1908.[9] Four types of human dental stem cells have been isolated and characterized as follows:

i. Dental pulp stem cells (DPSCs)
ii. Stem cells from human exfoliated deciduous teeth (SHED)
iii. Stem cells from apical papillae (SCAP)
iv. Periodontal ligament stem cells (PDLSCs).

Following sources of stem cells have been recognized in human dental pulp:[4]

I. Permanent teeth: DPSCs
II. Deciduous teeth: SHED
III. SCAP
IV. PDLSCs
V. Dental follicle progenitor cells
VI. Stem cells from supernumerary tooth – mesiodens
VII. Stem cells from teeth extracted for orthodontic purposes
VIII. Dental follicle progenitor cells
IX. Stem cells from human natal dental pulp.

Scaffolds

To provide a suitable environment for cell growth and differentiation, a scaffold should meet the following requirements:

a. Should be porous to allow placement of cells and growth factors
b. Should allow effective transport of nutrients, oxygen, and waste
c. Should be biodegradable, leaving no toxic byproducts
d. Should retain the shape and form of the final tissue structure and replaced by regenerative tissue
e. Should be biocompatible
f. Should have adequate physical and mechanical strength.[10]

The types of scaffold materials available are natural or synthetic, biodegradable, or permanent.[11]

Synthetic materials

- Polylactic acid
- Polyglycolic acid
- Polycaprolactone
- Tricalcium phosphate
- Synthetic hydroxyapatite
- Injectable hydrogels - polyethylene glycol.

Natural materials

- Collagen
- Chitosan (poly-N-acetyl glycosaminoglycans)
- Silk
- Fibrin.

Mineral scaffolds

- Hydroxyapatite
- Calcium phosphate.

Others

- Blood clot
- Platelet-rich plasma
- Platelet-rich fibrin
- Natural nanotoliths
- Nanofibers with the microalga Spirulina
- Bacterial cellulose nanocomposite
- Nanofiber scaffold
- Various fibrin gels.

Growth Factors/Morphogens

Growth factors are biological modulators that are able to promote cell proliferation and differentiation. Growth

factors are extracellular matrix (ECM) molecules which are involved in signaling and regulating dentogenic events during tooth development, and application of these exogenous signaling factors has been recommended for regenerative therapies although a number of challenges in the methods of delivery should be addressed before they are to be used in regular clinical practice.

Some of the naturally occurring and commercially available osteogenically active growth factors are:

- Bone morphogenic/morphogenetic protein (BMP)
- Platelet-derived growth factor
- Insulin-like growth factor
- Fibroblast growth factor
- Transforming growth factor-β
- Dentine sialoprotein
- Dentine phosphoprotein
- Enamel matrix derivative (Emdogain®, Biora Inc., Chaska, MN, USA).[12]

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.[13] Along with this safety, quantity and time of delivery of the growth factors pose a significant challenge. This problem can be overcome by use of the biomimetic ECM-embedded scaffold that can be produced in large quantities and is patient specific without complications of immune response and do not require any exogenous growth factor delivery.[14]

Another drawback is application of higher loading levels of growth factors to compensate their physiologic solubility can result in unwanted side effects and limited spatial control. Microencapsulation or binding of these factors to the scaffold can relieve these problems. Furthermore, microparticles containing growth factors can be used to control the activity of cells.[11]

Regenerative Endodontic Techniques

Basically, body tissue is composed of two components: cells and the surrounding environment. The latter includes the ECM for cell proliferation and differentiation (natural scaffold). Revascularization approach in young permanent infected teeth with immature root apex and apical periodontitis was first attempted in 1971 by Nygaard-Ostby and Hjortdal. However, it was not successful due to limitations in technologies, material, and instruments available in those times. However, with the currently available technologies, several case reports have documented revascularization of necrotic root canal systems by disinfection followed by establishing bleeding into the canal system through overinstrumentation.[15,16]

Following areas come under the category of regenerative endodontic principles:

1. Revascularization through blood clotting
2. Postnatal stem cell therapy
3. Pulp implantation
4. Scaffold implantation
5. Injectable scaffold delivery
6. 3D cell printing
7. Gene therapy.

The revascularization studies have established following prerequisites:

- Revascularization occurs most predictably in teeth with open apices and necrotic pulp secondary to trauma
- Apex open >1.5 mm
- Bacteria should be removed from canal by any of the following methods:
  - Triple antibiotic paste consisting of ciprofloxacin, metronidazole, and minocycline
  - Calcium hydroxide.[6]
- Effective coronal seal
- Matrix into which new tissue can grow
- Patients should be young
- Use of anesthetic without a vasoconstrictor when trying to induce bleeding
- No instrumentation of the canals
- NaOCl is used as an irrigant
- Formation of a blood clot probably serves as a protein scaffold permitting 3D ingrowth of tissue.[4]

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. 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, and odontoblasts). Technical procedures include the development of methods for harvesting and any necessary *ex vivo* methods required to purify and/or expand cell numbers sufficiently for regenerative endodontic applications.[17]

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. Stem cells are grown in two dimensions in all of these cases. In theory, to take two-dimensional cell cultures and make them 3D, the pulp cells can be grown on biodegradable membrane filters. To form a 3D pulp tissue, many filters will be required to be rolled together which can be implanted into disinfected root canal systems. The advantages of

this delivery system are that the cells are relatively easy to grow on filters in the laboratory. The growth of cells on filters has been accomplished for several decades, as this is how the cytotoxicity of many test materials is evaluated.[18]

Dental pulp tissue engineering was first tested by Bohl *et al*. (1998) and reported that culturing pulp cells grown on polyglycolic acid *in vitro* resulted in high cell density tissue similar to the native pulp.

Scaffold implantation

Pulp stem cells must be organized into a 3D structure that can support cell organization and vascularization to create a more practical endodontic tissue engineering therapy. This can be accomplished using a porous polymer scaffold seeded with pulp stem cells. Scaffold must contain certain growth factors for the proliferation and differentiation of stem cell, which might leads to improved and faster tissue development.[19-21]

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. However, in root canal systems, a tissue-engineered pulp is not required to provide structural support of the tooth. Due to this, tissue-engineered pulp tissue to be administered in a soft 3D scaffold matrix such as a polymer hydrogel can be practical. Hydrogels are a type of injectable scaffolds that can be delivered by syringe. Due to their unique compositional and structural similarities to natural ECM, hydrogels have been received a considerable interest as leading material for engineered tissue scaffolds. It also benefits their desirable framework for cellular proliferation and survival. Hydrogel scaffolds have created new opportunities to overcome various challenges in tissue engineering such as vascularization, tissue architecture, and simultaneous seeding of multiple cells because of its ability to control the porosity, surface morphology, shape, and size. Hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure.[22]

Gene Therapy

All human cells contain a 1-m strand of DNA containing 3 billion base pairs, with the sole exception of nonnucleated cells, such as red blood cells. The DNA contains genetic sequences (genes) that control cell activity and function; one of the most well-known genes is p53. New techniques involving viral or nonviral vectors can deliver genes for growth factors, morphogens, transcription factors, and ECM molecules into target cell populations, such as the salivary gland. Viral vectors are modified to avoid the possibility of causing disease but still retain the capacity for infection. Some viruses have been genetically modified to deliver genes, including adenovirus, adeno-associated virus, retroviruses, herpes simplex virus, and lentivirus. Nonviral gene delivery systems include plasmids, peptides, gene guns, DNA-ligand complexes, electroporation, sonoporation, and cationic liposomes. The choice of gene delivery system depends on the accessibility and physiological characteristics of the target cell population.[17]

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 that there has been little or no research in this field, except for the work of Rutherford. 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.[23] The Food and Drug Administration did approve research into gene therapy involving terminally ill humans, but approval was withdrawn in 2003 after a 9-year-old boy receiving gene therapy was found to have developed tumors in different parts of his body.[24] Gene therapy is a relatively new field, and evidence is lacking to demonstrate that this therapy has the potential to rescue necrotic pulp. At this time, the potential benefits and disadvantages are largely theoretical.[25-27]

Three-dimensional Cell Printing

The alternative approach for creating replacement pulp tissue may be to create it using a 3D cell printing technique. This technique helps to precisely position cells to create tissue constructs that mimic the natural tooth pulp tissue structure.[28] Theoretically, an inkjet-like device is used to dispense layers of cells suspended in a hydrogel to recreate the structure of the tooth pulp tissue. The ideal positioning of cells in a tissue engineering construct would include placing odontoblastoid cells around the periphery to maintain and repair dentin, with fibroblasts in the pulp core supporting a network of vascular and nerve cells.[29]

Summary

- Tissue regeneration in postnatal life recapitulates events that have occurred in the normal course of embryonic development and morphogenesis
- Both embryonic development and tissue regeneration are equally regulated through the interaction of selected and highly conserved families of proteins and gene products
- It is now accepted that the dental pulp harbors several niches of multipotential stem cells capable of self-renewal and differentiation

- Techniques to isolate and characterize human pulp stem cells and manipulate their growth under defined *in vitro* conditions have to be established and optimized before cell therapy
- At present, research is exploring the more suitable and advanced formula for a reliable autogenous stem cell source, appropriate signaling molecule(s), and a scaffold that will promote controlled cell growth and differentiation
- Tissue engineering using the triad of dental pulp progenitor/stem cells, morphogens, and scaffolds may provide an innovative and novel biologically based approach for generation of clinical materials and/or treatments for dental disease.

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Conflicts of interest

There are no conflicts of interest.

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