CHAPTER NAME - CURRENT TRENDS IN REGENERATIVE ENDODONTICS THERAPY (RET)

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1. INTRODUCTION

The developing dentition is at the risk of pulp necrosis due to caries, trauma, and developmental anomalies such as Dens evaginatus and invaginatus. [1] Caries is a multifactorial disease involving bacteria and their by-products, which can penetrate the pulp, causing inflammation and pulp tissue fibrosis. [2] If the bacteria are not removed, chronic inflammation may develop, and some pulp tissue will get necrosed. Long-term inflammation or repeated insults reduce the pulp's ability to repair it, and eventually, necrosis will spread to the entire pulp. [2]

Dental trauma is another common aetiological factor for loss of vitality with an incidence of 30% trauma in young permanent teeth. [3] It is well established that pulp necrosis of immature permanent teeth causes a cessation in root development. [2] The result of arrested root development leads to a low crown-root ratio, fragile walls, increased fracture risk, and an open apex. The treatment of an immature tooth with necrotic pulp and an open apex is considered a challenge to the dentist. [4] The traditional treatment of immature teeth with necrotic pulps relied on apexification procedures or the use of apical barriers. [3]

Apexification is defined as *"A method to induce a calcified barrier in a root with an open apex or the continued apical development of an incomplete root in teeth with necrotic pulp"*. [3] Historically, long-term calcium hydroxide treatment was used to induce apexification of the immature tooth with pulpal necrosis before placing an obturation material such as gutta-percha in the root canal system. [5] While the clinical success rate of calcium hydroxide ranged from 87% to 100%, with a radiographic success rate of 87% to 93%. [6] There are several associated problems

These include:

1. The time required for the formation of the calcified barrier (3-24 months). [5]

2. Multiple appointments needed for reapplication of calcium hydroxide. [5]

3. The effect of long-term application of calcium hydroxide (>30 days) decreases the fracture resistance of root dentin. [7]

It has been proposed that alkalinity of calcium hydroxide causes a disruption of the link between the hydroxyapatite crystals and the collagenous network in dentin due to neutralization, dissolution, or denaturing the acid proteins and proteoglycans, leaving the dentin structure with reduced organic support making the tooth prone to cervical fracture. [7]

Mineral trioxide aggregate (MTA) used as root-end filling offers an alternative material of choice in apexification. When placed adjacent to the periradicular tissues, it induces cementum-like hard tissue formation and offers several advantages over calcium hydroxide apexification. It includes a reduction in treatment time and fewer appointments for patients, which facilitates the timely restoration of the tooth. [8] MTA's clinical success rate range from 93% to 100%, with a radiographic success rate of 100%. [6] *In a systematic review, Cheng Lin et al.* reported that the success rates of calcium hydroxide to MTA are comparable. [6] However,

both of the apexification treatment fosters further root development and immature teeth remain vulnerable to cervical root fracture. [8]

Millions of teeth are saved each year by root canal therapy. Although current treatment modalities offer high levels of success for many pulpal 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. [9] Regenerative endodontics is an exciting and developing field in treating immature teeth with infected root canals that have been described as a "paradigm shift" in management of pulpless young permanent teeth that can result in continued root maturation and apical closure. [10]

The concept of regenerating pulpal tissue was promulgated by the classic studies of *Nygaard-Ostby (1961) and Hjortdal (1971).* They evaluated the effects of evoked bleeding by over instrumentation of human root canal systems. Unfortunately, histological analysis revealed tissue repair (e.g., fibroblasts, collagen, sparse vascularity) without histological evidence of the pulp-dentin complex's regeneration. [11]

In *1981 Skoglund & Tronstad* performed in vivo studies on dogs and evaluated the capacity of ischemic pulp tissue to revascularize after being replanted or auto transplanted due to avulsion. They observed that some teeth developed revascularization of the pulp tissue, and in others, tooth resorption was initiated without revascularization. These authors also found that a minimal extra-alveolar time could improve the prognosis of success in avulsed teeth. [12]

Kling et al. in 1986 correlated some factors in the prognosis of replanted teeth, i.e., frequency of pulp revascularization of replanted incisors, apical foramen diameter, extra alveolar time, storage medium, and post-operatory prescription of antimicrobials. It was demonstrated that immature teeth maintained in favorable extra-alveolar conditions for less than 45 minutes could provide a greater possibility of pulp revascularization. [13]

Hoshino & Sato in 1996 evaluated the effect of root canal disinfection using a mixture of antibiotics, ciprofloxacin, and metronidazole. Their treatment resulted in eliminating clinical symptom/sign and apical periodontitis and promoted thickening of the canal walls and apical closure of immature permanent teeth. [14]

In 2001, a new treatment option termed as "Revascularization" was introduced and first used by *Iwaya et al.* to manage immature teeth with necrotic pulp and apical periodontitis with a sinus tract. [15] *In 2004 Banchs and Trope* proposed a new protocol for a revascularization procedure. The author claimed that if revascularization is not achieved in three months, conventional treatment is then indicated. [16]

The term *"Regenerative endodontics"* was adopted by the American Association of Endodontists (AAE) in 2007. It was suggested by *Murray et al.* based on the concept of tissue engineering. Regenerative endodontics is defined as *"Biologically based procedures designed to replace damaged tooth structures, including dentin and root structures, as well as cells of the pulp-dentine complex"*.[9] The term Regenerative Endodontic Therapy (RET) denotes the treatment for the regeneration of pulp tissue. Whereas, Regenerative Endodontic Procedures

(REPs) denotes various type of procedures that are carried out to achieve regeneration of pulp tissue. The main objectives of Regenerative Endodontic Procedures (REPs) are to

- Regenerate pulp-like tissue; ideally, the pulp-dentin complex
- Regenerates damaged coronal dentin, such as following a carious exposure; and
- Regenerate resorbed root, cervical or apical dentin. [9]

Huang and Lin in 2008 proposed the term *"Revitalisation"* instead of revascularization because the tissues regenerated in the root canal space was not only blood vessels but also hard and soft tissues. [17] The term revitalization was used by the European Society of Endodontology (ESE) position statement in 2016.[18] In the endodontic literature, revascularization, revitalization, and regenerative endodontics are used synonymously and interchangeably. [15]

In most regenerative endodontic procedures, clinicians rely on creating bleeding from the apical region that passively fills the root canal space and forms a blood clot. However, it was not until *2011* that *Lovelace and Hargreaves et al***.** in a clinical study demonstrated that the influx of apical blood into disinfected root canals was accompanied by a clinically significant transfer of mesenchymal stem cells into the root canal system. [19]

This was an important demonstration in the field of regenerative endodontics because it established the stem cell-based procedures. The realization that autogenous stem cells can be delivered clinically into root canals without the need for ex vivo stem cell expansion propelled the researchers and clinicians to consider tissue engineering principles to improve treatment protocols and develop the next generation of procedures. [20]

The goal of REPs is to regenerate a fully functional pulp-dentin complex that fosters continued root development for immature teeth and prevents or resolves apical periodontitis. The degree of success of regenerative endodontic procedures is largely measured by the extent to which it is possible to attain primary, secondary, and tertiary goals. Regardless of this outcome, it is often beneficial to consider REPs to have a tooth to act as a space maintainer until a suitable therapeutic option is available. In some cases, the achievement of the primary goals is all that is necessary for the procedure to be considered a success (Figure- 1).[21]

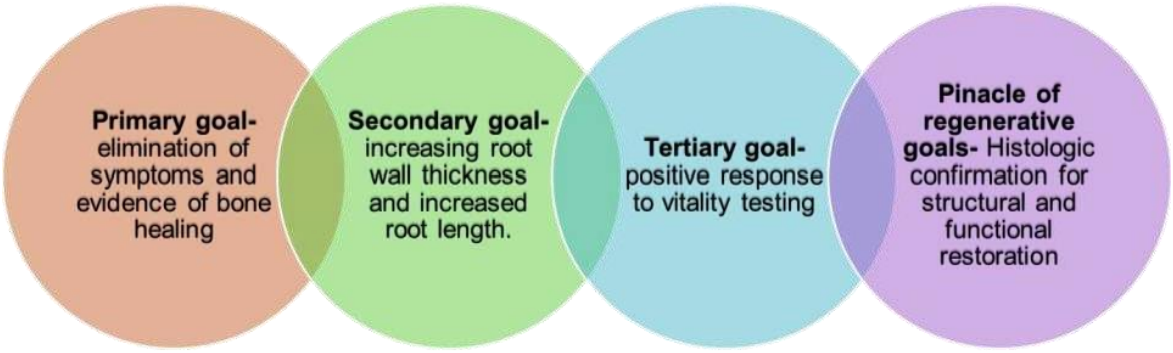


Figure-1: *Goals of regenerative endodontic therapy.*

Courtesy- *Geisler TM. Clinical considerations for regenerative endodontic procedures: Dental Clinics 2012 Jul 1; 56(3):603-26.*

The ultimate goal of REPs is to achieve histological confirmation of dental pulp with an intact odontoblastic layer and restoration of a functional pulp. [21] The dental pulp is a soft tissue of mesenchymal origin consisting of specialized cells with a collagenous matrix. It consists of 75% water and 25% organic matrix (Type I, Type III collagen, and non-collagenous proteins). It contains odontoblasts, a post-mitotic cell with a unidirectional secretory function arranged peripherally in direct contact with the dentin matrix. This close spatial relationship between the odontoblast and dentin is sometimes referred to as the dentin-pulp complex. The structure and biological properties of dentin and pulp are anatomically and physiologically complex. The identification of the dentin's bioactive nature's and its ability to facilitate the regenerative process following injury has led to the intense speculation that the natural biochemical and physiological properties of the tissue can be harnessed for the development of novel regenerative treatment modalities. [22]

Classically, dentine composition has focused on the tissue's structural properties, with its predominantly collagenous matrix mineralized with hydroxyapatite crystals. A host of molecules is known to be contained within dentine and predentine, which are likely to be important in regulating the dental tissue regenerative process. Apart from the mineral phase of dentine (hydroxyapatite), an extracellular matrix (ECM) comprises of collagenous and noncollagenous proteins (NCPs), which are present in the dentin play an essential role in the regenerative process. [23] This NCPs component contains bioactive regulatory molecules such as Dentine sialoprotein (DSP), Dentin phosphoprotein (DPP), Bone sialoprotein (BSP), Dentine Matrix protein-1 (DMP-1), Osteopontin, and Matrix Extracellular Phosphoglycoprotein (MEPE). This shows that the dentine matrix contains a broad spectrum of growth factors that help in the recruitment and expansion of stem cells to the site of injury, assisting in tissue regeneration. [23]

Thus, the regeneration approach uses a combination of scaffolds, stem cells, growth factors, tissue engineering, organ tissue culture, transplantation, and tissue grafting. [24] This chapter provides a brief overview of key elements in regenerative endodontics and its current trends.

2. CASE SELECTION FOR REGENERATIVE ENDODONTIC THERAPY (RET)

According to the "Clinical Considerations for Regenerative Procedure" suggested by AAE, Regenerative Endodontic Therapy is recommended for teeth with necrotic pulp and an immature apex. [15] *Cvek* proposed a treatment plan based on stages of root development (Figure 2). [25]

(i) Stage 1 (less than 1/2 of root formation with open apex),
(ii) Stage 2 (1/2 root formation with open apex)
(iii) Stage 3 (2/3 of root development with open apex). The above three are the suitable candidates for RET.
(iv) Stage 4 (nearly completed root formation with open apex can be managed with either RET or an apical MTA plug and root canal filling because the root canal walls have enough thickness and strength.

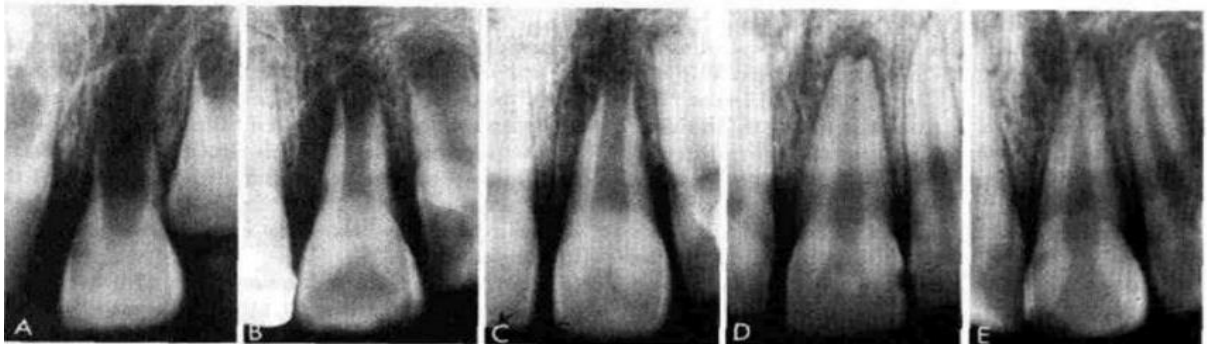


Figure -2; A-D: Immature apex; E- Stage V – Mature apex

Courtesy: *Prognosis of nonvital maxillary incisors treated with calcium hydroxide and gutta-percha – A retrospective clinical study –Cvek's et al-Endod Dental Traumatol-1992*

Estefan et al reported that younger individual's age ranges from 9 to 18 years and with preoperative apical diameter of ≥1mm showed greater increase in root thickness, length and root narrowing. [26] Immature permanent teeth with a necrotic pulp requiring post [15] and primary/deciduous teeth are not suitable candidates for RET. [27] A little evidence is available to support the use of revascularization on avulsed and replanted tooth [28] and no studies in the literature to date have shown revascularization of a tooth in children below 7 years of age. [27]

3. CUURENT STRATEGIES IN REGENERATIVE ENDODONTIC THERAPY

Many strategies have been evolved to engineer new tissues and organs, but virtually all combine material with either bioactive molecules that induce tissue formation or cells grown in the laboratory. The regenerative strategies for both mature and immature teeth are explored in two ways. (29)

CELL BASED APPROACH:

E.g. Ex vivo transplantation of stem cells along with/ without growth factors in scaffolds.

CELL FREE APPROACH:

E.g., Cell homing by molecules that recruit the patient's endogenous stem cells.

The quartet of regenerative endodontics is Stem cells, Growth factors, Scaffolds, and Disinfection (fig-3), which is a fundamental part interacting with the interplay between the other factors. [20]

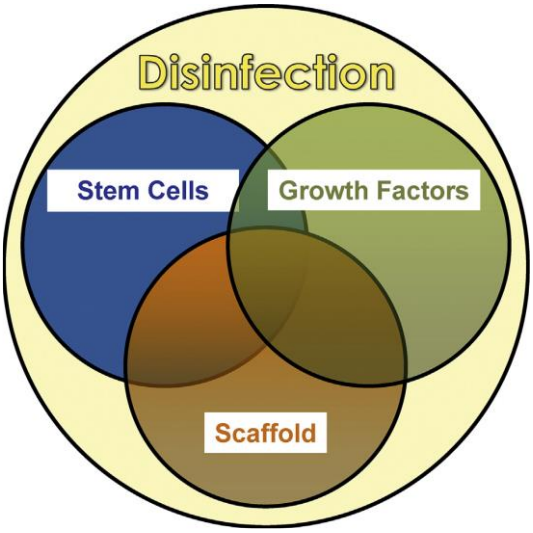


Figure – 3; Courtesy: *Diogenes A, Ruparel NB, Shiloah Y, Hargreaves KM. Regenerative endodontics: a way forward. The Journal of the American Dental Association. 2016 May 1; 147(5):372-80*

4. ROLE OF STEM CELLS IN REGENERATIVE ENDODONTIC THERAPY

Stem cells, also known as "progenitor or precursor" cells, are defined as" *Clonogenic cells capable of both self-renewal and multi-lineage differentiation".* (30) In 1868, the term "stem cell" was first introduced from German biologist Haeckel's works. Later the term stem cell was proposed for scientific use by Russian histologist *Alexander Maksimov* in 1909 and further research by Canadian scientists in the 1960s. In 1998, the first human embryonic stem cell line was derived at the University of Wisconsin- Madison. (30) In 1963, hematopoietic stem cells giving rise to blood cells were identified in the bone marrow. (31) Since then, it has been established that bone marrow is also the primary source for multipotent Mesenchymal Stem Cells (MSCs). Bone Marrow Mesenchymal stem cells (BMMSCs) can differentiate into osteogenic, chondrogenic, adipogenic, myogenic, and neurogenic lineages. MSCs are found in many other tissues in the body, including umbilical cord blood, adipose tissue, adult muscle, and dental tissues. They can differentiate into at least three cell lineages such as osteogenic, chondrogenic, and adipogenic. They can also differentiate into other lineages such as odontogenic when grown in a defined microenvironment in vitro. (32) *Crisan and colleagues* demonstrated that human perivascular cells from diverse and multiple human tissues give rise to multi-lineage progenitor cells that exhibit the features of MSCs. (33) BMMSCs have been identified by their capacity to form adherent colonies, morphologically similar to fibroblasts (colony-forming unit – fibroblastic, CFU-F) when placed at low densities in the presence of media supplemented with mitogenic growth factors or serum. (32)

CLASSIFICATION OF STEM CELLS:

Stem cells are broadly classified as follows (Figure -4). (34)

Based on the origin:

1. Embryonic/fetal stem cells

2. Adult/Postnatal stem cells

Based on their differentiation potential:

1. Totipotent,

2. Pluripotent,

3. Multipotent, and

4. Unipotent

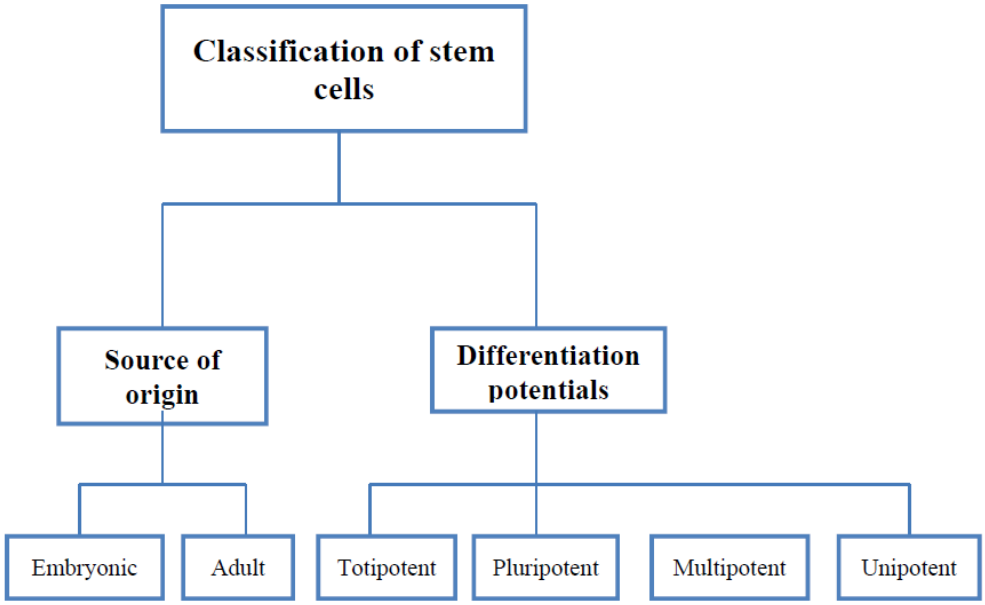


Figure 4: Classification of Stem cells

Courtesy: *Multipotent Stem Cell and Current Application-Aligholi Sobhani et al.; Acta Med Iran 2017; 55(1):6-23.*

Following are the sources of stem cells that have been recognized in human dental pulp (35), (36)

1. Dental pulp stem cells (DPSCs; Gronthos et al., 2000)
2. Stem cells from human exfoliated deciduous teeth (SHED; Miura et al., 2003)
3. Stem cells of the apical papilla (SCAP; Sonoyama et al., 2006).
4. Periodontal ligament stem cells (PDLSCs; Seo et al., 2004)
5. Dental follicle progenitor cells. (Figure-5, 6) (35), (36)
6. Induced Pluripotent Stem cells (iPSC)

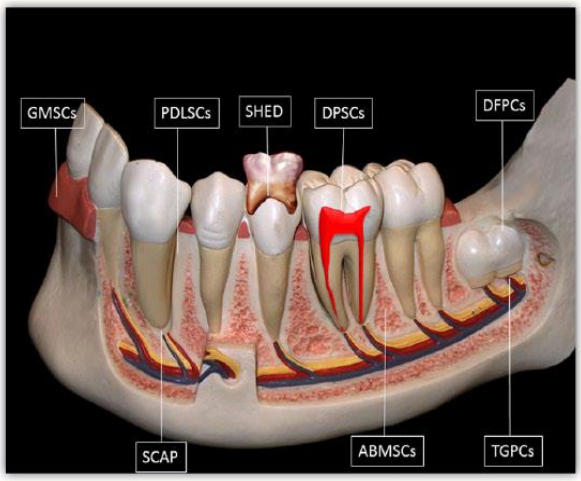


Figure- 5

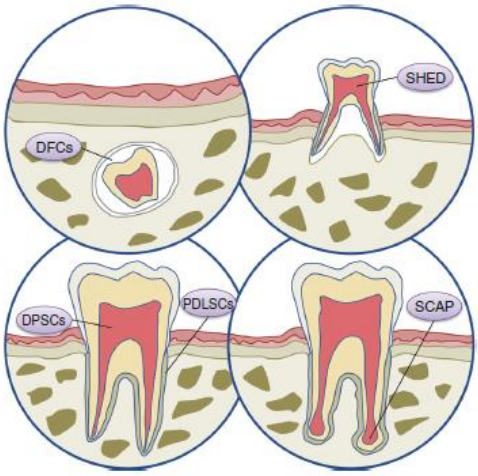


Figure- 6

Figure 5 & 6: Schematic drawing illustrating potential sources of post-natal stem cells in the oral environment. Cell types include tooth germ progenitor cells (TGPCs), dental follicle stem cells (DFSCs), salivary gland stem cells (SGSCs), stem cells of the apical papilla (SCAP), dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), bone marrow stem cells (BMSCs) and as illustrated in the insert oral epithelial stem cells (OESCs) and gingival-derived mesenchymal stem cells (GMSCs).

Figure 5- Courtesy: *Chalisserry EP, Nam SY, Park SH, Anil S. Therapeutic potential of dental stem cells. Journal of tissue engineering. 2017 May 20; 8.*

Figure 6-Courtesy: *Zheng C, Chen J, Liu S, Jin Y. Stem cell-based bone and dental regeneration: a view of microenvironmental modulation. International journal of oral science. 2019 Aug 19; 11(3):1-5.*

CHARACTERISTICS OF STEM CELLS:

- Undifferentiated Embryonic/Adult cells that continuously divide.
- Self-renewal of the ability to go through numerous cycles of cell division while maintaining the undifferentiated state.
- They can produce intermediate cells (progenitor/precursor cells- capacity to differentiate into different cell types).
- Differentiation occurs when stem cells acquire the features of specialized cells. (32)
-

DENTAL PULP STEM CELLS:

DPSCs were the first type of Dental stem cells (DSC) derived from dental pulp. *Gronthos et al., in 2000,* from pulp tissue of the human-impacted third molars through enzymatic digestion. These multipotent stem cells exhibited a typical fibroblast-like morphology. (37)

DPSCs are a putative candidate for dental tissue engineering due to (38)

a. Easy surgical access to the collection site and very low morbidity after extraction of the dental pulp.

b. DPSCs can generate much more typical dentin tissues within a short period than non-dental stem cells.

c. Can be safely cryopreserved and recombined with many scaffolds.

d. Possess immuno-privilege and anti-inflammatory abilities favourable for the allotransplantation experiments. (38)

IDENTIFICATION OF DPSCs:

Four commonly used stem cell identification techniques are [9]

1. Fluorescent antibody cell sorting: Stem cells can be identified and isolated from mixed cell populations by staining the cells with specific antibody markers and using a flow cytometer.
2. Immunomagnetic bead selection.
3. Immunohistochemical staining.
4. Physiological and histological criteria, including phenotype, proliferation, chemotaxis, mineralizing activity, and differentiation. [9]

STEM CELLS OF THE APICAL PAPILLA:

A new unique population of mesenchymal stem cells residing in the apical papilla of permanent immature teeth known as stem cells from the apical papilla (SCAP) was first discovered by *Sonoyama et al. in 2006.* (39) Histologically apical papilla is precisely seen apical to the epithelial diaphragm, and there is a cell-rich zone lying between the apical papilla and the pulp (figure – 7). (40)

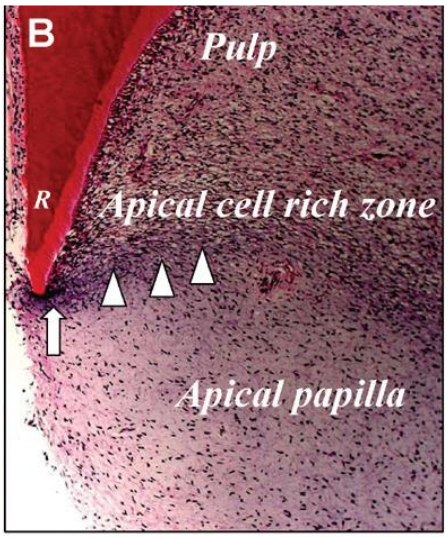


Figure 7- Courtesy: *The Hidden Treasure in Apical Papilla: The Potential Role in Pulp/Dentin Regeneration and Bio Root Engineering - George T.-J. Huang, Sonoyama et al.: (J Endod 2008; 34:645– 651)*

SCAP has somewhat different characteristics than DPSCs assessed by histologic, immunohistochemical, cellular, and molecular analyses. SCAP appears to be the source of primary odontoblasts responsible for the formation of root dentin, whereas DPSCs are likely the source of replacement odontoblasts. When treating immature teeth, conservation of these stem cells may allow the continuous formation of the root to completion. Because of its location, the apical papilla is benefited by collateral circulation, enabling it to survive during the pulp necrosis. (39)

ISOLATION OF SCAP:

The apical papilla and consequently SCAP can be easily isolated following tooth extraction by separating the tissue at the tips of the developing roots by tweezers. This tissue is then dissected into smaller pieces and digested using a cocktail of collagenase and dispase in a well-established protocol to isolate single-cell suspensions, which are then cultured (Figure –8).(40)

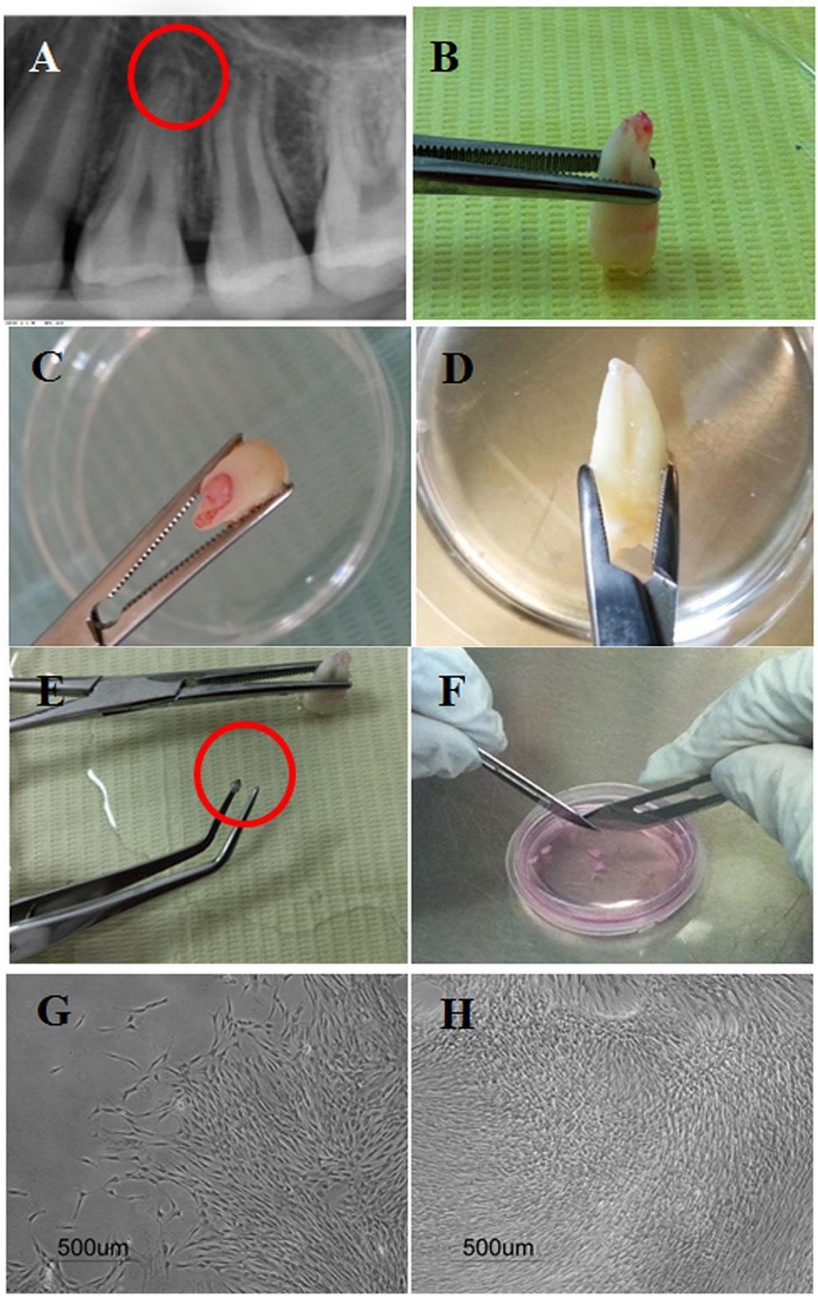


Figure – 8: Isolation of stem cells from the apical papilla showing:

(A) Periapical radiographs of the apical papilla related to the apex of a human premolar

(B–D) Extracted premolars with intact apical papilla

(E) Apical papilla separated gently using tweezers;

(F) Scalpel dissection of apical papilla tissue in a cell culture dish with culture medium.

***Figure 8- Courtesy**: Nada OA, El Backly RM. Stem cells from the apical papilla (SCAP) as a tool for endogenous tissue regeneration. Frontiers in bioengineering and biotechnology. 2018 Jul 24;6:103.*

PROPERTIES OF STEM CELLS: (32)

Table - 1

PROPERTIES	DPSC	SCAP	SHED	PDLSC	DFSC	IPSC
BONE FORMATION	+		+	+	+	
DENTIN/ PULP	+	+	+		+	
COLLAGEN FORMATION	+		+			
ADIPOGENIC FORMATION		+	+		+	
NEUROGENIC DIFFERENTIATION	+	+	+		+	+
PERIODONTAL TISSUE FORMATION	+			+	+	+
VASCULOGENESIS	+					

Adapted from- *Sedgley CM, Botero TM. Dental stem cells and their sources. Dental Clinics. 2012 Jul 1;56(3):549-61.*

5. EFFECT OF GROWTH FACTORS IN PULP REGENERATION

Growth factors are peptide molecules that transmit signals to control cell behavior and activity. They act through interaction with specific receptors located on the surfaces of cells. Several growth factors have been evaluated for their ability to trigger the differentiation of selected mesenchymal stem cell populations into odontoblast-like cells. (41)

Growth factors may act on adjacent cells (paracrine functions), on the cell producing the growth factor (autocrine function), act within the producing cell (intracrine function), and between the cells (juxtacrine function), highlighting the complexity of control of cellular activities in the body. They are present only in minute concentrations (picogram) yet exert a powerful influence on wound healing and repair. (42)

Functions:

- To stimulate the division of neighbouring cells and those infiltrating into the defect, e.g., platelet-derived growth factor (PDGF).
- To stimulate the differentiation of individual cells along a specific pathway, e.g., differentiation factor – Bone Morphogenic protein (BMP).
- To stimulate angiogenesis – Vascular endothelial growth factor (VEGF)
- To serve as chemoattractants for specific cell types. (41)

The growth factors that participate in dental pulp regeneration through two sources, namely: (43)

I. Endogenous molecules and

II. Exogenous molecules

(I) ENDOGENOUS BIOLOGICAL MOLECULES:

Dentin contains a variety of biological molecules, including growth factors, noncollagenous proteins, and glycosaminoglycans. The growth factors such as

- Transforming growth factor – β(TGF- β)
  - Bone morphogenic protein (BMP)
  - Growth/differentiation factor(GDF)
- Platelet-derived growth factor(PDGF)
- Fibroblast growth factor(FGF)
- Vascular endothelial growth factor(VEGF)
- Insulin-like growth factor(IGF) (43)

Non-collagenous proteins in the dentin include

- Dentin sialoprotein (DSP)
- Dentin phosphoprotein(DSPP)
- Dentin matrix protein (DMP)
- Bone sialoprotein BSP)
- Osteopontin (OP) (43)

Sulfated glycosaminoglycans

- Chondroitin sulfates
- Dermatan sulfates. (43)

Other than this, several key mobilization factors have also been identified, including Granulocyte- Colony Stimulating Factor (G-CSF), *Cytokines* such as interleukin (IL)-8 and Fms-like tyrosine kinase-3 (Flt-3) ligand, *Chemokines* such as Stromal cell-derived factor-1 (SDF-1). (44) During dentin mineralization, these bioactive growth factors become embedded and immobilized in the dentin matrix. Since active proteins and growth factors have a short half-life, binding them to extracellular matrix components may be required to maintain their bioactivity by protecting them from proteolytic degradation and prolonging their life span. Because there is no turnover in the dentin extracellular matrix, regulatory molecules can be reactivated much later in life on release from their bond. During dental caries, bacterial lactate exposes the organic component of dentin and releases bioactive factors, modifying immune response, cell recruitment, and differentiation. Application of dental materials namely calcium hydroxide or mineral trioxide aggregate and self-etching dental adhesives release bioactive factors. (45)

Whereas during the regenerative endodontic procedures, organic acids or chelating agents such as EDTA/citric acid is used to demineralize dentin before the influx of endogenous stem/progenitor cells so that the biological cues embedded in the dentin matrix can be released and direct the cellular activities towards regeneration (Figure- 9) .(45) Presumably, apical bleeding delivers the biological molecules to the root canal space along with stem/progenitor

cells because blood clots contain blood-derived growth factors such as PDGF, TGF-b, FGF, VEGF, and IGF. The endogenous factors are thought to fuel the activities of recruited cells and pulp regeneration. (43)

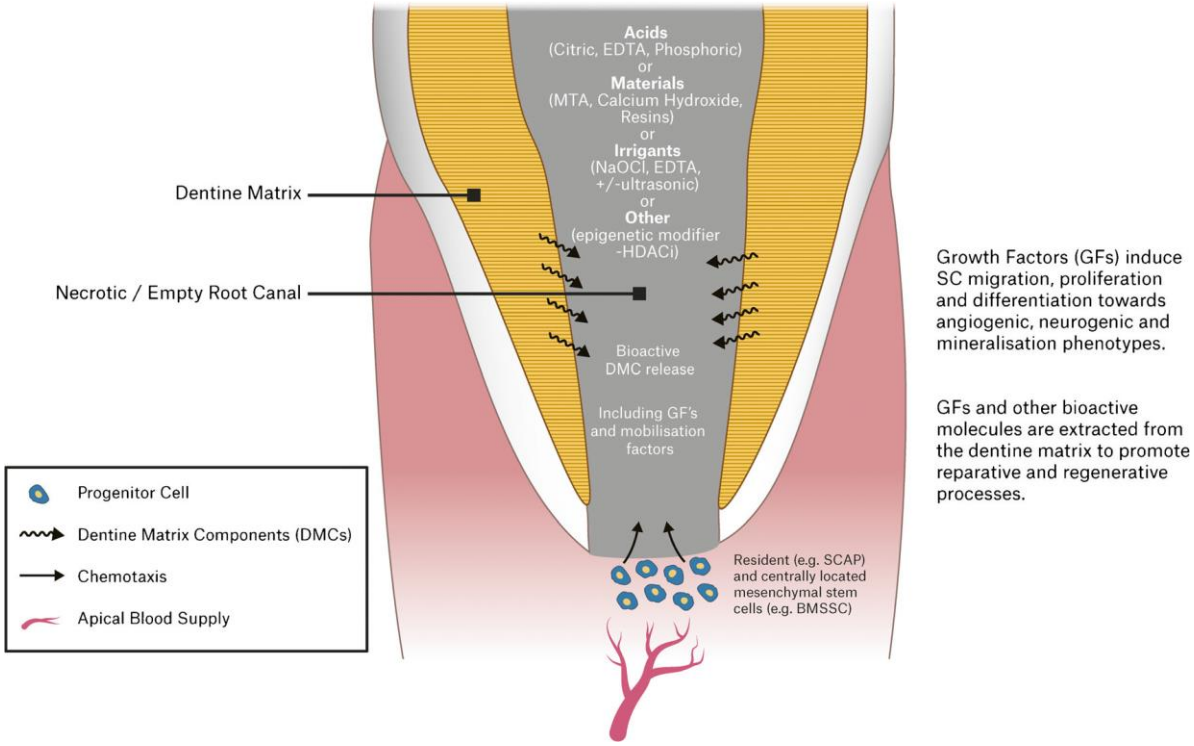
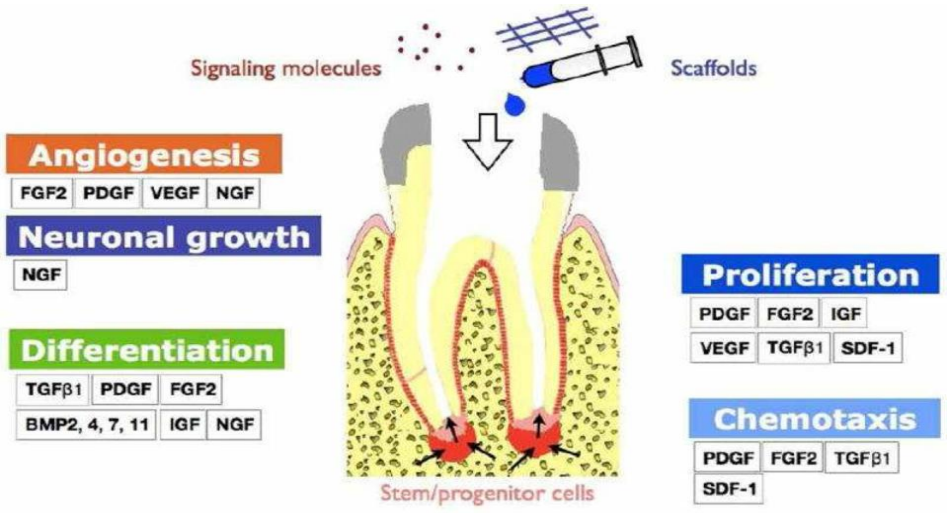


Figure- 9: Schematic drawing representing the influence of dentin matrix components (DMCs) extracted by a range of etchants, irrigants, dental materials, and epigenetic modifying agents (histone deacetylase inhibitors [HDACi]) on the promotion of cell migration, angiogenesis, neurogenesis, mineralization, and regenerative events.

Courtesy: *Duncan HF, Kobayashi Y, Shimizu E. Growth factors and cell homing in dental tissue regeneration. Current oral health reports; 2018 Dec 1; 5(4):276-85.*

EFFECT OF GROWTH FACTORS IN PULP REGENERATION: (Figure-16)



Courtesy: *Kim SG, Zhou J, Solomon C, Zheng Y, Suzuki T, Chen M, Song S, Jiang N, Cho S, Mao JJ. Effects of growth factors on dental stem/progenitor cells, Dental Clinics: 2012 Jul 1:56 (3):563-75.*

6. SCAFFOLDS

Scaffolds are three-dimensional (3D) porous stable biomaterials designed in such a manner which will: (46)

1. Provide a spatially correct position of cell location.
2. Promote cell-biomaterial interactions, cell adhesion, and ECM deposition.
3. Permit sufficient transport of nutrients and regulatory factors to allow cell survival, proliferation, and differentiation.
4. Biodegrade at a controllable rate that approximates the rate of tissue regeneration.
5. Provoke a minimal degree of inflammation or toxicity *in vivo*. (47)

Apart from blood cells, most of the normal cells in human tissues are anchorage-dependent, residing in a solid matrix called the extracellular matrix (ECM). The best scaffold for an engineered tissue should be the ECM of the target tissue in its native state. *Laminin,* an Extracellular matrix protein, promotes odontoblast differentiation. (47)A recent study by Howard and colleagues demonstrated a vital factor in dental pulp stem cell migration. (48) *Fibronectin* has been shown to increase ameloblast growth and its differentiation, while *vitronectin* provides a structural framework. (49)

Ideal requirements of a scaffold:

a. High porosity and adequate pore size facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients.
b. It should allow the effective transport of nutrients, oxygen, and waste.
c. Biodegradability is essential since scaffolds need to be absorbed by the surrounding tissues without the necessity of surgical removal.
d. The rate at which biodegradation occurs has to coincide with the rate of tissue formation.
e. It should be biocompatible.
f. Should have adequate physical and mechanical strength. (50)

CLASSIFICATION OF SCAFFOLDS : (46)

- Based on the degradability of matrices
- Based on the form
- Based on the presence or absence of cells
- Based on origin

Table- 2:

Based on the degradability of matrices	Based on forms	Based on the presence or absence of cells
Biodegradable scaffolds	Solid blocks	Cell-free scaffolds
Permanent or biostable scaffolds	Sheets Porous sponges Hydrogels (injectable scaffolds)	Scaffolds seeded with stem cells

Table 3:

BASED ON ORIGIN	
Biological or natural scaffolds	**Artificial or synthetic scaffolds**
Platelet-rich plasma	**Polymers**
Platelet-rich fibrin	Polylactic acid (PLA)
Collagen	Polyglycolic acid (PGA)
Glycosaminoglycans/ hyaluronic acid	Polylactic co glycolic acid (PLGA)
Demineralized or native dentin matrix	Polyepsiloncaprolactone (PCL)
Blood clot	**Bioceramics**
Silk	Calcium/ phosphate material
	Bioactive glasses
	Glass-ceramics

Adapted from- *Gathani KM, Raghavendra SS. Scaffolds in regenerative endodontics: A review. Dent Res J 2016; 13:379-86.*

7. ROOT CANAL IRRIGANTS IN RET:

Elimination of bacteria from the root canal system is necessary for pulpal and periapical healing. However, because of the lack of mechanical debridement in immature teeth during regenerative endodontic procedures, chemical debridement must be solely relied on. This differs from conventional root canal therapy in which the canals are chemo mechanically debrided. Tissue healing, whether it is repair or regeneration, occurs in a sterile or highly disinfected microenvironment. (51)The host immune defense system does not promote tissue-

destructive pro-inflammatory processes but can stimulate tissue-forming processes to replace inflammatory tissues with native or ectopic tissues. It is suggested that the determination of the tissues formed during the wound healing process strongly depends on a multitude of local parameters such as the local dynamics of available constructive cells nearby, the remaining three-dimensional tissue structures, and the degree and chronicity of prior infection. It is important to stress that the degree and chronicity of prior infection significantly affect wound healing by devastating essential host structures and available constructive cells—stem/progenitor cells and resident cells from periapical tissues and apical papilla. Long-term infection with large periapical lesions may eliminate the stem cell population near the periapical areas such as stem cells of the apical papilla (SCAP), the mesenchymal cells /progenitor cells from the remote sites due to their smaller quantities and slower proliferation compared to resident tissue forming cells from the periodontal ligament and alveolar. Therefore, it is crucial to prevent the migration of these mature resident cells of periodontal origin and stimulate the recruitment of stem/progenitor cells at the apical tissues for dental pulp-dentin regeneration. Furthermore, the root canal dentin microstructures, especially dentinal tubules, are altered by chronic infection might become unfavorable for cell attachment and differentiation because of invaded microorganisms (figure 10). (51)

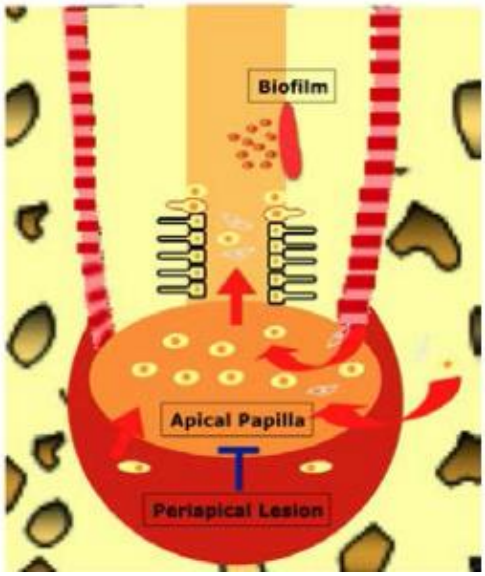


Figure 10: The regeneration of the pulp-dentin complex. Stem/progenitor cells at the apical tissues, including stem cells of the apical papilla (SCAP), inflamed periapical progenitor cells, periodontal ligament stem cells, and bone marrow mesenchymal stem cells, should be mobilized into the root canals. Long-term infection may cause detrimental effects on the migration and differentiation of the stem/progenitor cells. The mature resident cells of periodontal origin competitively participate in tissue healing processes and may form tissues of periodontal origin in the root canal space

Courtesy*: Infection and Pulp Regeneration-Sahng G. Kim; Dent. J. 2016: 4:*

SODIUM HYPOCHLORITE:

Sodium hypochlorite (NaOCl) has been used as a disinfecting agent in most reported cases. [(2)] NaOCl is an alkaline material with a pH ranging from 10.9 to 12. It has several desirable

characteristics, including excellent bactericidal efficacy, tissue dissolution capacity, and adequate lubrication for endodontic instruments. The first 2 beneficial properties are crucial for the disinfection of immature teeth in regenerative procedures, which typically involve minimal to no mechanical preparation. (52) Though it is a go-to irrigant for a clinician to attain maximum disinfection in the root canal, it has some effects on stem cell survival, attachment, and proliferation. (53) A study by *Trevino et al***.** evaluated the survival of stem cells of the apical papilla (SCAPs) cultured in an organotypic root canal model previously irrigated with various combinations of commonly used chemical agents. It was found that dentin conditioning with 17% EDTA promoted more remarkable SCAP survival, whereas 6% of NaOCl had a profound detrimental effect on SCAP survival. Significantly, the use of 17%EDTA after 6% NaOCl attenuates its undesirable effects. (54)

The adverse effects of NaOCl do not appear to be directly related to residual NaOCl in the dentinal tubules, resulting in direct toxicity because neutralization with sodium thiosulfate (5%) did not reverse this effect. (53) Thus, NaOCl has a profound effect on dentin, resulting in diminished stem cell survival and differentiation. These effects can be minimized by using 1.5% NaOCl followed by 17% EDTA. (55)

American Association of endodontics (AAE) recommends 20ml of 1.5%NaOCl with a total exposure time of 5 minutes. The guidelines suggest disinfection with NaOCl using a technique that minimizes the possibility of its extrusion into the periapical space. Some of the suggested techniques to this effect include using an irrigating needle with a closed-end and side-vents, or Endovac. Subsequently, irrigation is completed with saline or C10H16N2O8 (EDTA) (20 mL/canal for 5 min) with the irrigating needle positioned about 1 mm coronally from the root end to minimize cytotoxicity to the stem cells in the apical tissues. (56)

ETHYLENE DIAMINE TETRA ACETIC ACID (EDTA):

In 1951, the first reports on the demineralizing activity of EDTA on dental hard tissues were published. Chelators were first introduced in endodontics by Nygaard-Ostby in 1957. A variety of growth factors are present in the EDTA-soluble fraction of demineralized human dentine extracellular matrix, including transforming growth factor-b1 (TGF-b1), fibroblast growth factor-2 (FGF-2), bone morphogenetic protein-2 (BMP-2), platelet-derived growth factor (PDGF), placenta growth factor (PIGF) and epidermal growth factor (EGF) in addition to angiogenic factors such as vascular endothelial growth factor (VEGF). These molecules are useful at deficient concentrations and still elicit cellular responses at the picogram level, modifying immune defense, angiogenesis, cell recruitment, proliferation, and differentiation as well as mineralization. (57)

EDTA's demineralizing effect is commonly used in root canal treatment. The smear layer's removal after root canal preparation by EDTA allows for more thorough disinfection and dentinal tubules before canal filling. During this procedure, the effect on cells in the surrounding tissue is negligible. During a clinical regenerative endodontic procedure, the first critical step is root canal disinfection, a prerequisite for regeneration. Although NaOCl is essential to combat infection; its effects on cells are undesirable. The second critical step is to

recruit stem cells from the apical papilla, which are flushed into the canal after initiation of bleeding Pre-treatment of dentine after disinfection and before induction of bleeding might have beneficial effects on the behavior of this cells.(41) *Galler et al* showed that a significantly higher amount of induced cell migration by EDTA compared to other irrigants like NaOCl and H_2O_2 after incubation for 24hrs and 48hrs. (58)

In the same study, *Galler et al* evaluated the cell viability on dentin after pretreating with different irrigating solutions; they found that cell viability on EDTA conditioned dentin is similar to that of untreated dentin disks and statistically higher than other irrigating solutions. (58)

Taweewattanapaisan et al has evaluated the chelating effect of EDTA on dentin contacted blood clot in root canal dentin walls observed a clumping of platelets in EDTA irrigated groups. This may be due to the loss of fibrinogen binding function (Figure- 11). (59)

Also, their results showed that fiber density in the coronal, middle, and apical portions of the root canal did not significantly differ in each group of the irrigation protocols, also found the use of EDTA for 1min showed no significant difference in fibrin formation as in 5mins of irrigation. This showed the extensive effect of EDTA on fibrin formation. After EDTA irrigation, flushing with NSS could enhance fibrin formation because it reduced residual EDTA in the root canal. Based on this study's results, using EDTA followed by normal saline solution (NSS) did not affect fiber density, but it might affect the amount of growth factor released from root dentin. (59) The American Association of Endodontics and the European Society of Endodontology recommend clinicians use 17% EDTA solution for 1 minute as a final irrigation. (56)

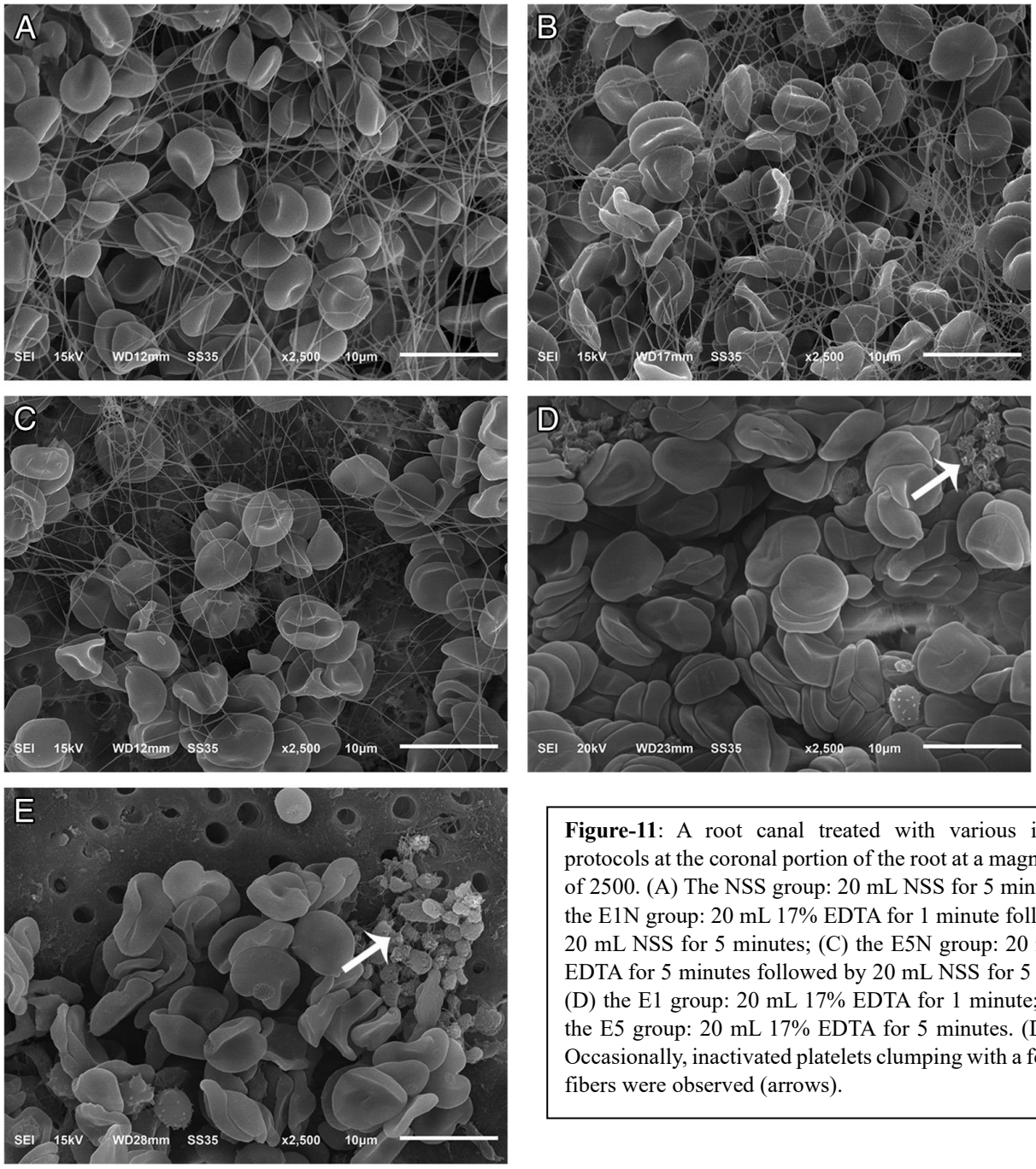


Figure-11: A root canal treated with various irrigation protocols at the coronal portion of the root at a magnification of 2500. (A) The NSS group: 20 mL NSS for 5 minutes; (B) the E1N group: 20 mL 17% EDTA for 1 minute followed by 20 mL NSS for 5 minutes; (C) the E5N group: 20 mL 17% EDTA for 5 minutes followed by 20 mL NSS for 5 minutes; (D) the E1 group: 20 mL 17% EDTA for 1 minute; and (E) the E5 group: 20 mL 17% EDTA for 5 minutes. (D and E) Occasionally, inactivated platelets clumping with a few fibrin fibers were observed (arrows).

Courtesy: *Taweewattanapaisan P, Jantarat J, Ounjai P, Janebodin K. The Effects of EDTA on Blood Clot in Regenerative Endodontic Procedures. Journal of endodontics. 2019 Mar 1;45(3):281-6.*

8. INTRACANAL MEDICAMENTS IN RET

CALCIUM HYDROXIDE:

Calcium hydroxide is recommended as intracanal medication in RET because of its good antimicrobial property. [174],[187] Calcium hydroxide has a high pH of 12.5-12.8, which is not a favorable environment for most bacteria to survive. (62) Also, calcium hydroxide can hydrolyze the lipid moiety of gram-negative bacteria lipopolysaccharide (LPS), resulting in the release of free hydroxy fatty acids and degradation of LPS. (63) Calcium hydroxide in RET was tested against stem cells survival from apical papilla in vitro [187] rather than killing the intracanal bacteria in vivo. (52) A recent study has shown that human apical cells attachment to root dentine is higher when treated with calcium hydroxide rather than Triple antibiotic paste in vitro. Furthermore, water-based calcium hydroxide slightly increased the amounts of (TGF)-β1 compared with the use of EDTA alone, although this finding was not statistically significant. (64)

TRIPLE ANTIBIOTIC PASTE: (TAP)

Information regarding microbial ecology in the canals of immature permanent teeth with infected necrotic pulp is scarce. *Nagata et al* reported that microbial ecology in the canals of traumatized immature permanent teeth with infected necrotic pulp was similar to that of mature permanent teeth. [187] Biofilms were also formed on the radicular canal walls, and bacteria penetrated the dentinal canal tubules of immature permanent teeth with infected necrotic pulp. Antibiotics, also known as antimicrobials, are developed to selectively target microbes in infection rather than the host's normal cells. Grossman first described the use of a topical antimicrobial agent to sterilize the infected root canal in 1972. Later *Hoshino and Sato et al*(65) also used triple antibiotic paste to sterilize infected root canals in vitro.[14] In antimicrobial therapy, the mechanism of action and side effects of single antimicrobial agents is well described by the manufacturer. However, when the antimicrobial agents are combined, such as a triple or double antibiotic paste, their mechanisms of action and side effects are unknown. It is assumed that antimicrobial combination would prevent polymicrobial infection and have synergistic effects. (66)

In RET of immature permanent teeth with infected necrotic pulp, triple antibiotic paste (minocycline, ciprofloxacin, and metronidazole) has been recommended as an intracanal medication, based on its excellent antimicrobial activity to kill all species of bacteria in the infected root canals in vitro. The triple antibiotic paste was also tested for its effect on stem cells' survival from the apical papilla in vitro. However, some have suggested that one antibiotic Augmentin may be as effective as a triple antibiotic paste in RET. Augmentin has been shown to kill 100% of microorganisms isolated from the infected root canal associated with an apical abscess in vitro. Unlike other antibiotics targeting bacterial protein or DNA synthesis, Augmentin inhibits bacterial cell wall synthesis. Human cells do not have cell walls; therefore, Augmentin only affects bacterial cells and not human cells.(67)

Composition and preparation:

According to *Hoshino et al* antibiotic 3 mix – ratio is 1:1:1 (68)

- Ciprofloxacin- 200mg
- Metronidazole- 400mg
- Minocycline – 100mg

Carrier Macrogol ointment and propylene glycol (MP) ratio: 1:1

9. PROTOCOL FOR REGENERATIVE ENDODONTIC THERAPY

Factors evaluated to select ideal candidates include the stage of root development and etiology of pulp necrosis. Based on the classic trauma literature, children with underdeveloped teeth with apical diameters ≥ 1 mm are potential for REP. However, a more recent study comparing successful REP outcomes between young (9–13 years) and old (14–18 years) children as well as apical diameters, namely narrow (0.5–1 mm) and broad (≥ 1 mm) demonstrated that patients between the ages of 9 and 13 with an apical diameter of ≥ 1 mm had increased success with regards to root length, thickness, and apical diameter. [27] Despite a non-existent apical papilla in mature teeth, evoking bleeding by lacerating the periapical tissues in mature teeth in adults led to successful clinical delivery of Mesenchymal stem cells (MSCs) into the root canal system. (69) This influx of MSCs is comparable to the clinical delivery of MSCs, and the cells also expressed genes related to stem cell homing, angiogenesis, and odontoblastic differentiation, all components critical to dental pulp regeneration. Another crucial factor in the determination of an ideal candidate is the etiology of pulp necrosis. Trauma compared to dental anomalies such as dens evaginatus has shown less than ideal clinical outcomes for root development. (70)

HOW DOES REVASCULARIZATION HAPPEN?

Several theories explain the revascularization mechanism. The periapical region of immature teeth presents multipotent periodontal cells with great potential for differentiating into new fibroblasts and cementoblasts. So, it has been suggested that differentiated cementoblasts and fibroblasts are responsible for increasing dentinal wall thickness and apical closure. (70) Another hypothesis suggests that residual multipotent stem cells from pulp tissue may be abundant in young, immature teeth, adhering to dentinal walls to generate odontoblast-like cells for root-end development. A third possibility involves the ingrowth of stem cells from apical papilla that could proliferate inside root canals through the blood induction of periapical tissues, since these cells have the high proliferative capacity, probably being transported inside root canals in association with bleeding induced from the periapical tissue (figure-12, 13).(71)

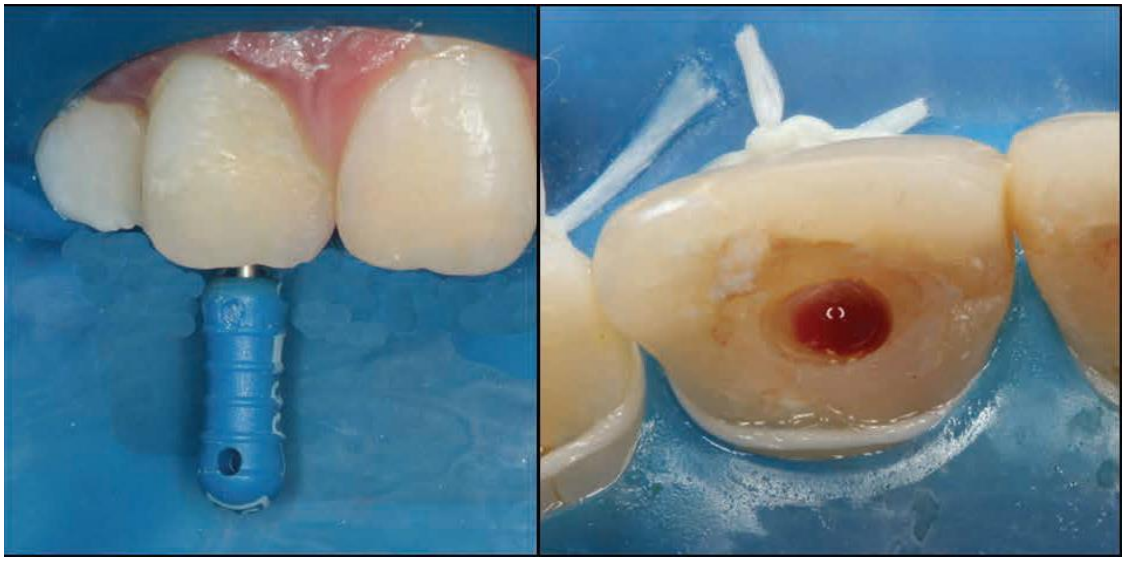


Figure 12: Blood clot stimulation with a manual endodontic file.

***(Courtesy:** ALBUQUERQUE MT, NAGATA JY, SOARES AD, ZAIA AA. Pulp revascularization: an alternative treatment to the apexification of immature teeth. RGO-Revista Gaúcha de Odontologia. 2014 Dec;62(4):401-10).*

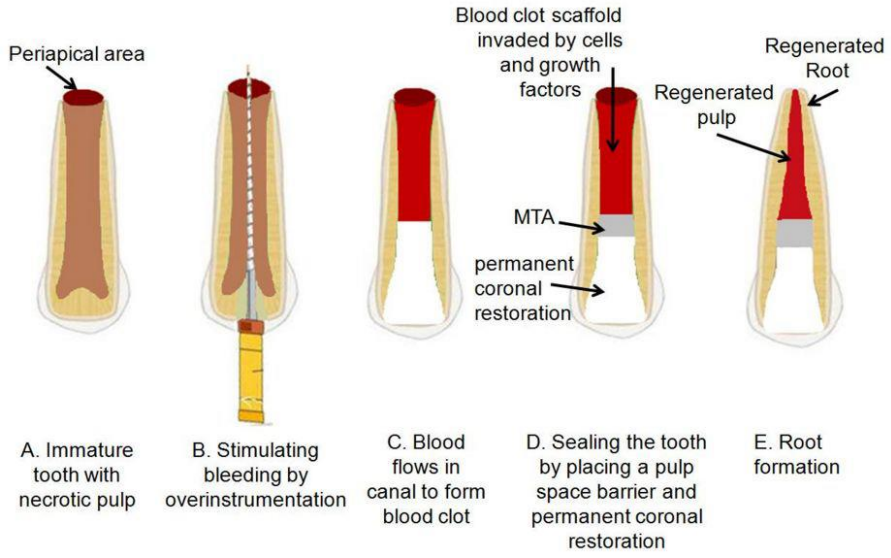


***Figure- 13:** Diagrammatic representation of root canal revascularization procedure*

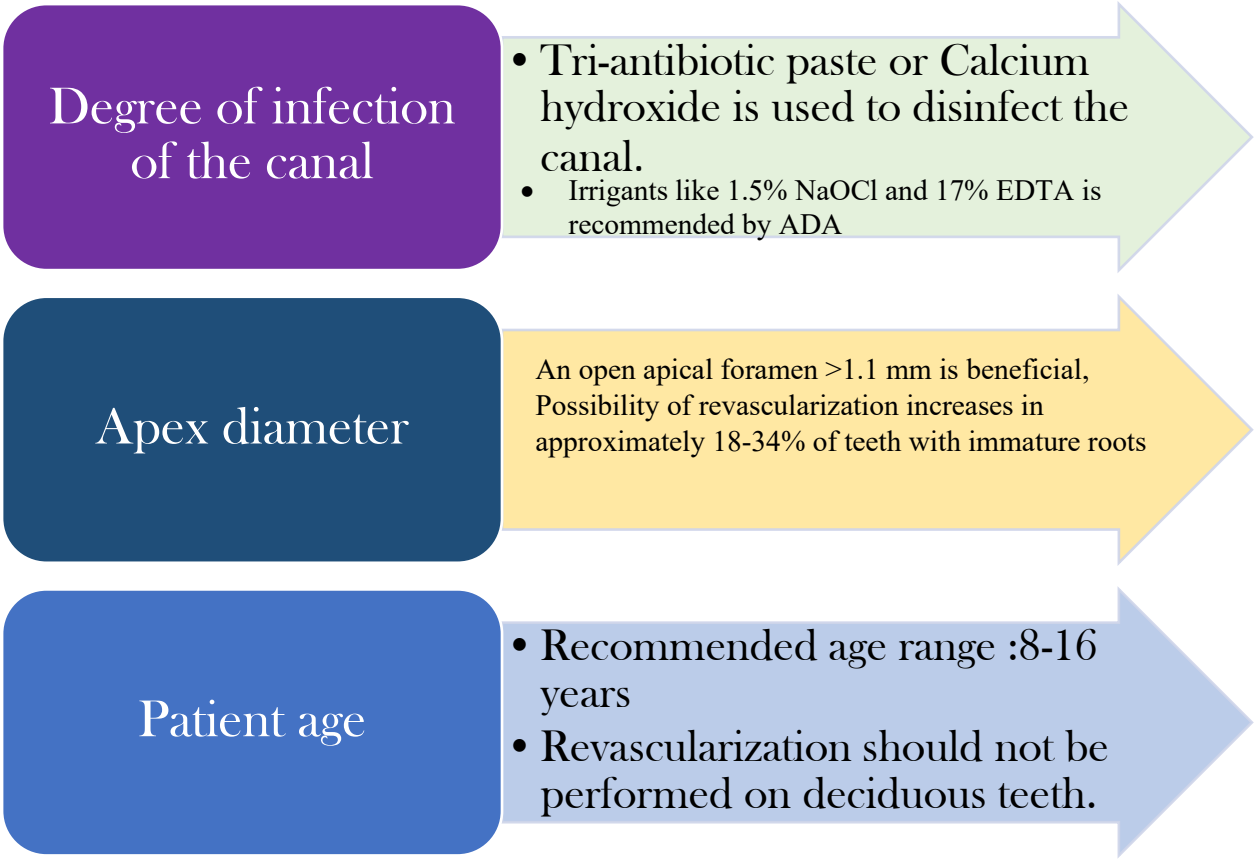
***Courtesy:** Bansal R, Jain A, Mittal S, Kumar T, Kaur D. Regenerative endodontics: a road less traveled. Journal of clinical and diagnostic research: JCDR. 2014 Oct;8 (10):ZE20.*

LIMITATION OF REVASCULARIZATION:

- Discoloration of teeth due to the use of TAP/grey MTA. [30]
- The nature of tissue formed inside is uncertain. [30]
- Success rate more in young individuals rather than elderly. [29]
- Calcification of canal space is also noted. [29]

FACTORS THAT AFFECTING THE REVASCULARIZATION PROCESS:

To achieve successful results of the treatment procedure, a thorough understanding of these factors is fundamental. The first factor is the disinfection of the canal. During the early stage of research, the regenerative endodontic treatment of pulp tissue in an infected tooth was impossible. The first attempt at the regenerative endodontic treatment of an avulsed immature tooth with necrotic non-infected pulp was successful. If the canals were effectively disinfected and the coronal access was effectively sealed, regenerative endodontic treatment should occur as in an avulsed tooth. To disinfect the canal, many types of medications are used nowadays. The second factor is the apex diameter. A tooth with an open apex allows the migration of mesenchymal stem cells into the root canal space, and this could allow host cell homing to form new tissue in the root canal space. An apical opening of 1.1 mm in diameter or larger is beneficial, with natural regenerative endodontic treatment occurring in approximately 18% to 34% of teeth with immature roots. The third factor is patient age. Several case reports of regenerative endodontic treatment procedures have generally been limited to patients reaching adolescence, mostly aged from 8 - 16 years. Regenerative endodontic treatment procedure should not be performed on deciduous teeth because of the possible risk of impairing the eruption pattern of permanent teeth. The three factors that affect the results of regenerative endodontic treatment are summarized in[10] (Figure: 14)



Courtesy: *Lin LM, Kahler B. A review of regenerative endodontics: current protocols and future directions. Journal of Istanbul University Faculty of Dentistry. 2017;51(3 Suppl 1):S41*

AAE CLINICAL PROTOCOL FOR RET

The American Association of Endodontists (AAE) has suggested the "Clinical Considerations for a Regenerative Procedure" to help clinicians manage immature permanent teeth with necrotic pulp/apical periodontitis in 2016. However, the AAE further suggested that these considerations should be seen as one possible source of information. Given the rapidly evolving nature of this field, "clinicians should also actively review new findings elsewhere as they become available." A standardized clinical protocol and strict outcome criteria are necessary for RET from both clinical and research perspective. (56)

Case Selection:

- A tooth with necrotic pulp and an immature apex.
- Pulp space is not needed for post/core, final restoration.
- Compliant patient/parent.
- Patients were not allergic to medicaments and antibiotics necessary to complete the procedure (ASA 1 or 2).

Informed Consent

- Two (or more) appointments.
- Use of antimicrobial(s).
- Possible adverse effects: staining of crown/root, lack of response to treatment, pain/infection.
- Alternatives: MTA apexification, no treatment, extraction (when deemed non-salvageable).
- Permission to enter information into the AAE database (optional).

First Appointment

- Local anesthesia, dental dam isolation, and access.
- Copious, gentle irrigation with 20ml NaOCl using an irrigation system that minimizes the possibility of extrusion of irrigants into the periapical space (e.g., a needle with a closed-end and side-vents, or Endovac™). Lower concentrations of NaOCl are advised [1.5% NaOCl (20mL/canal, 5 min) and then rinsed with saline or EDTA (20 mL/canal, 5 min), with irrigating needle positioned about 1 mm from root end, to minimize cytotoxicity to stem cells in the apical tissues.
- Dry canals with paper points.
- Place calcium hydroxide or low concentration of triple antibiotic paste. If the triple antibiotic paste is used: 1) consider sealing pulp chamber with a dentin bonding agent [to minimize the risk of staining] and 2) mix 1:1:1 ciprofloxacin: metronidazole: minocycline to a final concentration of 0.1-1.0 mg/ml. Triple antibiotic paste has been associated with tooth discoloration. Double antibiotic paste without minocycline paste or substitution of minocycline for other antibiotics (e.g., clindamycin; amoxicillin; cefaclor) is another possible alternative as root canal disinfectant.
- Deliver into canal system via syringe
- If a triple antibiotic is used, ensure that it remains below CEJ (minimize crown staining).

- Seal with 3-4mm of temporary restorative material such as Cavit™, IRM™, glass ionomer, or another temporary material. Dismiss the patient for 1-4 weeks.

Second Appointment (1-4 weeks after 1st visit)

- Assess response to initial treatment. If there are signs/symptoms of persistent infection, consider additional treatment time with antimicrobial or alternative antimicrobial.
- Anesthesia with 3% mepivacaine without vasoconstrictor, dental dam isolation.
- Copious, gentle irrigation with 20ml of 17% EDTA.
- Dry with paper points.
- Create bleeding into the canal system by over-instrumenting (endo file, endo explorer) (induce by rotating a pre-curved K-file at 2 mm past the apical foramen the entire canal filled with blood to the level of the cementoenamel junction). An alternative to creating a blood clot is the use of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), or autologous fibrin matrix (AFM).
- Stop bleeding at a level that allows for 3-4 mm of restorative material.
- Place a resorbable matrix such as CollaPlug™, Collacote™, and CollaTape™ over the blood clot if necessary and white MTA as capping material.
- A 3–4 mm layer of glass ionomer (e.g., Fuji IX™, GC America, and Alsip, IL) is flowed gently over the capping material and light-cured for 40 s. MTA has been associated with discoloration. Alternatives to MTA (such as bioceramics or tricalcium silicate cement [e.g., Biodentine®, Septodont, and Lancaster, PA, USA]) should be considered in teeth where there is an esthetic concern.

Anterior and Premolar teeth - Consider the use of Collatape / Collaplug and restoring with 3mm of a non-staining restorative material followed by bonding a filled composite to the beveled enamel margin.

Molar teeth or teeth with PFM crown - Consider the use of Collatape / Collaplug and restoring with 3mm of MTA, followed by RMGI, composite, or alloy.

Follow-up

- Clinical and Radiographic exam
- No pain, soft tissue swelling, or sinus tract (often observed between first and second appointments).
- Resolution of apical radiolucency (often observed 6-12 months after treatment)
- Increased root walls (this is generally observed before the apparent increase in root length) often occurs 12-24 months after treatment).
- It increased root length.
- Positive Pulp vitality test response. (56)

10. OUTCOME ASSESSMENT: (Figure: 15)

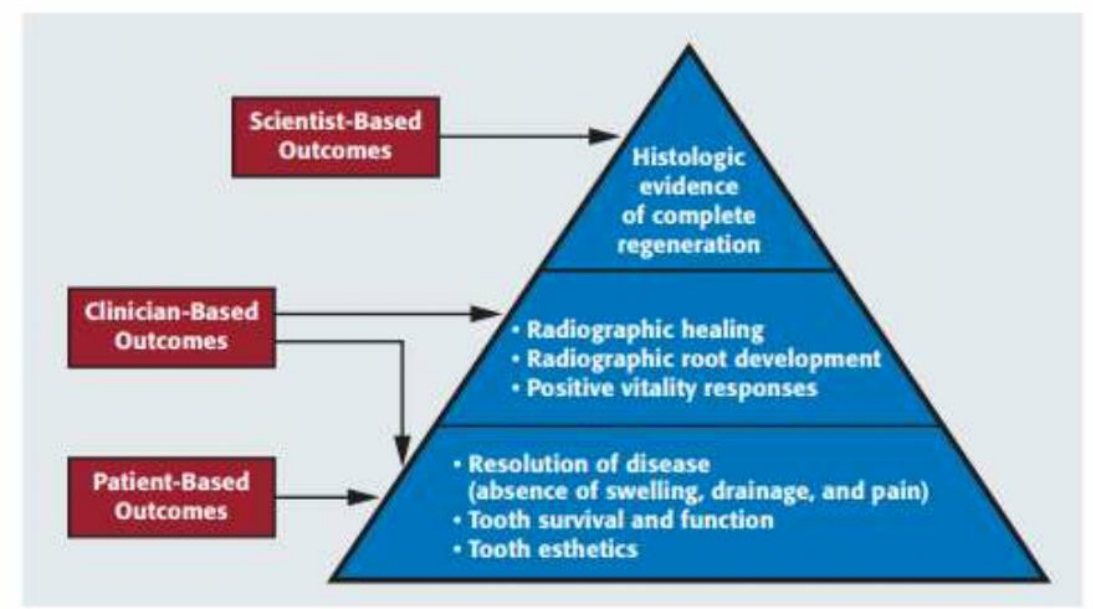


Figure 15- outcome assessment

Courtesy: *Diogenes A, Ruparel NB, Shiloah Y, Hargreaves KM. Regenerative endodontics: a way forward. The Journal of the American Dental Association. 2016 May 1;147(5):372-80.*

CLINICAL OUTCOMES

Since 2001, many RET clinical studies on human immature permanent teeth with necrotic pulp have been published.(72–75) The American Association of Endodontists clinical considerations for regenerative endodontic procedures define success by three measures:

- Primary goal (essential): The elimination of symptoms and the evidence of bony healing
- Secondary goal (desirable): Increased root wall thickness and increased root length
- Tertiary goal: a positive response to vitality testing. (56)

The primary goal of resolution of the sign/symptom of infection and bone healing is generally achievable. Two recent systematic reviews demonstrated that the primary goal of RET could be reliably achieved with high probabilities (91%-94% of periapical healing). (55,56)

11. ADVANTAGES AND DISADVANTAGES OF RET

ADVANTAGES:

The main advantages of the regenerative procedure are that

i. It allows continued root development. (9)
ii. It potentially increases the fracture resistance of the tooth by deposition of dentin on the lateral walls. (9)
iii. The tooth undergoing for RET procedure was able to regain its nociceptive response on vitality testing. (9)

DISADVANTAGES:

i. Case selection, Patient compliance and Long term follow up. (76)
ii. Technique sensitive. (76)
iii. Discoloration of crown due to triple antibiotic paste (i.e., Minocycline) and, calcium silicate cements (MTA or Biodentine). (76)
iv. Intracanal calcification (62.1%) after RET is possible. (77)
v. Retreatment of failed RET is difficult due to intracanal calcification. (77)

12. FUTURE PERSPECTIVES IN REGENERATION OF THE DENTAL PULP

There are only some examples of successful stem cell therapy: for examples, blood reconstitution, corneal regeneration, and skin regeneration. (78) The cell homing approach in tissue regeneration care often observed in normal tissue wound healing. When tissue is injured, chemotactic factor, stromal cell-derived factor 1 (SFF-1) is released, which may signal nearby perivascular stem cells and distant mesenchymal stem cells to the location of tissue damage supported studies of human and animal regenerative endodontics, regeneration of the dental could also be achieved by cell based and cell homing approaches. Basically, both cell-free and cell-based approaches of pulp tissue regeneration are still within the preclinical stage of experiments. However, from future prospect pulp tissue regeneration appears to be an attainable goal, supported the concept of stem cell-based pulp tissue engineering. (78)

CHALLENGES:

- Although the replacement pulp has the potential to revitalize teeth, it may also become susceptible to further pulp disease and may require retreatment; the implantation of engineered tissue also requires enhanced microbiological control methods required for adequate tissue regeneration. (79)
- The success of clinical applications of pulp stem cells is limited by the culture conditions and the nature of microenvironment in which the primitive multipotent pulp stem cells are maintained and expanded.
- To improve the ability of dental pulp constructs to adhere to root canal walls, it seems that the ideal scaffold design is in the same shape as gutta-percha cones. Researchers had used single-canal teeth and cylindrical scaffolds in an attempt to simplify the transplantation process. A more complex root canal anatomy will require more complex scaffolds or the use of more flexible scaffolds to perform regenerative endodontics.
- Dental pulp tissue constructs adhered more completely to the coronal aspects of the root canal and less completely to the middle and apical aspects. This likely was caused by the increasing complexity of root canal anatomy toward the apex and the physical constraints of the scaffold materials, as well as the placement method. (79)
- Since most of the tissue-engineered parts have been developed using very potent signal molecules to induce the transformation the growth of the stem cells, a way has to be

found to insure that these transformation and growth will not continue beyond control when implanted.

- Matching the aging of the implanted tissue-engineered parts with that of the surrounding tissues and organs is a great obstacle too. (79)

13. CONCLUSION

Regenerative endodontics presents a replacement era in biological and clinical endodontics. Our understanding of the clinical protocols has evolved to eliminate pulp infection and to also allow for stem cell potential to be induced within the canal and for the release of growth factors fossilized within the dentine walls. While repair rather instead of true regeneration is achieved with current protocols, it's hoped that further research within the area of stem cell-based pulp engineering will allow for true regeneration and improved treatment outcomes. RET is predicated on the concept of tissue engineering technology to regenerate the dentine-pulp complex in the canal space of immature permanent teeth damaged by caries or trauma, thus restoring development of the arrested tooth root. RET is in a position to eliminate patient's clinical symptom/signs and resolve apical periodontitis, which is that the primary goal of endodontic therapy. Continued root development (thickening of the canal walls and/or apical closure) after RET isn't predictable. However, in contrast to apexification, RET has the potential to encourage continued root maturation of immature permanent teeth with necrotic pulp/apical periodontitis. The tissue formed in the canal space after RET is not pulp-like tissue but periodontal-like tissues (cementum and bone). Although the vitality of damaged tissue in the canal space is restored, the biological function as a dental pulp is lost after RET. However immature permanent tooth with a necrotic pulp can be successfully and reliably treated with RET where the tooth remains intact with normal form, function and patient centered outcomes can be achieved.

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