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RESEARCH ARTICLE

REGENERATIVE ENDODONTICS: A REVIWE

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ABSTRACT

Scientific advances in the creation of restorative biomaterials, *in vitro* cell culture technology, tissue grafting, tissue engineering, molecular biology and the human genome project provide the basis for the introduction of new technologies into dentistry. Traditionally, apexification has been used to treat immature permanent teeth that have lost pulp vitality. This technique promotes the formation of an apical barrier to close the open apex so that the filling materials can be confined to the root canal. Because tissue regeneration cannot be achieved with apexification, a new technique called regenerative endodontic treatment was presented recently to treat immature permanent teeth. Regenerative endodontic treatment is a treatment procedure designed to replace damaged pulp tissue with viable tissue which restores the normal function of the pulp-dentin structure. After regenerative endodontic treatment, continued root development and hard tissue deposition on the dentinal wall can occur under ideal circumstances. Restoration of vitality of non-vital tooth is based on tissue engineering and revascularization procedures. The purpose of this article is to review these biological procedures and the hurdles that must be overcome to develop regenerative endodontic procedures.

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INTRODUCTION

Regenerative endodontic procedures can be defined as biologically based procedures designed to create and deliver tissues to replace diseased, missing and traumatized pulp-dentin complex. Presently, two concepts exist in regenerative endodontics to treat non-vital infected teeth –

Tissue engineering technology

(active pursuit of pulp-dentine regeneration to implant or regrow pulp), and

Revascularization

(new living tissue is expected to form from the tissue present in the teeth itself, allowing continued root development).

Tissue engineering technology

Tissue engineering can be defined as 'an interdisciplinary field that applies the principles ofengineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function.' (2)

The three key components for tissue engineering are:

Stem Cells

They are 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. (3) They differentiate into one daughter stem cell and one progenitor cell.

There are basically two types of stem cells: (4)

- **Embryonic stem cells-** located within the inner cell mass of the blastocyst stage of development.
- **Postnatal stem cells-** that have been isolated from various tissues including bone marrow, neural tissue, dental pulp and periodontal ligament.

Since the sourcing of embryonic stem cells is controversial and is surrounded by ethical and legal issues, many researchers are now focussing attention on developing stem cell therapy using postnatal stem cells donated by the patients themselves or their close relatives.

Stem cells are often categorized by their source

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

For endodontic regeneration, the most promising cells are autologous postnatal dental stem cells because there are less chances of immune rejection (2).

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

They show more striking odontogeniccapability (typical tooth-shaped tissue with balanced amelogenesisand dentinogenesis) as compared to non-dental stem cell population like bone marrow stromal stem cell. (5),(6) Various sources for postnatal dental stem cells have been successfully studied:

Permanent teeth - Dental pulp stem cells (DPSC): derived from third molar. (2)

Deciduous teeth - Stem cells from human-exfoliated deciduous teeth (SHED): stem cells are present within the pulp tissue of deciduous teeth. (7)

Periodontal ligament - Periodontal ligament stem cells (PDLSC). (8)

Stem Cells from apical papilla (SCAP).(9)
Stem cells from supernumerary tooth - Mesiodens. (10)
Stem cells from teeth extracted for orthodontic purposes.(11)
Dental follicle progenitor cells.(12)
Stem cells from human natal dental pulp- (hNDP). (13)

Scaffold

A scaffold used for regeneration should provide the framework for cell growth, differentiation and organization at a local site. A scaffold should be porous to allow for placement of cells and also be biocompatible with host tissue. (42) It should be biodegradable and should degrade gradually so that it is replaced by regenerative tissue. (43) It should be effective for transport of nutrients and waste. (44) Most tissue engineering efforts use biomaterials for scaffolds already approved by the FDA. They can be natural (collagen, dentin, fibrin, silk, alginate) or synthetic (various polymers like PLA, PGA, etc.). Synthetic polymers are generally degraded by simple hydrolysis while natural polymers are mainly degraded enzymatically.

Collagen is the most widely studied natural scaffold. The most widely used synthetic scaffolds are polymers of lactide and glycolide. In regenerative endodontics, a tissue-engineered pulp is not required to provide structural support to the tooth. So, engineered pulp tissue can be administered in a soft three-dimensional scaffold matrix, such as polymer hydrogel, (59) which can be injected at the site (injectable scaffold delivery). Hydrogels have similar physical properties as that of living tissue, which is due to their high water content, soft and rubbery consistency and low interfacial tension with water or biological fluids. Research is focusing on making hydrogels photopolymerizable (60) or self-hardening e.g., silanized hydroxyl-propyl-methyl cellulose, (61) so that they form rigid structures once they are implanted into the tissue sites. Another injectable scaffold studied is ?-tricalcium phosphate.β-tricalcium phosphate.(62) It is alginate in gel phase and forms beads in solid phase. Treated dentin matrix also provides suitable environment for regeneration of dental tissue. (63) Silk scaffolds may be used for mineralized osteo-dentin formation. The size and shape of silk scaffold pores guide mineralized tissue. (54) Enamel matrix derivatives (Emdogain), whose major component is amelogenins, have also been used as potential scaffolds. (58). The seeding of cells on tissue engineering scaffolds is known as 'creating a tissue construct'. To promote the formation of higher order tissue structures, tissue constructs are maintained in cell culture in the presence of bioactive molecules called growth factors-the third component of tissue engineering.

Growth Factors

Growth factors are proteins that bind to receptors on the cell and induce cellular proliferation and/or differentiation. (64) Many growth factors are quite versatile, stimulating cellular division in numerous cell types, while others are more cell specific. (65) Growth factors play a role in signalling many events in pulp-dentine regeneration. Two important families of growth factor that play a vital role are transforming growth factor (TGF) and bone morphogenetic protein (BMP). TGF-β1 and β3 are important in cellular signalling for odontoblast differentiation and stimulation of dentin matrix secretion. These growth factors are secreted by odontoblasts and are deposited within the dentin matrix, where they remain protected in an active form through interaction with other components of the dentin matrix. (66) The addition of purified dentin protein fractions stimulates an increase in tertiary dentin matrix secretion suggesting that TGF-β1 is involved in injury signalling and tooth-healing reaction. BMPs induce higher quantity and more homogeneous reparatory dentin with the presence of many tubes with defined odontoblastic process as compared to that with calcium hydroxide. BMP-2, BMP-4 and BMP-7 have been shown to direct stem cell differentiation into odontoblasts and result in dentin formation making the BMP family the most likely candidate as growth factors. Some natural materials like dentin are also used because they release bio-active molecules. Enamel matrix derivative is also capable of inducing dentin formation when applied to dentin pulp complex. Poor angiogenesis is a major roadblock for tissue regeneration. Following approaches are currently being studied for the development of vasculature to support the metabolic needs of engineered tissue:

Transplanted endothelial cells can increase the vasculature in polymer scaffolds and integrate with growing host capillaries. (88). Localized delivery of inductive angiogenic factors (VEGF, PDGF, EGF) at the site of the engineered tissue. (89) Co-transplantation of hematopoietic and mesenchymal stem cells.(90) Although we are aware of the role played by these growth factors, for tissue engineering to be successful it is critical to deliver appropriate growth factors to the desired site at the appropriate dose and for appropriate time for which further research is required. Many of these proteins have short half life in the body, yet they need to be present for an extended period to be effective. For this, an alternate approach is to deliver a gene that encodes for the growth instead of delivering factor itself, called gene therapy.

Revascularization

Regeneration of tissue from cells in teeth itself.

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, (105) but it was not successful due to limitations in technologies, material and instruments available in those times. But with the currently available technologies, several case reports (106),(107) have documented revascularization of necrotic root canal systems by disinfection

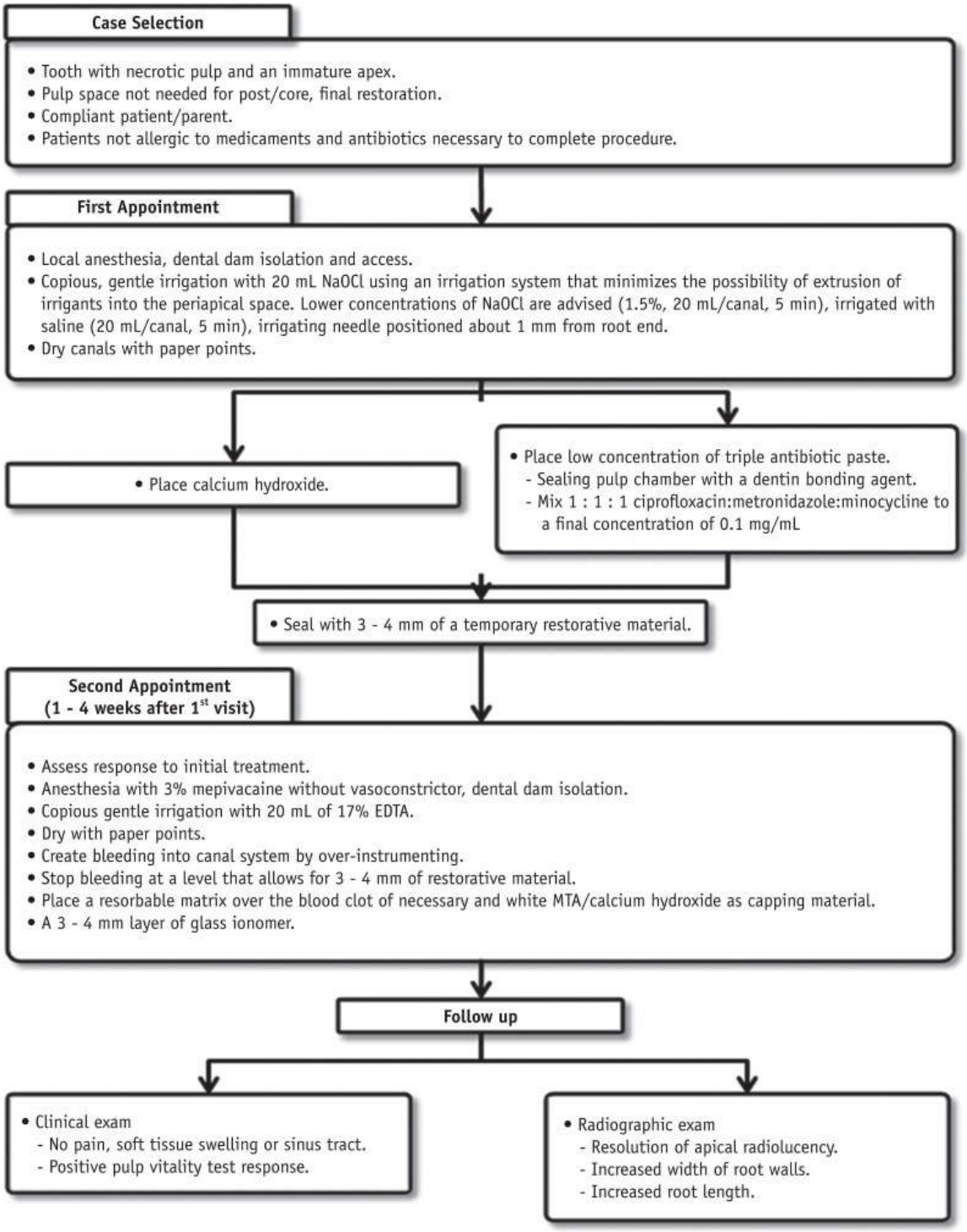
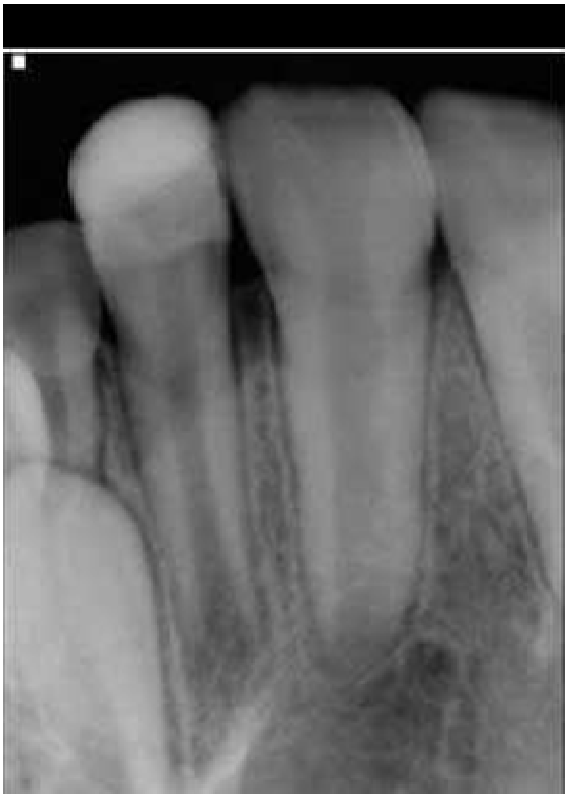
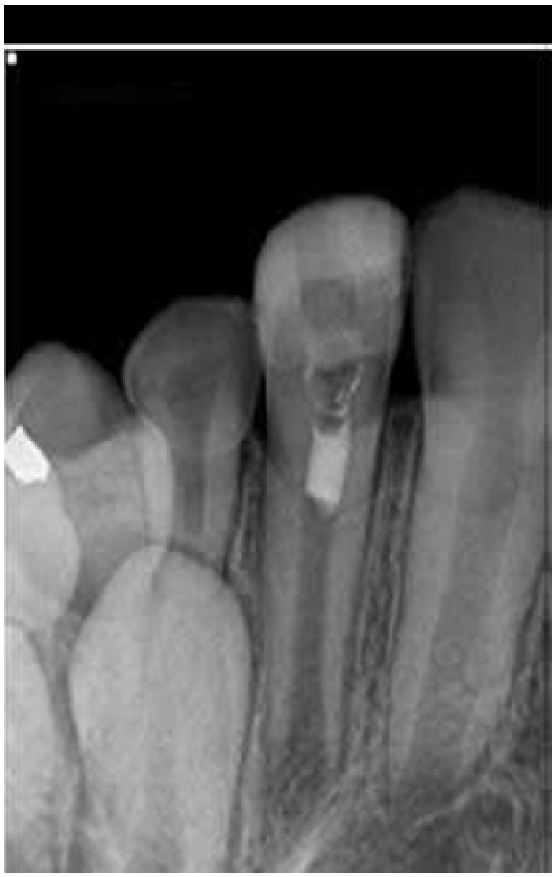


Figure. Procedure for regenerative treatment in tooth

followed by establishing bleeding into the canal system via over-instrumentation. The revascularization method assumes that the root canalspace has been disinfected and that the formation of blood clot yields a matrix (e.g., fibrin) that traps cells capable of initiating new tissue formation. It is different from apexification because not only the apex is closed but the canal walls are thicker as well. It is also different from apexogenesis which also accomplishes a closed apex and thicker dentinal walls, but, by the use of remaining vital root pulp. The revascularizationstudies have established following prerequisites: The success of root canal revascularization is mainly due to the following facts: firstly, the immatureavulsed tooth has an open apex, short root and intact but necrotic pulp tissue. Therefore, the new tissue has easy access to the root canal system and a relatively short distance for proliferation to reach the coronal pulp horn. The speed with which the tissue completely revascularizesthe pulp space is important because bacteria from outside are continually attempting to enter the pulp space. The ischemically necrotic pulp acts as a scaffold into which the new tissue grows, and the fact that the crown is usually intact slows bacterial penetration because their only access to the pulp is through cracks or enamel defects. Thus, the race between proliferation of new tissue and infection of the pulp space favors the new tissue. Secondly, minimum instrumentation preserves viable pulp tissue which contributes to further development of open apex root.

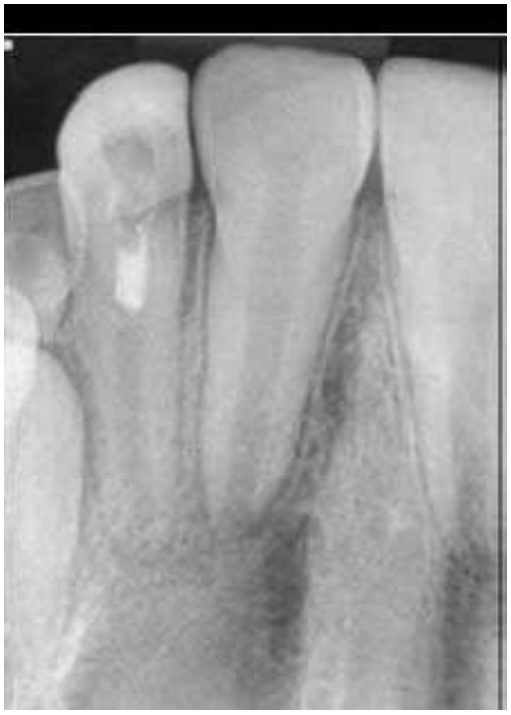


Tooth No. 7 showing incompletely formed apices and apical periodontitis. The patient also presented with pain to percussion and swelling.

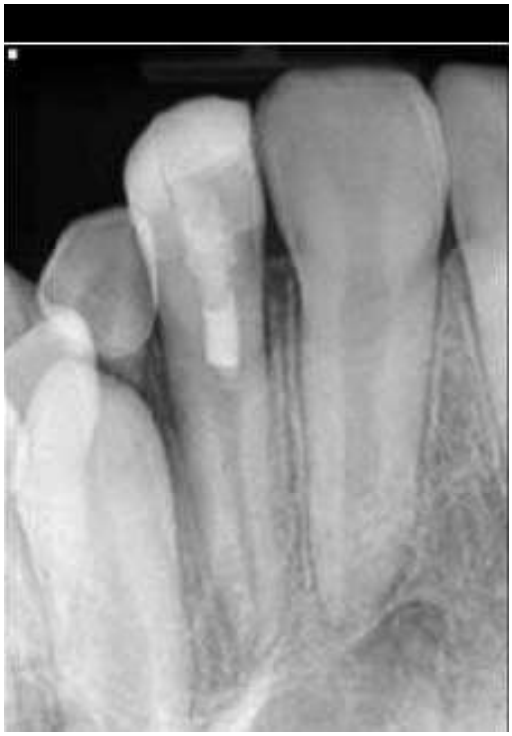


Tooth No. 7 after second visit showing placement of the MTA barrier.

Thirdly, young patients have greater healing capacity and more stem cell regenerative potential.



Eight-month recall. Note the resolution of the apical periodontitis and continued development of the root walls and apical closure



15-month recall. Note the continued apical development and intact and uniform PDL and LD. Also note the hard-tissue barrier apical to the MTA

Advantages of root canal revascularization

The greatest benefit of these biological approaches for dental tissue restoration over many conventional dental materials is that the reparative matrices become an integral part of the tooth, overcoming any of the problems of retention of a restoration and possible marginal bacterial microleakage. This treatment approach strengthens the root walls of immature teeth.

Conclusion

Future regenerative endodontics may involve the cleaning and shaping of root canals followed by the implantation of vital dental pulp tissue constructs created in laboratory. The success of regenerative endodontic therapy is dependent on the ability of researchers to create a techniquethat will allow clinicians to create a functional pulp tissue within cleaned and shaped root canal systems. The source of pulp tissue may be from root canal revascularization, stem-cell therapy and pulp implantation.

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