

Tissue Engineering : Is it the future of Endodontics?

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Abstract:

Pulpal regeneration after tooth injury is not easy to accomplish, because of the infected pulp requires tooth extraction or root canal therapy. Current treatment modalities offer high levels of success for many conditions; an ideal form of therapy might consist of regenerative approaches in which diseased or necrotic pulp tissues are removed and replaced with healthy pulp tissue to revitalize teeth.

This review describes the possible techniques that will allow regenerative endodontics to become a reality. These potential approaches include root canal revascularization, postnatal stem cell therapy, pulp implant, scaffolding implant, three dimensional cell printing, injectable scaffolds and gene therapy.

Key Words: Tissue engineering, Regenerative endodontics, Pulp revascularization, Dental pulp stem cells, Morphogens, Scaffolds.

Introduction:

There is a high rate of success in retention of teeth by endodontic therapy. However, many teeth are not restorable because of apical resorption, fracture, incompletely formed roots or carious destruction of coronal structures. A novel approach to restore tooth structure is based on biology ie, regenerative endodontic procedures by the application of tissue engineering.

Tissue engineering is an emerging multi disciplinary field that applies the principles of engineering and life sciences for the development of biological substitutes that can restore, maintain, or improve tissue function (Langer & Vacnati, 1993).

Regenerative endodontic procedures can be defined as biologically based procedures, designed to predictably replace damaged, diseased, or missing structures, including dentin and root structures as well as cells of the pulp dentin complex with live viable tissues preferably of the same origin that restore the normal physiologic functions of the pulp dentin complex (Murray et al, 2007).

Hermann (1952) was the first to carry out regenerative endodontic procedure, when he applied calcium hydroxide in vital pulp amputation. Subsequent regenerative dental procedures included guided tissue or guided bone regeneration (GTR, GBR) procedures and distraction osteogenesis (Block et al, 1995), the application of platelet rich plasma (PRP) for bone augmentation (Kassolis et al, 2000), emdogain for

periodontal tissue regeneration (Heijl et al, 1997), recombinant human bone morphogenic protein (rhBMP) for bone augmentation (Fjuimura et al, 1995), and preclinical trails on the use of fibroblast growth factor 2 (FGF2) for periodontal tissue regeneration (Takayama et al, 2001; Lin et al, 2010).

A counter argument to the development of regenerative endodontic procedure is that although the replaced pulp has potential to revitalize the teeth, it may also become susceptible to further pulp disease and may require retreatment.

Regenerative endodontics comprises of research in:

1. Adult stem cells
2. Growth factors
3. Organ tissue culture
4. Tissue engineering materials

Adult stem cells:

All tissues originate from stem cells. A stem cell is defined as a cell that has the ability to continuously divide to either replicate itself (self replication) or produce specialized cells that can differentiate into various other types of cells or tissues (Rao, 2000).

Types of stem cells are:

1. Early embryonic stem cells
2. Blastocyst embryonic stem cells
3. Fetal stem cells
4. Umbilical cord stem cells
5. Adult or postnatal stem cells

The plasticity of stem cell defines its ability to produce cells of different tissues. Stem cells are commonly subdivided into totipotent, pluripotent and multipotent categories according to their plasticity.

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To accomplish endodontic regeneration, the most promising cells are autologous postnatal stem cells, because these appear to have the minimum disadvantages. Postnatal stem cells have been found in almost all body tissues, including dental tissues. Four types of human dental stem cells have been isolated:

- (a). Dental pulp stem cells (DPSCs; Gronthos et al, 2000)
- (b). Stem cells from Human exfoliated deciduous teeth (SCHED; Miura et al, 2003)
- (c). Stem cells from apical papillae (SCAP; Sonoyama et al, 2006)
- (d). Periodontal ligament stem cells (PDLSCs; Seo et al, 2004)

Odontoblasts are postmitotic terminally differentiated cells which cannot proliferate to replace subjacent irreversibly injured odontoblasts. The ability of both young and old teeth to respond to injury by induction of reparative dentinogenesis suggests that a small population of competent progenitor pulp stem cells may exist within the dental pulp throughout life. Information on the mechanism by which these cells are able to detect and respond to tooth injury is a scarce, but this information will be valuable for use in developing tissue engineering and regenerative endodontic therapies.

Stem cell lines are usually grown in medium containing animal products. Fetal bovine serum is an important additive for cell growth, however, the allergenic potential and the possibility of contamination while using a medium containing serum would be a barrier to transplantation and consequently to the introduction of cell therapy methods into clinical applications.

Studies have shown that serum free growth medium consisting of Dulbecco modified Eagle medium with antibiotic and antimycotics which was supplemented with 1 % Insulin-Transferring-Selenium-X and 100 µg/ ml of embryotropic factor showed an acceptable survival rate, the highest proliferation rate and the strongest expression of all the stem cell markers. It also proved to be a suitable medium for the culture of human dental pulp stem cells and to preserve pluripotent in differentiation (Hirata et al, 2010).

The regeneration of dental tissue relies on the ability of stem cells to produce extracellular matrix proteins encountered in the dental pulp tissue.

The distribution and expression of extracellular matrix proteins differ among the DPSCs. These differences seem to be related to the donor tooth

condition like deciduous or permanent, retained or erupted and degree of root resorption (Harumi Miyagi et al, 2010).

However, it is not yet clear which type of stem cell source are most potent and best for targeted therapy. Lack of understanding of nature of these cells and their lineage specific propensity might hinder their full potential. Studies have demonstrated that gene variations occur within the different sources of the same cells and these variations determine their lineage propensity towards specific destination. Stem Cells of Deciduous teeth retained their plasticity over the passages, whereas Permanent Stem Cells lost their plasticity and were shown to be more committed towards neuronal lineage (Govindasamy et al, 2010). It seems evident that the dental pulp might be used only as a source of progenitor cells with dentinogenic competence for the regeneration of dentin pulp complex.

The differentiation potential of apical papilla progenitor cells has not been established yet. The nature of all embryonic dental papilla, mature dental pulp and apical papilla progenitor cell populations remain to be characterized further (Tziafas & Kodonas, 2010).

Recent evidence suggests that stem cells are localized in areas with low oxygen tension. Work on hemopoietic and neural stem cells showed that culturing progenitors in hypoxic conditions increases the number of multipotent clones when compared with normoxic cultures. In addition to effects on differentiation and cell fate, hypoxia promotes survival and increases the proliferation of multipotent precursors. This phenomenon may depict clinical situations in which pulp tissues are affected by noxious stimuli such as mechanical pulp exposure or trauma that leads to localized ischemia. The secondary dentin bridge that formed under the injury site is possibly the product of differentiated progenitors from deciduous pulp stem cell reservoir. Further studies are required to understand whether DPSCs react differently to signaling molecules after hypoxic treatment, which might alter their differentiation potential (Sakdee et al, 2009).

Growth factors:

Growth factors are proteins that bind to receptors on the cell and induce cellular proliferation or differentiations. Many growth factors are quite versatile, stimulating cellular division in numerous cell types, while others are more cell specific. Bone morphogenic proteins (BMPs) are important growth

factors required in tooth development and regeneration. Recombinant BMP-2,-4,-7 induce formation of reparative dentin *in vivo* (Nakashima, 1994).

The application of recombinant human insulin like growth factor-1 together with collagen has been found to induce complete dentin bridging and tubular dentin formation (Lovschall et al, 2001). This indicates the potential of adding growth factors before pulp capping or incorporating them into restorative and endodontic materials to stimulate dentin and pulp regeneration. The therapeutic effect of calcium hydroxide may be because of its extraction of growth factors from dentin matrix (Smith et al, 1995). Once released, these growth factors may play key roles in signaling many of the events of tertiary dentinogenesis, a response of pulp dentin repair.

Data suggest that FGF2 plays a role not only as a differentiation inducing factor in the injury repair process of pulpal tissue but also as a positive regulator of chemokine expression, which may help in tissue engineering and pulp regeneration using Human DPSCs. However, the fate of odontoblastic or osteoblastic differentiation, effective local delivery for FGF2 interaction of chemotactic and odontogenic factor limitations need to be overcome (Kim et al, 2010).

Ability of MTA to induce useful cellular response to achieve suitable tissue wound healing is by promoting by adhesion, supporting cellular proliferation and by inducing migration of human mesenchymal stem cells.

Mesenchymal stem cells are usually involved in tissue and bone remodeling, and local environment is thought to play an important role in the commitment and differentiation of mesenchymal derived stem cells (D'Anto et al, 2010).

Scaffold:

The scaffold provides a physico-chemical and biological three dimensional micro environment for cell growth and differentiation, promoting cell adhesion and migration. The scaffold serves as a carrier for morphogen in protein therapy and for cells in cell therapy.

Types of scaffolds:

- (a). Biological or Natural eg. Collagen, Glycosaminoglycan
- (b). Artificial or Synthetic eg. Poly lactic acid (PLA) Poly glycolic acid (PGA), Poly ethylene glycol (PEG), Arginine, Hydroxyapatite, Tricalcium Phosphate

Gene therapy:

New techniques involving viral or non viral vectors that can deliver genes for growth factors, morphogens, transcription factors and extracellular matrix molecules into target cell populations has been developed.

The use of gene delivery in endodontics would be to deliver mineralizing genes into pulp tissues to promote tissue mineralization. Dr. Rutherford 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 (Rutherford, 2001).

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.

Potential technologies for regenerative endodontics:

Following are the areas of research that might have application in the development of regenerative endodontic techniques:

1. Root canal revascularization via blood clotting
2. Postnatal stem cell therapy
3. Pulp implantation
4. Scaffold implantation
5. Injectable scaffold delivery
6. Three – Dimensional cell printing
7. Gene therapy

A study has found that inducing bleeding of pulp was easier and effective when an anesthetic solution did not contain a vaso constrictor (Petrino et al, 2010).

Research priorities for developing regenerative endodontic techniques:

(a). *Improved methods to disinfect and shape root canal systems:-*

The majority of the available evidence suggests that necrotic and infected tooth pulp does not heal. It will be necessary to disinfect the root canal systems and remove infected hard and soft tissues before using regenerative endodontic treatments.

To successfully attach and adhere to root canal dentin, the stem cells must be supported within a polymer or hydrogel scaffold. Furthermore, it has been observed that pulp stem cells, periodontal stem cells, and fibroblasts neither adhere nor grow in infected root canal system. Chlorhexidine gluconate has been studied for its various properties like antimicrobial activity and

biocompatibility, with the objective of evaluating it as an alternative to sodium hypochlorite (Trojani et al, 2005).

(b). Smear layer removal:-

The presence of a smear layer on root canal walls may inhibit the adherence of implanted pulp stem cells, potentially causing the regenerative endodontic treatment to fail. Improved methods to remove the

smear layer from the root canal walls appear to be necessary to help promote the success of regenerative endodontics.

Recently MTAD, which is an aqueous solution of 3% doxycycline, 4.25% citric acid and 0.5% polysorbate detergent, has been studied for its biocompatibility, broad spectrum antibacterial activity and effective removal of endodontic smear layer.

Table I: Developmental approaches for regenerative endodontic techniques

TECHNIQUE	ADVANTAGES	DISADVANTAGES
Stem Cell Therapy (Rao, 2000) Autologous or allogenic stem 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
Growth Factors (Kim et al, 2010) FGF2, BMPs	<ul style="list-style-type: none"> • Helps in differentiation • Modulates repair process in pulp tissues 	<ul style="list-style-type: none"> • Interaction of chemotactic and odontogenic factor limitations need to be over come
Pulp Implan (Helminger et al, 1997) 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 (Nakashima, 2005) 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 (Baron et al, 2005) Inkjet like device dispenses layers of cells in a hydorgel 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 <i>in- vivo</i>
Injectable scaffolds (Trojani et al, 2005) 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
Bioactive materials (D'Anto et al, 2010) Mineral trioxide aggregate	<ul style="list-style-type: none"> • Promotes cellular adhesion • Supports cellular proliferation • Induces migration of human mesenchymal cells 	<ul style="list-style-type: none"> • Reaction with other local factors are unknown
Gene therapy (Rutherford, 2001) 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
Coenzymes (Okamoto et al, 2009) 3-hydroxy-3-methyl glutaryl coenzyme A	<ul style="list-style-type: none"> • Known to induce anigogenesis • Increases neurogenesis • Anti inflammatory effect 	<ul style="list-style-type: none"> • Cell death observed with high concentration
Root canal Revascularization (Banchus, & Trope, 2004) open up tooth apex to 1mm 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"> • Potential risk of necrosis if tissue becomes re infected

However, its interaction with regenerating pulpal tissue is unknown.

(c). Delivery of regenerative endodontic procedures:-

The cells may then be seeded in the apical 1 to 3 mm of a tissue engineered scaffold with the remaining coronal 15+ mm containing an acellular scaffold that supports cell growth and vascularization. This tissue construct may involve an injectable slurry of {hydrogel+ cells + X (growth factors); Torbinejad et al, 2003}.

Studies have shown that clinically compromised dental pulp might contain putative cells with certain stem cell properties. Further characterization of these cells will provide insight regarding whether they could serve as a source of endogenous multipotent cells in tissue regeneration based dental pulp therapy (Wang et al, 2010).

Challenges and future direction:

Despite the impressive progress in tissue engineering approaches to regenerative pulp therapy, numerous challenges remain. The associated broad spectrum of responses in pulp includes neural and vascular regeneration.

(a) Nerve regeneration:

Dental pulp is richly innervated. The main nerve supply enters the pulp through apical foramen along with the vascular elements. They include both sensory and sympathetic nerves. Pulpal nerves play a key role in regulation of blood flow, dentinal fluid flow, and pressure. The innervation of the pulp has a critical role in the homeostasis of the dental pulp. The pulpal nerve fibers contribute to angiogenesis, extravasation of immune cells and regulate inflammation to minimize initial damage, maintain pulp tissue, and strengthen pulpal defense mechanisms. The increasing interest in tissue engineering of tooth must take into account neuro-pulpal interactions and nerve regeneration (Nosrat et al, 2004).

(b) Vascular regeneration:

Pulp vasculature plays an important role in regulating inflammation and subsequent repair and regeneration of dentin. There is an intimate association of the neural elements with vascular supply of the dental pulp suggesting the interplay of neural and vascular elements and involvement in pulp homeostasis. The vascular endothelial growth factor (VEGF) is an excellent regulator of angiogenesis and is known to

increase vascular permeability. VEGF induces chemotaxis, proliferation and differentiation of human dental pulp cells. The utility of gene therapy in stimulation of vascular growth permits local stimulation of vascularization during regeneration (Matsushita et al, 2000).

The recent advances in vascular biology and VEGF and techniques of gene transfer and gene therapy will be of potential clinical utility in dentistry, specially in endodontics.

Statin, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, is known to promote bone formation. Pulp tissue contains a large amount of blood vessels and peripheral nerves. Statin is known to induce angiogenesis and to regulate the survival and increase neurogenesis of neuronal cells, indicating the possible effectiveness of statin in pulp regeneration along with dentin regeneration. Furthermore, statin has an anti-inflammatory effect in various tissues. This could help to restore the inflamed pulp tissue. Taken together, results suggest that statin might be an ideal active ingredient in pulp capping material to accelerate reparative dentin formation. However, at the same time attention has to be paid to the cell death observed in the cells treated with high concentration of statin. Therefore, a careful evaluation of the suitable concentration is required before its use in pulp regeneration (Okamoto et al, 2009).

(c) To measure appropriate clinical outcomes we have to find out the following:

- Vascular blood flow
- Mineralizing odontoblastoid cells
- Intact afferent innervations
- Lack of signs or symptoms

Conclusion:

Tissue engineering using the triad of dental pulp stem cells, morphogens and scaffolds may provide an innovative and biologically based approach for generation of clinical materials and treatment of dental diseases.

The challenges of introducing endodontic tissue engineered therapies are substantial; the potential benefits to patients and the profession are groundbreaking.

Better understanding of cell interactions and growth along with further research can make endodontic tissue engineering a reality in the near future.

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