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Current Protocols in Regenerative Endodontics: A Review

[1]Dr. Nihal R Kothari, Post Graduate, Dept of Paediatric and Preventive dentistry, Government Dental College and Research Institute, Bengaluru 560002

[2]Dr. N S Sahana, MDS, PhD, Professor and Head, Dept of Oral and Maxillofacial pathology, Government Dental College and Research Institute, Bengaluru 560002

[3]Dr. S K Srinath, MDS, PhD, Professor and Head, Dept of Paediatric and Preventive dentistry, Government Dental College and Research Institute, Bengaluru 560002

Corresponding Author: Dr. Nihal R Kothari, Post Graduate, Dept of Paediatric and Preventive dentistry, Government Dental College and Research Institute, Bengaluru 560002

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Abstract

Due to short comings of the traditional apexification procedures, Regenerative endodontics uses principles of tissue engineering and is a promising approach in immature permanent teeth with necrotic pulps. Over the past 2 decades, in-vitro and in-vivo studies have given strong evidences for the success of regenerative endodontics. However, the approach is not widely practiced. This review outlines the biological basis and current protocols used in the treatment. We also discuss the scope for future prospective in the field.

Keywords: Immature permanent teeth, Regenerative endodontics, Tissue engineering, Revascularization.

Introduction

What is Regenerative Endodontics?

Apexification procedures using calcium hydroxide or apical MTA plugs before root canal filling are traditional treatment options for immature permanent teeth with necrotic pulp/apical periodontitis. The multiple treatment visits over an extended period of time and prolonged calcium hydroxide dressing could increase the risk of root fracture [1]. Immature permanent teeth with pulpal necrosis are often difficult to debride and considering thin dentinal walls, they are at an increased risk of a subsequent cervical fracture [2]. Maintaining vitality of pulp is essential in case of immature permanent teeth for continuous root development and apical closure. This goal can be accomplished either by maintaining pulpal health or regenerate necrosed pulpal tissue[3].

Regenerative Endodontic Procedures (REP) is an alternative approach based on the principles of regenerative medicine and tissue engineering. These challenging cases can be successfully treated by regenerating functional pulpal tissue utilizing **REP**[2].

Regenerative endodontic therapy has been defined as **"biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex''[2].**

Regenerative endodontics applies the concept of the triad of tissue engineering including stem cells, biomimetic scaffold and bioactive growth factors (FIG 1) in the canal space to regenerate the pulp tissue damaged by infection, trauma or developmental anomalies. In the literature revascularization, revitalization and regenerative endodontics are used interchangeably [1].

What is Tissue Engineering?

An interdisciplinary field that integrates the principles of biology and engineering to develop biological substitutes that replace or regenerate human cells, tissue or organs in order to restore or establish normal function[4]. There are three key elements for tissue engineering: **stem cells, scaffolds** and **growth factors.**

Stem Cells: Stem cells are undifferentiated cells that continuously divide. Stem cells can be embryonic or adult/postnatal. Embryonic stem cells are "pluripotent" or "omnipotent" capable of developing more than 200 cell types. Adult stem cells are "multipotent" can divide and create another cell like itself, and also a cell more differentiated than itself, but the capacity for differentiation into other cell types is limited. One of the primary source for multipotent stem cells (MSC) is Bone marrow. MSCs are found in many other tissues in the body such as umbilical cord, blood, adipose tissue, adult muscle, and dental tissues. These MSC's can differentiate into osteogenic, chondrogenic, adipogenic, myogenic, neurogenic and odontogenic lineages, when grown in a defined microenvironment in vitro [4]. Various types of dental stem cells in table 1

Scaffolds: The scaffold provides support for proliferation, differentiation and vascularization for cells. Current REPs have utilized dentin as well as the blood clot or platelet-rich plasma as scaffolds in the root canal [4].

Growth Factors: Signaling proteins bind to receptors on the cell to induce either cellular proliferation and/or differentiation. Bone morphogenetic protein, transforming growth factor–beta and fibroblastic growth factor are key examples of growth factors in pulp and dentin formation. Currently REPs utilize growth factors found in platelets and dentin [4].

Goals of Regenerative endodontics

- Healing of apical periodontitis
- Increased thickness of the root canal wall
- Restoration of pulpal function
- Continued development of the root apex

Historical Background And Evolution: Regeneration of body parts is not a new concept. Early 330 BC, Aristotle observed that the lost tip of the tail of a lizard could grow back. Spallanzani in the late 1700s, reported the capability of newt to regenerate complete limb. Regeneration in lower life forms has laid the groundwork for understanding the potential of regeneration in humans [5]. Immense number of species are capable of regeneration of body parts. Amphibians have ability to regenerate almost any structure that can be cut off [6].

Cancer specialist Stevens in 1950s and 1960s, suggested that some cells might be pluripotent and that surrounding cells might provide the necessary environmental signal needed to stimulate differentiation [7].

In 1998 a team of scientists at the University of Wisconsin isolated the first human embryonic stem cells [7].

The consequences of bleeding from the periapical tissues and formation of blood clot in animals and humans was studied by the pioneers in regenerative endodontics, **Nygaard – Ostby B (1961)**. They observed that blood clot

in the root canal was organized and gradually transformed into fibrous connective tissue that later deposited cellular cementum on the root canal walls [8].

Forty-eight anterior teeth in four monkeys were studied by **Myers WC, Fountain SB (1974).** They inferred that dental pulp regeneration was aided by blood and blood substitutes in periapical infection and necrotic root canals [9].

The antibacterial effect of a mixture of ciprofloxacin, metronidazole and minocycline, on bacteria taken from infected dentine of root canal walls was studied by Hoshino et al (1996) who concluded that the mixture eliminated bacteria from the infected dentine of root canals [10].

Based on previous work by Nygaard et al (1961, 1974), Myers et al (1974), Hoshino et al (1996), in 2001 Iwaya et al applied the concept of disinfection and revascularization in a necrotic immature mandibular second premolar with periapical involvement in a 13-year-old patient. Antimicrobial agents (ciprofloxacin + metronidazole) were used in the canal. After 5 months, radiographic examination showed the start of apical closure. Canal wall thickening and complete apical closure was achieved after 30 months [11].

Banchs and Trope in 2004, presented a new technique to revascularize immature permanent teeth with apical periodontitis. They demonstrated disinfection of canals with copious irrigation and a combination of antibiotics (ciprofloxacin + metronidazole + minocycline). Bleeding was initiated into the canal by mechanically irritating the apex and blood clot was produced to the level of CEJ. Mineral trioxide aggregate (MTA) was used as an intra-canal barrier instead of glass ionomer cement. The combination of a disinfected canal, a matrix into which new tissue could grow, and an effective coronal seal appears to have produced the environment necessary for successful revascularization [12].

This protocol has been broadly adopted in many subsequent studies in the literature and the Clinical Considerations for a Regenerative Procedure (AAE 2016)

Over the years, several authors emphasized a re-emergence of a biological, or regenerative approach for endodontic treatment. Several case reports where immature permanent teeth with pulp necrosis followed by REP were published with successful clinical outcome of resolution of sinus tracts, pain, swelling and increase in radiographic root length, over a period of 0.5–2 years [13].

Biological Basis For Regeneration: Principle of tissue engineering, include source of stem/progenitor cells, growth factors and scaffolds.

Source of cells: Stem cells can be found in dental pulp, apical papilla and even the inflamed periapical tissue [13]. During REP the induced bleeding reveals a massive influx MSCs into the root canal space [14]. Sonoyama et al in their pilot study isolated stem cells from the apical papilla (SCAP) and found them to be as potent in osteo/dentinogenic differentiation as MSCs from bone marrows. Their study discussed the implications of SCAPs in root development and apexogenesis [15]. An inflow of blood into the root canal space after laceration of the apical region has a 400 to 600 fold greater concentration of MSC markers (CD73 and CD105) compared to concentrations of the patient's systemic blood. Thus, these local sources of stem cells can be introduced into the root canal system of patients [16].

Growth factors or other tissue-inducing mediators: Stem cells differentiate into a number of cell phenotypes depending on their lineage and exposure to environmental stimuli such as growth factors, extracellular matrix or other conditions [17]. Exposure of the same population of dental pulp cells to three different combinations of growth

factors results in cells that express a mineralizing phenotype, an adipocyte phenotype or a cartilage-like phenotype [17]. Thus, only by lacerating the apical papilla may not be sufficient to guide the stems cell differentiation into pulp-dentin complex. Growth factors are important adjuncts [14]. Several growth factors can have one target cell and one growth factor can have several target cells [23]. Growth factors, noncollagenous proteins and glycosaminoglycans are found in the dentine matrix. Disinfection irrigants and medicaments influence release of growth factor from dentin. These biological molecules can direct the behaviors of cells mobilized into root canals [24]. Table 2 depicts Growth factors released from dentine matrix and their functions.

Scaffold: Scaffolds form a three dimensional tissue structure and regulate stem cell differentiation by local release of growth factors or by signaling cascade [18]. Currently, dentin, blood clot or platelet-rich fibrin are used as scaffolds in the root canal. Many types of biodegradable or permanent scaffolds made of natural or synthetic materials are available [19]. Figure 2 depicts classification of scaffolds [21].

Platelet-rich fibrin (PRF), a second-generation platelet concentrate also facilitates the condensation of overlying MTA. Rationally, PRF is expected to be an optimal bio-scaffold for tooth revascularization/revitalization [20].

Table 3 & 4 shows various scaffolds in REP. Multiple research need to be conducted to evaluate efficiency of the scaffolds.

When to Do REP?

As per Clinical Considerations for a Regenerative Procedures suggested by AAE, REP is recommended for teeth with a necrotic pulp and an immature apex at Cvek stage 1, stage 2 and stage 3 (Table 5) In these stages, teeth have short root, thin canal walls and wide-open apex and apexification has no potential for root maturation [1].

Table 5 depicts the classification of root development given by Cvek's in 1992.

Canal walls in teeth at stage 4 have enough thickness and strength, can be managed with RET or an apical MTA plug. Teeth requiring post for adequate coronal restoration are better treated with apical MTA plug [1].

Size of apical diameter: Immature permanent teeth with preoperative apical diameter wider than 1 mm demonstrated greater root maturation. REP was also successful with apical diameter as small as 0.5 mm. The typical size of human cells ranges from 10 to 100 µm thus, stem cells and endothelial cells can easily enter the canal space through the apical foramen even smaller than 0.5 mm in diameter. However, REP were suitable for the patients ranging from age 9 to 18 years [1].

Clinical Outcomes [22]: The American Association of Endodontists (AAE, 2016) define success of REP by three measures:

Primary goal (essential): The elimination of signs and symptoms and the evidence of bony healing.

Secondary goal (desirable): Increased root wall thickness and/or increased root length.

Tertiary goal: positive response to vitality testing.

Current Clinical Protocol: The first appointment includes access and disinfection of the pulp space. Second appointment involves removal of the medicament, release of growth factors from the dentin (by irrigating with EDTA), delivering stem cells into the root canal by stimulating bleeding and creating a scaffold (blood clot or PRF), sealing the tooth by placing a pulp space barrier (MTA) and permanent coronal restoration to prevent bacterial re-infection [22].

Root canal disinfection: The presence of infection in root canal systems, renders the treatment unsuccessful as pulp stem cells, periodontal stem cells, and fibroblasts do not adhere to infected dentin and differentiate [4]. Chemical

disinfection of the root canal system should be bacteriocidal/bacteriostatic properties and should not damage the survival and proliferative capacity of the patient's stem cells [25]. AAE recommends copious, gentle irrigation with 1.5% NaOCl (20mL/canal, 5 min) using a needle with closed end and side-vents to minimise extrusion of irrigants. With needle positioned about 1 mm from root end, canals are then irrigated with saline or EDTA (20 mL/canal, 5 min)[26]. After EDTA treatment, growth factors embedded in the dentin matrix are released into the canal space. These growth factors signal pulp stem cells to differentiate into odontoblast-like cells and produce reparative dentin [27]. A final rinse with EDTA before creation of a blood clot is advised as it promotes the adhesion, migration and differentiation of dental pulp stem cells. It also increases the adherence of newly formed mineralized tissue to the root walls [28].

MTAD is an aqueous solution of 3% doxycycline, 4.25% citric acid, and 0.5% polysorbate 80 detergent [29]. MTAD is reported to be effective in removing endodontic smear layers, provide sustained antimicrobial activity against microbes that are resistant to conventional irrigants [4].

Placement of an intra-canal medicament: '3mix' or 'triple antibiotic paste' (TAP) was a combination of metronidazole, ciprofloxacin and minocycline introduced by Banchs(2004). The rationale for the use of TAP was that it completely eliminated cultivatable bacteria from infected root canals with diffusion of the drugs throughout the entire root dentine [30,31]. AAE guidelines suggests combination of ciprofloxacin: metronidazole: minocycline in 1:1:1 to a final concentration of 1-5 mg/ml. TAP is associated with tooth discoloration thus paste without minocycline or substitution with clindamycin, amoxicillin or cefaclor can be an alternative. To minimize crown staining ensure that it remains below CEJ.[25].

Root canal revascularization: Creation of a blood clot or protein scaffold in canal [22]: On the second appointment, after administration of local Anesthesia without vasoconstrictor, dental dam isolation and copious, gentle irrigation with 20ml of 17% EDTA, the root canal is dried with paper points. Bleeding is induced by over-instrumenting and rotating a K-file at 2 mm past the apical foramen with the goal of having the entire canal filled with blood to the level of CEJ. Platelet-rich plasma (PRP), platelet rich fibrin (PRF) or autologous fibrin matrix (AFM) can also be used as scaffold. Bleeding is stopped at a level that allows for 3-4 mm of restorative material. White MTA is used as capping material. Owing to discoloration with MTA, Biodentine/EndoSequence should be considered in teeth with esthetic concern. The access cavity is filled with RMGIC, composite or alloy [22].

The patient is then followed up for 6, 12, 24 months for clinical and radiographical evaluation which includes resolution of pain, swelling or sinus tract, apical radiolucency (6-12 months), increased width of root walls (12-24 months), increased root length and positive pulp vitality test response. CBCT is highly recommended for initial evaluation and follow-up visits [22].

REP Techniques under Research And Scope In Future:

Postnatal stem cell therapy: This technique involves injecting postnatal stem cells into disinfected root canal systems. Postnatal stem cells derived from skin, buccal mucosa, fat, and bone, can be delivered to the canals using an injectable matrix [32].

Advantages: Easy to harvest the cells, quick and easy delivery.

Disadvantages: higher risk for complications and the stem cells do not produce functioning pulp.

Pulp implantation: Pulp cells can be grown on biodegradable membrane to form a three dimensional pulp tissue, which can be implanted into disinfected root canal systems [4].

Advantages: more stable than injecting dissociated cells

Disadvantages: lack vascularity and must be engineered to fit root canal precisely.

Scaffold implantation: Pulp stem cells must be organized into a three-dimensional structure to support cell organization and vascularization. A porous polymer scaffold can be seeded with pulp stem cells. Some immune reactions to various scaffold materials may be a concern for future use in REP[4].

Injectable scaffold delivery: Injectable scaffolds in form of Hydrogels can be delivered in root canals by syringe. They are noninvasive and easy to deliver. Although the system is promising, it is yet to be proven to be functional in vivo [4].

Three-dimensional cell printing: Layers of cells are dispensed using an ink-jet-like device in a hydrogel to recreate the structure of pulp tissue. The technique can be used to precisely position cells, and create tissue that mimic the natural dental pulp. Early research has yet to show that three-dimensional cell printing can create functional tissue in vivo [4].

Gene therapy: New techniques involving vectors can deliver genes for growth factors, morphogens, transcription factors, and extracellular matrix molecules into target cell populations. In endodontics, gene therapy can be used to deliver mineralizing genes into pulp tissue. However, there has been little or no research in this field [4].

Conclusion

REP presents a new era in clinical endodontics. Currently, REP is recognized as the first treatment choice for immature teeth with pulp necrosis. While clinical reports with current protocols shows favourable outcomes, we are hopeful that further research in this area gives improved treatment outcomes. There is much to learn, including procedures for older adults, previously treated teeth, and teeth with mature apices. Thus there is a broad scope for research in the regenerative endodontics.

Figure 1: Domains in REP

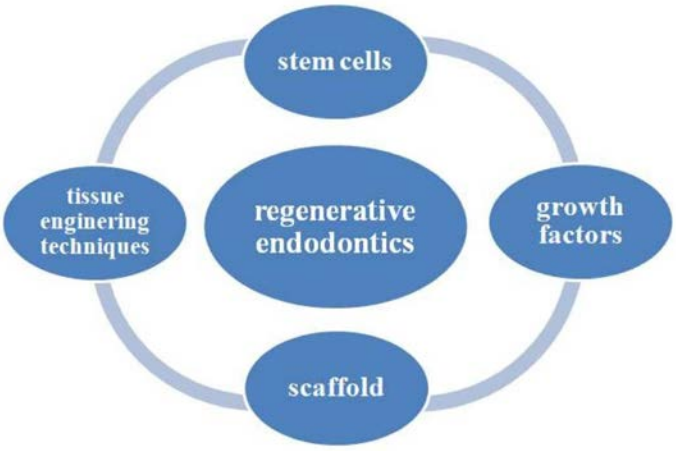

Figure 2: Classification of Scaffolds [21] ssification of Scaffolds [21]

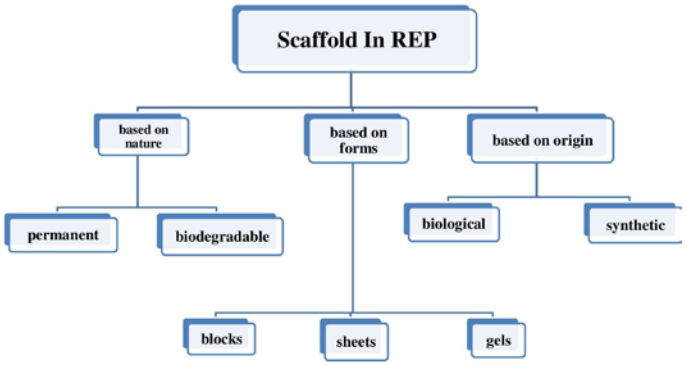

Table 1: Various post natal stem cells isolated from teeth

DPSCs	Dental pulp stem cells
SHEDs	Stem cells from human-exfoliated deciduous teeth
PDLSCs	Periodontal ligament stem cells
DFPCs	Dental follicle progenitor stem cells
SCAPs	Stem cells from apical papilla

Table 2: Growth factors released from dentine matrix and their functions [24]

Growth factors	Role in REP
TGF-β1 fibroblast growth factors 2 (FGF2) platelet derived growth factors (PDGF)	enhance cell migration
PDGF vascular endothelial growth factors (VEGF)	control angiogenesis
TGF-β1 FGF2 VEGF insulin-like growth factors	stimulate cell proliferation
Bone morphogenetic proteins FGF2	promote dentinogenesis

Table 3: types of scaffold based on origin [21]

Scaffold based on origin		
Biological	Natural	Synthetic
Blood clot:	Collagen	polymers such as polylactic acid (PLA), poly-l-lactic acid (PLLA), polyglycolic acid (PGA), PLGA, and polyepsiloncaprolactone (PCL)
Platelet rich plasmin(PRP)	Chitosan	Bioceramics
Platelet rich fibrin(PRF)	Dentin chips	

Table 4: Advantages and disadvantages of various scaffolds used in REP [21]

Scaffold	Advantages	Disadvantages
Blood clot	• Easy technique • Fairly good results	• Slower healing • Less growth factors
PRP	• More cytokines than PRP	• Requires addition of thrombin • Slower healing

Collagen	• biocompatible and biodegradable • simulates natural ECM of dentin • allows soft tissue and hard tissue formationCan be processed into porous sponges, gels, and sheets	• mechanically weak • undergoes rapid degradation
Chitosan	• nontoxic • gel forming ability • fibroblast and odontoblastic proliferation. Porous, can be molded into any shape	• low strength requires a vehicle for delivering the material

Table 5: Cvek's classification of root development 1992 [1]

Stage	Root development	Treatment recommended
Stage 1	less than 1/2 of root formation with open apex	REP
Stage 2	1/2 root formation with open apex	REP
Stage 3	2/3 of root development with open apex	REP
Stage 4	nearly completed root formation with open apex	REP/Apexification
Stage 5	Mature tooth	RCT

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