

REGENERATIVE ENDODONTICS

- A New Frontier - A Review



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The goal of endodontics is to prevent or treat apical periodontitis an optimal way to accomplish this is to either maintain pulp health in cases of pulpal inflammation or to regenerate healthy pulp tissue in case of pulpal necrosis. Simple meaning of regeneration lies in the growth of a lost tissue or destroyed part or organs. Regenerative endodontics is also based on the similar factor. It is defined "as biologically based procedures designed to create and deliver tissue to replace diseased, missing and traumatized pulp-dentin complex".

- a. The pulp-dentin regeneration by tissue engineering technology.
- b. The new living tissue is expected to form from the tissue present in the teeth itself, allowing continued root development (revascularization).

The three key elements for tissue engineering are:

- A) Stem cells - to respond to growth factors.
- B) Scaffold of extracellular matrix (ECM).
- C) Growth factors (signals for morphogenesis).

A) Stem cells: It is defined as clonogenic cells capable of both self-renewal and multilineage differentiation since they are thought to be undifferentiated cells with varying degrees of potency and plasticity.¹

They differentiate into one daughter stem cell and one progenitor cell.

There are basically two types of stem cells:

- Embryonic stem cells - located within the inner cell mass of the blastocyst stage of development.
- Post natal stem cells - that have been isolated from various tissues including bone marrow, neural tissue, dental pulp and periodontal ligament. Because of the ethical and legal issues for using an embryonic stem cells, many researchers are now shifted their attention towards postnatal stem cells, donated by the patients themselves or their close relatives.

Stem cells are often categorized by their source:

- a. **Autologous stem cells** - are obtained from the same individual to whom they will be implanted.
- b. **Allogenic stem cells** - originate from a donor of the same species.
- c. **Xenogenic cells** - are those isolated from individuals of another species.

- 1) Autologous stem cells: autologous post natal dental stem cells are promising for Regenerative endodontics as they share

Advantages:

- a. Less chances of immune rejection and pathogen transmission.
- b. Harvesting the patient's own cells makes them the least expensive.
- c. Avoids legal and ethical concerns.

They show more striking odontogenic capability (typical tooth-shaped tissue with balanced amelogenesis and dentinogenesis) as compared to non-dental stem cell population like bone marrow stromal stem cell.

Disadvantages:

- a. Harvesting cells from patients is that surgical operations may lead to postoperative sequelae such as donor site infection.¹⁶
- b. Autologous postnatal stem cells also must be isolated from mixed tissues and possibly expanded in number before they can be used.

This consumes time, so certain autologous regenerative may not be very quick. To accomplish endodontic regeneration, the most promising cells are autologous post natal stem cells.

These appear to have few disadvantages, but that would not prevent them from being used clinically.



2) **Allogenic stem cells:** Cells originate from a donor of the same species however, the most serious disadvantage of immune rejection and pathogen transmission.¹⁵

3) **Xenogenic stem cells:** Are those isolated from individuals of another species. However many problems remain such as the high potential for immune rejection and pathogen transmission from the donor animal to the human recipient. The future use of xenogenic stem cells is uncertain, and largely depends on the success of the other available stem cell therapies.

Autologous stem cells are relatively easy to harvest and easy to inject by syringe (injectable postnatal stem-cell therapy). But in this technique, cells have low survival rate and they might migrate to different locations within the body possibly leading to aberrant patterns of mineralization.²

A solution to the above problem is to apply the cells together with scaffold material-the second component of tissue engineering.

B) Scaffold :

A scaffold provide the framework for cell growth, differentiation and organization at a local site.

- a. A scaffold should be porous to allow for placement of cells and also be biocompatible with host tissue.
- b. It should be biodegradable and should degrade gradually so that it is replaced by regenerative tissue.
- c. It should be effective for transport of nutrients and waste.
- d. Should be biodegradable, leaving no toxic by-products.
- e. Should be biocompatible.
- f. Should have adequate physical and mechanical strength.

Scaffold can be classified as

- i. natural
- ii. synthetic

Natural scaffold: It includes collagen, glycosaminoglycans, demineralized or native dentin matrix³ and fibrin. Considering the clinical applications, platelet rich plasma appears to satisfy criterias such as it is autologous, fairly easy to prepare, rich in growth factors, degrades over time, and forms a three dimensional fibrin matrix.

Synthetic scaffold : It includes polylactic acid, polyglycolic acid, polylactic - coglycolic acid, poly epsilon caprolactone, hydroxyapatite/tricalcium phosphate, Enamel matrix derivatives (Emdogain), porous ceramic, fibrous titanium mesh and hydrogels such as alginate or variants of polyethylene glycol. Synthetic polymers are generally degraded by simple hydrolysis while natural polymers are mainly degraded enzymatically.

C) Growth factors

Growth factors are proteins that bind to specific cell membrane receptors and induce the generation of a new tissue.⁴ Growth factors control the activity of stem cells, e.g. by regulating the rate of proliferation, inducing differentiation into another cell type, or by stimulating cells to synthesize mineralizable matrices.⁵ Such molecules play a key role in the formation and repair of dentin and pulp. Notably, the dentin matrix is a reservoir of growth factors capable of stimulating tissue response after being mobilized.¹⁴ Once released, these molecules play a key role in several signaling events such as the formation of tertiary dentin and repair.⁵

Although the mechanisms underlying the reparative dentin formation have not been completely elucidated. It is known that proteins, such as Bone morphogenetic proteins (BMP), have an important role.⁶ BMP are members of the transforming growth factor (TGF)-beta family. They were originally identified as regulators of cartilage and bone formation and they play an important role in embryogenesis and morphogenesis of various organs and tissues, including teeth.⁷

It has been demonstrated that human recombinant BMP (rhBMP-2, rhBMP-7) induce dentinogenesis.⁶ The response of dental pulp cells to BMPs suggests that the cells present receptors for these bioactive molecules. BMP receptors (BMPR) are serine/threonine kinases that include type I receptors (BMPR-IA, BMPR-IB) and the type II receptor (BMPR-II). It was demonstrated that dental pulp cells (SHED, DPSC, fibroblasts) express BMPR-IA, BMPR-IB and BMP-II receptors.⁸

Gene Therapy

Gene therapy using vectors is recently used as a means of delivering genes for growth factors, morphogens, transcription factors, extracellular matrix molecules locally into the target cell population. The gene can stimulate or induce a natural biological process by expressing molecules involved in regenerative response for the tissue of interest.

Precise delivery and efficient transfer of genes into target tissue cells, prompt assessment of gene expression at required time, appropriate levels and the minimization of undesirable systemic toxicity are essential for successful gene therapy. Both an in-vivo and an ex-vivo approach can be used for gene therapy. In the in-vivo approach the gene is delivered systemically into the bloodstream or locally to target tissues by injection or inhalation¹⁰. The ex-vivo approach involves genetic manipulation of cells in vitro, which are subsequently transplanted to the regeneration site. The cells play a role not only in the repair process but also in secretion of growth factors locally to stimulate host cells.¹¹

The choice of in-vivo or ex-vivo approach depends on morphological and physiological characteristics of target tissue, the vector used, nature of affected disease and the safety of the procedure.¹⁰

Either viral or non-viral vectors are used to enable the cellular uptake and expression of genes.

Viral vectors are genetically altered to eliminate ability of causing disease without losing infectious capacity to the cell. The viruses can replicate genes of interest together with their own genome through the use of the host cell genetic machinery. At present, adenoviral, retroviral, adeno associated virus, herpes simplex virus, lentivirus are being developed.¹⁰

Non-viral delivery systems of plasmids, peptides, cationic liposomes, DNA-ligand complex, gene gun, electroporation, and sonoporation have been developed to address safety concerns such as immunogenicity and insertional mutagenesis¹⁰. Most of the risks of gene therapy may arise from the vector system rather than the gene expressed.¹⁰ The FDA 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. Because of the apparent high risk of health hazards, the development of a gene therapy to accomplish endodontic treatment seems very unlikely in the near future.

Revascularization

Revascularization was first attempted in 1971 in young permanent infected teeth with immature root apex and apical periodontitis.

Revascularization of necrotic root canal systems is done by disinfection followed by establishing bleeding into the canal system by over-instrumentation and use of anaesthetic without a vasoconstrictor when trying to induce bleeding.¹²

The revascularization studies have established following conditions:

Revascularization occurs most predictably in teeth with open apices(> 1.5 mm) and necrotic pulp secondary to trauma. Sodium hypochlorite is used as an irrigant. Bacteria should be removed from canal by using '3 mix-MP' triple antibiotic paste (ciprofloxacin, metronidazole and minocycline, Calcium hydroxide, formocresol) there should be an effective coronal seal and matrix into which new tissue can grow.

Formation of a blood clot probably serves as a protein scaffold permitting 3-dimensional ingrowth of tissue.

Studies have shown the continued thickening of the dentinal walls and subsequent apical closure. The root length is increased by the growth of cementum. Connective tissue similar to periodontal ligament was also present in the canal space.¹³








The following represents an initial framework to identify major research priorities in developing regenerative endodontic techniques.

Conclusion:

Several developmental issues have been described to accomplish endodontic regeneration. Each one of the regenerative techniques has advantages and disadvantages, and some of the techniques are hypothetical, or at an early stage of development. The proposed therapies involving stem cells, growth factors, and tissue engineering all require pulp re-vascularization, in itself an enormous challenge.

Importantly, these advances will likely be primarily focused on the biological regeneration of vital tissue. The next decade will certainly be an exciting time for dental and craniofacial research.

TABLE 3. Developmental approaches for regenerative endodontic techniques

Technique	Image	Advantages	Disadvantages
Root-canal revascularization: open up tooth apex to 1 mm to allow bleeding into root canals		<ul style="list-style-type: none"> ✓ Lowest risk of immune rejection ✓ Lowest risk of pathogen transmission 	<ul style="list-style-type: none"> ➤ Minimal case reports published to date ➤ Potential risk of necrosis if tissue becomes reinfected
Stem cell therapy: autologous or allogenic stem or cells are delivered to teeth via injectable matrix		<ul style="list-style-type: none"> ✓ Quick ✓ Easy delivery ✓ Least painful ✓ Cells are easy to harvest 	<ul style="list-style-type: none"> ➤ Low cell survival ➤ Cells do not produce new functioning pulp ➤ High risk of complications
Pulp implant: pulp tissue is grown in the laboratory in sheets and implanted surgically		<ul style="list-style-type: none"> ✓ Sheets of cells are easy to grow ✓ More stable than an injection of dissociated cells 	<ul style="list-style-type: none"> ➤ Sheets lack vascularity so only small constructs are possible ➤ Must be engineered to fit root canal precisely
Scaffold implant: pulp cells are seeded onto a 3-D scaffold made of polymers and surgically implanted		<ul style="list-style-type: none"> ✓ Structure supports cell organization ✓ Some materials may promote vascularization 	<ul style="list-style-type: none"> ➤ Low cell survival after implantation ➤ Must be engineered to fit root canal precisely
3-D cell printing: ink-jet-like device dispenses layers of cells in a hydrogel which is surgically implanted		<ul style="list-style-type: none"> ✓ Multiple cell types can be precisely positioned 	<ul style="list-style-type: none"> ➤ Must be engineered to fit root canal precisely ➤ Early-stage research has yet to prove functional in vivo
Injectable scaffolds: polymerizable hydrogels, alone or containing cell suspension are delivered by injection		<ul style="list-style-type: none"> ✓ Easy delivery ✓ May promote regeneration by providing substitute for extracellular matrix 	<ul style="list-style-type: none"> ➤ Limited control over tissue formation ➤ Low cell survival ➤ Early-stage research has yet to prove functional in vivo
Gene therapy: mineralizing genes are transfected into the vital pulp cells of necrotic and symptomatic teeth		<ul style="list-style-type: none"> ✓ May avoid cleaning and shaping root canals ✓ May avoid the need to implant stem cells 	<ul style="list-style-type: none"> ➤ Most cells in a necrotic tooth are already dead ➤ Difficult to control ➤ Risk of health hazards ➤ Not approved by the FDA

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