EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 68, 4053:4062, OCTOBER, 2022
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
Submit Date : 20-08-2022 • Accept Date : 06-09-2022 • Available online: 01-10-2022 • DOI : 10.21608/edj.2022.156249.2218

PULP REVASCULARIZATION: THE BEGINNING OF A NOVEL AGE IN ENDODONTICS

Mohamed Ahmed ElBanna* , Tarek Aly Ezzat** *and* Mai Abdelbari***

ABSTRACT

For patients with apical periodontitis, the development and maintenance of regenerative endodontic procedures can provide them with various biological and clinical benefits. These include the ability to improve the immune system's response to tooth decay, the establishment of a functional pulp-dentin complex, and the development of root formation. The mechanism by which this type of treatment is performed is regarded as a model for the restoration of a damaged tooth.

Numerous studies have been conducted on the various components of pulp tissue engineering, including the development of scaffolds, biomolecules, and stem cells. Unfortunately, these studies have revealed that the current clinical method for revascularization can lead to unfavorable outcomes.

The development and maintenance of regenerative endodontic procedures can provide them with various clinical and biological benefits. It can lead to the establishment of a stronger and more stable tooth, which can reduce the risk of fracture. Currently, the preferred method of treating patients with non-vital pulps is to undergo apexification. However, this procedure may no longer be the best option for those with severe apical periodontitis. Revascularization is a promising treatment method that has been shown to be safe and effective.

This review aims to provide a comprehensive overview of the various challenges that stem from the development and maintenance of regenerative endodontic procedures. It also highlights the current research findings that can help improve the clinical practice of this type of treatment.

KEY WORDS: Apexification, apexogenesis, immature teeth, open apices, regenerative endodontics, revascularization.

* Endodontics, Faculty of Dental Medicine, Cairo University, Cairo, Egypt
** Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Alexandria University, Alexandria, Egypt.
*** Operative, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt.

INTRODUCTION

Pulpal and periapical disease may develop in teeth after trauma. Most dental injuries happen to children aged 7 to 10 who have insufficient apical root growth. 1,2

In 0.9 to 13% of all dental traumas, complicated crown fractures involving the enamel, dentin, and pulp take place. 3,4

In addition, physicians find it difficult to handle immature, necrotic permanent teeth. It produces a situation where standard root canal treatment goals are not only difficult to achieve, but even when they are, the root is left short, weak, and brittle. 5

Calcium hydroxide and MTA apexification 1, 2 are two more potential treatments, however none promotes continuous root development and increases the risk of root fracture. And tooth mobility is another drawback for the reason that of poor crown root ratio.6,7

The preferred treatment for wounded immature teeth with exposed pulp is vital pulp therapy (VPT). 6,8 VPT permits root development to continue, which results in apical closure and stronger root structure. Mineral trioxide aggregate (MTA) single visit apexification replaced the earliest method of calcium hydroxide apexification. 10,11

However, this method had a number of negative effects as well, which eventually inspired the creation of a biological strategy known as pulp revascularization or regenerative endodontic therapy. 12 The subject of endodontic science known as "regenerative endodontics" is new and intriguing and is characterized as biologically based treatments intended to restore damaged dentine, root structure, and pulp-dentine complex cells. 12

HISTORY

Revascularization is not a recent phenomenon. Ostby5 first described it in 1961, and Rule and Winter6 first noted root growth and apical barrier formation in cases with pediatric pulpal necrosis in 1966. In 1972, Ham et al. 13 showed that young pulpless teeth in monkeys could close at the apex. The benefits of this treatment approach were demonstrated in 2001 by Iwaya et al. 14 and in 2004 by Banchs and Trope 15. This led to a radiographically evident normal maturation of the entire root.

Revascularization's Validation

The following factors are crucial for the effective revascularization of developing teeth with apical periodontitis:

1. Canal disinfection is thought to be essential to effective treatment.
2. Creating a framework in the canal for the developing tissues: After the canal has been cleaned and disinfected, the apex is mechanically irritated to cause clot formation. This clot will act as a scaffold for the development of new tissues.
3. Bacteria-tight sealing of the access aperture: Three key elements, including stem cells, signaling molecules, and a three-dimensional physical structure, have been studied to ensure the effectiveness of this treatment. The in-growth of new tissues from the periapical area in an empty canal depends on all three factors.

Immature necrotic permanent teeth can be treated by revascularization. In fact, it would give a necessary tooth, aid in root growth that is still ongoing, and thicken the dentinal walls. 16

Revascularization as a notion is not brand-new. It was first described by Ostby 17 in 1961, and Rule and Winter 18 confirmed root growth and the emergence of the apical barrier in pediatric pulpal necrosis cases in 1966.

The search for the possibility of regeneration of the whole pulp tissue in a necrotic, infected

tooth was sparked by rare reports of regeneration of apical tissues following traumatic avulsion and replantation. Ham et al. published a 19

To determine the effectiveness of revascularization techniques for the management of traumatized immature, nonvital, infected permanent teeth, we therefore designed a prospective pilot clinical study. This study would include periodic follow-up appointments to evaluate the treatment response in terms of clinical and radiographic healing, root development, and thickening of lateral dentinal walls.

The Revascularization Mechanism

1. It's possible that a few vital pulp cells are still present at the root canal's apex. These cells may continue to divide and proliferate in the new matrix before differentiating into odontoblasts with the help of Hertwig's epithelial root sheath cells, which are highly resistant to oxidation even in the presence of inflammation. The newly generated odontoblasts can lay down atubular dentin on the lateral sides of the dentinal walls of the root canal, strengthening and fortifying the root. This causes apexogenesis, or the extension of the root. 20

2. Multipotent dental pulp stem cells may also be a factor in ongoing root growth. The existing tissue may be seeded with these cells from the apical end. Apical end cells may be rooted onto the dentinal walls already present, where they may develop into odontoblasts and deposit tertiary or atubular dentin. 21

3. The existence of stem cells in the periodontal ligament, which have the capacity to proliferate, develop into the apical end and inside the root canal, and deposit hard tissue at both the apical end and on the lateral root walls, may be responsible for the third potential mechanism. 21

4. Apical papilla or bone marrow stem cells may be responsible for the fourth potential process of root formation. Mesenchymal stem cells from the bone can also be transplanted into the canal lumen by using instruments outside the boundaries of the root canal to cause bleeding. These cells have a high proliferative potential. 22

5. The blood clot itself, which is a rich source of growth factors, may also play a significant part in regeneration. This is another theory. These could promote the differentiation, growth, and maturation of fibroblasts, odontoblasts, cement oblasts, etc. from the immature, undifferentiated mesenchymal cells in the newly formed tissue matrix. These include platelet-derived growth factor, vascular endothelial growth factor (VEGF), platelet-derived epithelial growth factor, and tissue growth factor. 23

6. The root structure of developing teeth may encourage connection between the canal space and periodontal tissue to promote apical healing with periodontal tissue (e.g., open apex, large root canal, and thin radicular dentin walls). Revascularization appears to be less likely to happen in apical apertures less than 0.3 mm and more predictable when the apical diameter is more than 1 mm. 24

Scaffold for Regenerating Pulp Tissue

Blood clot

Ostby was the first to employ the use of a blood clot to regenerate dental pulp tissues, which led to the development of granulation tissues, fibrous tissues, or cementum-like tissues inside the root canals. In 1974, Myers and Fountain16 used blood clots to successfully create 0.1–1.0 mm of soft connective tissues into the root canal. The fibrin matrix that makes up the blood clot confines the cells required for tissue repair. 25

Additionally, it offers a favorable route for fibroblasts and macrophages from the periapical region to migrate into the root canal and promote the formation of new tissue. 26

The blood clot can play a significant role in cell differentiation and, as a result, in promoting

tissue regeneration thanks to its high growth factor concentration. 27

Dentin

Fully enclosing the root canal space is an acellular dentin matrix that is loaded with growth factors28. Growth hormone, IGF-1 and -2, 29, bone morphogenetic protein-2 (BMP-2), -4 and -6, 27, and TGF—1, -2, and -3 are a few of them. 28

These growth factors have a critical role in the regulation of the inflammatory response, tissue repair and regeneration, and odontoblast differentiation when released from the dentin matrix. 29

Complete dentin tissues could regenerate in vivo when dentin was treated with EDTA 28.

RPF and PRP

Whitman brought platelet-rich plasma (PRP) to the dental community in 1997. 30 It was proposed that PRP has the capacity to draw periapical tissues' surrounding stem cells. 31 Increased critical tissue regeneration was seen in the root canals of puppy teeth treated with PRP and dental pulp cells. 32

Clinically, PRP creates vital tissue in the root canal more quickly than other methods. 31 The PRF (platelet-rich fibrin) is referred to as a second-generation platelet concentrate, while PRP is referred to as a first-generation platelet concentrate. Choukroun et al. created PRF initially (2001). It offers the advantage of a prolonged (7–14 days) gradual release of growth factors. It is therefore better than PRP, which exhibits quick release growth factors in 7–14 hours. 33

Synthetic polymers

Vacanti et al. made the initial suggestion that synthetic biodegradable polymers like polyglycolic acid (PGA), polylactic acid (PLA), and poly-lactic-coglycolide may be used as matrix for cell transplantation. 34

PGA was used in conjunction with human pulpal fibroblasts to complete the first in vitro attempt at pulp tissue engineering. A new tissue-like construct that resembled normal pulp tissue in terms of cellularity could be seen 35.

Using human dermal microvascular endothelial cells 36 or stem cells from human exfoliated deciduous teeth (SHED), the poly-L-lactic acid (PLLA) scaffold was able to create tissue that was comparable in architecture and cellularity to dental pulp tissue when implanted into immunodeficient animals. 37

The regeneration of a dental crown with enamel, dentin, and a clearly defined pulp chamber on a PGA/PLLA scaffold utilizing tooth bud cells that were transplanted into rats was also successful. 38

For the regeneration of tooth pulp, the synthetic open-cell PLA (OPLA) is yet another promising polymer. Human removed teeth with cleansed and shaped canals that had been seeded with SHED were able to cling to the root canal dentin. 39

Bioactive ceramics

The dentin marker dentin sialo phosphoprotein could be expressed by cells cultivated on porous ceramics, and they could also attach and multiply 40. It has been proposed that HA (hydroxyapatite) [(Ca10 (PO4)6(OH)2] is an efficient scaffold for dentin and dentin-pulp complex regeneration. When pulp-derived cells were combined with HA or HA/TCP and transplanted subcutaneously in naked mice, despite the fact that HA is a non-biodegradable ceramic and -TCP (Tricalcium phosphate) [-TCP Ca3(PO4)2] is considered to be biodegradable 41, bone and dentin-like mineralized tissues were produced 42.

Clinical technique for pulp-dentin regeneration

Ostby first presented the idea of renewing dental pulp tissue by introducing blood into the root canal in the 1960s, 17 but it was abandoned for more than

20 years with no apparent results. The notion that revascularization, or the restoration of a circulatory network within the root canal, is necessary for the completion of root formation, which started in traumatology, came to the fore in the 1970s. 43,44

Initial case reports on regenerative endodontic therapy then used the word "revascularization" to describe the process. 14,15 Following the first case report, several techniques for pulp dentin regeneration were introduced throughout the course of more than ten years. The American Dental Association adopted this brand-new therapy in 2011. However, based on numerous clinical and basic research investigations, evidence-based recommendations that produce the best outcomes are still being steadily produced.

Distinctions From Traditional Endodontic Therapy

The fundamental objective of REPs as an endodontic treatment is the resolution of apical periodontitis, just like traditional root canal therapy. However, there are some variations in the fundamental idea and associated practices. First off, juvenile permanent teeth with thin walls and widely opened apices are the teeth to which REPs were first applied. For the purpose of preventing infection during endodontic therapy, aggressive filing is done.

However, mechanical debridement with endodontic files is not advised in REPs in order to preserve the health of the stem cells in the apical tissue and prevent additional thinning of the already fragile root canal wall. 14,45 Instead, it is suggested to use an irrigant and intracanal medications to do sufficient chemical cleaning.

Second, when disinfecting REPs, careful consideration of cell cytotoxicity should be made. In spite of the fact that sufficient disinfection can produce a sterile environment, pulp tissue regeneration necessitates a delicate balance between disinfection and the microenvironment required for cell viability in order to encourage stem cell survival and differentiation. For disinfection, sodium hypochlorite (NaOCl) solutions in concentrations ranging from 0.5% to 6% have been utilized. 46 Recent research has shown that NaOCl concentrations more than 3% may be harmful to stem cells of the apical papilla (SCAP) and impair cell adhesion on the dentin surface. 47-49

Due to these factors, new research, including therapeutic recommendations from the American Association of Endodontists (AAE), suggests using REPs with lower NaOCl concentrations. At a similar vein, using calcium hydroxide or triple antibiotic paste (TAP) in lesser doses as intracanal medications was advised. 50

Since a recent study found that ethylenediamine tetra acetic acid (EDTA) solution may release diverse growth factors locked up in dentin, hence encouraging differentiation of dental pulp stem cells (DPSCs) into odontoblast-like cells, EDTA has been advised as the final irrigation. 55

Finally, tissue engineering is used by REPs to create a pulp dentin structure in the canal. The three components of tissue engineering—scaffolds, growth factors, and stem/progenitor cells—benefit from intracanal hemorrhage. 51-53 It is hypothesized that causing bleeding into the canal causes mesenchymal stem cells (MSCs) to be delivered to the area. 53 In addition to serving as a scaffold, the blood clot that forms is a rich supply of growth factors that could be crucial in the regeneration process. 51,54

Clinical procedure: revascularization

Interventions

The standardized operating technique of RET calls for two treatment visits, according to the most recent operational standards for RET published by the American Academy of Endodontics (AAE) 56 and the European Society of Endodontics (ESE) 57.

Infection is managed and inflammation is reduced on the initial visit. The second appointment is when the revascularization and regeneration of the pulp takes place.

With the exception of the anesthetic and rubber dam implantation processes, all RET procedures will be carried out under a dental microscope.

Appointment No. 1

in an effort to reduce inflammation and control infections. The following elements will be part of the initial appointment:

(1) Isolation and anesthesia. Following local anesthetic, the tooth is cleaned and isolated with a rubber dam.

(2) Access planning. The pulp chamber will be totally unroofed when the tooth's entire decaying portion has been removed.

(3) Making a root canal. It will be decided on the working length and the initial apical file.

(4) Irrigation of root canals. Due to the significant reliance on chemical irrigants for canal disinfection, a needle with side-port vents will be inserted into the apical third of the tooth, and irrigation will be carried out using needles with a slow infusion rate. Three steps of thorough irrigation and drying with sterile paper points are required for each canal.

- 20 mL of 1.0% sodium hypochlorite applied for 5 minutes as irrigation (NaOCl).
- 5-min irrigation with 20 mL 17% ethylene diamine tetra acetic acid (EDTA);
- 5-min irrigation with 20 mL 17% ethylene diamine tetra acetic acid (EDTA);

Intra-pulpal dressings with medication. The root canal will be homogeneously filled to the working length with calcium hydroxide paste or low concentration triple antibiotic paste to prevent bacterial recurrence and provide ongoing disinfection.

To reduce the danger of discoloration, seal the pulp chamber with a dentin bonding agent if the triple antibiotic paste is used.

Alternatively, mix the three antibiotics in a 1:1:1 ciprofloxacin: metronidazole: minocycline ratio to a final concentration of 0.1 mg/ml.

Syringe-deliver into the canal system. Make sure the level of triple antibiotic is kept below CEJ if it is used (minimize crown staining).

IRM, glass-ionomer, or similar temporary substance in the amount of 3–4 mm should be used to seal. Give the patient a 1-4 week break.

Appointment No. 2

Patients will come back for a follow-up appointment in two to four weeks.

The first session will be repeated if the symptoms are not reduced. Only those patients who are symptom-free will move on to the following RET treatment phase.

The following elements will be part of the second appointment:

(1) Operation field disinfection, cleaning, anesthetic, field isolation, and temporary seal removal

(2) irrigation in root canals. After flushing away the calcium hydroxide paste with 20 mL of 17% EDTA for 5 min, the canal will be irrigated with sterile saline solution to lessen the cytotoxicity of the EDTA. Using sterile paper points, dry the channel.

Both blood clots and platelet-rich fibrin (PRF) can revascularize an area:

(3) creating the PRF. The median cubital vein will be used to extract 10 mL of whole blood, which will then be placed into 10-ml plastic tubes without anticoagulant reagent and centrifuged for three minutes at 800 rpm using Centrifuge to make PRF. The upper yellow fluid liquid (i-PRF) will be

collected after centrifugation as near as feasible to the layer of red cells (Fig. 4).

(4) RET with an i-PRF. In order to cause bleeding, K-files will be purposefully employed to pierce the periapical tissues with instrumentation that extends up to 2-3 mm past the apical foramen. Blood will only be present in the root canal's top third, though. The root canal will get an injection of the i-PRF. 3 mm beneath the cement-enamel junction (CEJ). A 3-mm layer of MTA will be applied over the PRF following a coagulation time of 10-15 minutes.

K-files will be purposefully used to pierce the periapical tissues through over instrumentation up to 2-3 mm beyond the apical foramen in order to cause bleeding during RET with BC. The canal gap will be allowed to fill with blood to a level 3 mm below the CEJ. If necessary, cover the blood clot with a resorbable matrix, such as CollaPlug, Collacote, CollaTape, or another material, and use white MTA/CaOH as a capping material. MTA has a reputation for being discolored. When there is an aesthetic difficulty with a tooth, alternatives to MTA should be taken into account.

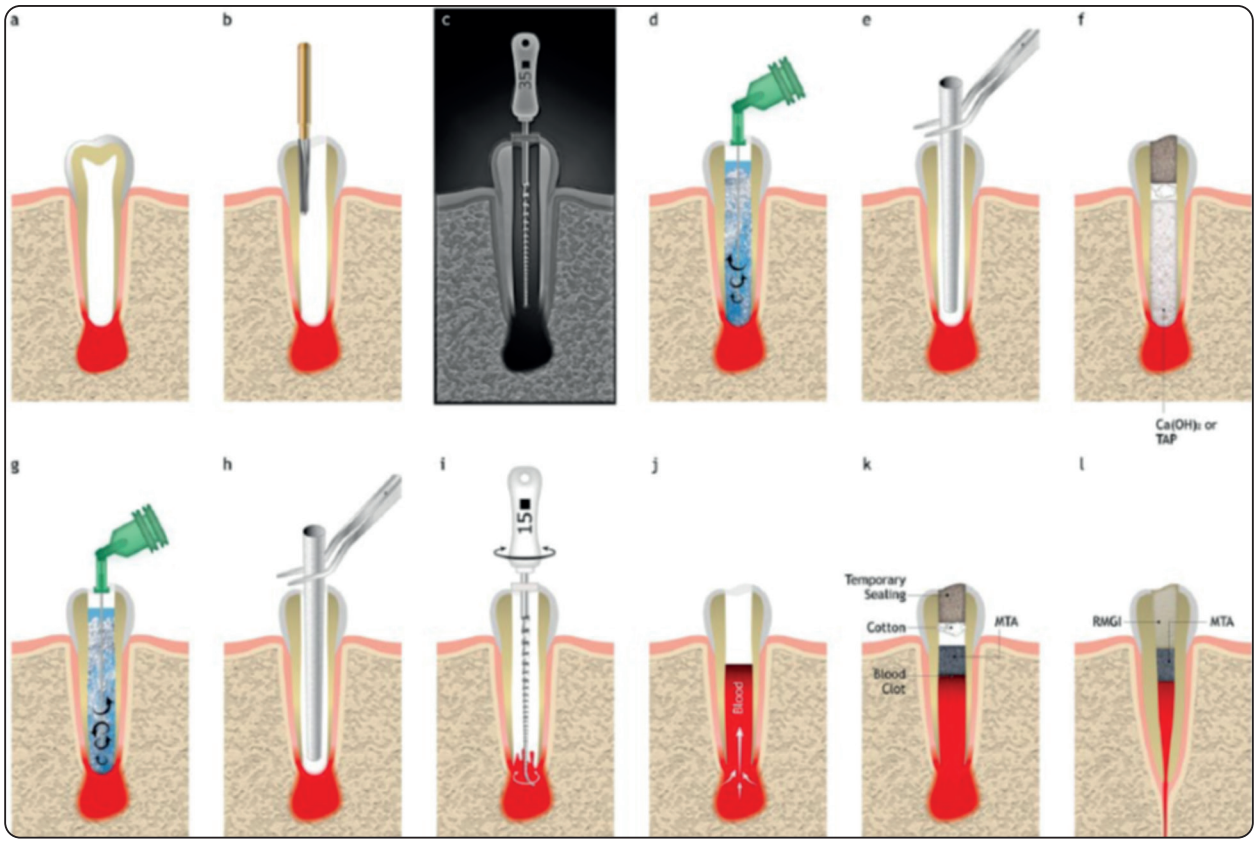

Fig. (1). Schematic illustration of revascularization procedure. Revascularization is considered for immature teeth with open apices (a). After accessing the opening (b), Gentle irrigation limited to coronal part of the chamber is performed. A radiograph with K-file insertion (c), Provides the approximate tooth length, which helps to determine working length. Low concentration of NaOCl (1.5 or less than 3%, 20 mL/canal, 5 min) is used for disinfection (d), Following which saline or 17% EDTA is used. After copious irrigation and canal drying with paper point (e) Intracanal medicaments, such as Ca(OH)2 or TAP were placed, and covered with temporary filling material (f) After confirming the absence of any signs of infection, the final step is initiated. Final irrigation is performed with sterile saline and 17% EDTA (g) After the canal has dried (h) Pre-curved K-file is introduced 2 mm past the apical foramen and rotated to induce bleeding (i) Blood fills the canal from the bottom and the blood clot can be identified after 15 min (j) After the blood clot is confirmed, capping materials such as MTA are placed over the blood clot (k) Regeneration of pulp-dentin leads to root development with thickening, lengthening, and apical closure, as well as maintenance of tooth vitality (l).

The MTA layer will be followed by a cavity-lining layer of self-adhering flowable composite, and the final filling will be completed with adhesive resin for long-lasting restoration.

Follow-up radiographic and clinical examination

* No discomfort, soft tissue edema, or sinus congestion (often observed between first and second appointments).

* Apical radiolucency resolution (often observed 6-12 months after treatment).

* Expanded root wall width (this is generally observed before apparent increase in root length and often occur 12-24 months after treatment).

* Pulp vitality test.

* Lengthened roots.

Limitations

There are not many restrictions with this strategy. 59 Although, Long-term clinical data are still unavailable, and the origin of the regenerated tissue is unknown. Another drawback is that a revived tooth may be vulnerable to recurrent pulp disease and may need retreatment. It's also possible that the entire canal may be calcified, which would compromise aesthetics and may make future endodontic procedures more challenging, should they be necessary.

Revascularization is not the best option when post and core are the only remaining restorative therapy options since the essential tissue in the apical two thirds of the canal cannot be violated for post placement.

The revascularization method assumes that the formation of a blood clot yields a matrix that traps the cells capable of forming new tissue. But the concentration and composition of cells trapped in the fibrin clot is unpredictable. This limitation can be overcome by use of platelet concentrates. Platelet rich plasma is an ideal scaffold for revascularization.

CONCLUSION

The pulp space's induced creation and regeneration of essential tissues can thicken the tooth's root structure, making it stronger and possibly less likely to fracture. The first-choice treatment for immature permanent teeth with non-vital pulp may no longer be the apexification surgery. Due to the preservation of biological principles and the potential to shorten the treatment time for developing teeth, revascularization is a relatively new and promising therapeutic option.

REFERENCES

1. Andreasen JO, Ravn JJ. Epidemiology of traumatic dental injuries to primary and permanent teeth in a Danish population sample. Int J Oral Surg 1972; 1:235- 239.
2. Bastone EB, Freer TJ, McNamara JR. Epidemiology of dental trauma: a review of the literature. Aust Dent J 2000; 45:2-9.
3. Canakci V, Akgul HM, Akgul N, Canakci CF. Prevalence, and handedness correlates of traumatic injuries to the permanent incisors in 13-17-year-old adolescents in Erzurum, Turkey. Dent Traumatol 2003; 19:248-254.
4. Tapias MA, Jimenez-Garcia R, Lamas F, Gil AA. Prevalence of traumatic crown fractures to permanent incisors in a childhood population: Mostoles, Spain. Dent Traumatol 2003; 19:119-122.
5. Deepak.S, Nivedhitha.M.S, Clinical Practice and Guidelines and Protocols for Revascularization Procedure – A Review, Deepak.S et al /J. Pharm. Sci. & Res. Vol. 9(11), 2017, 2089-2092
6. Rafter M. Apexification: a review. Dent Traumatol 2005; 21:1-8.
7. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. J Endod 1999; 25:197–205.
8. Witherspoon DE. Vital pulp therapy with new materials: new directions and treatment perspectives-permanent teeth. J Endod 2008;34: S25-28.
9. Katebzadeh N, Dalton BC, Trope M. Strengthening immature teeth during and after apexification. J Endod 1998; 24:256-259
10. Resenberg B, Murry PE, Namerow K. The effect of calcium hydroxide root filling on dentine fracture strength.

Dent Traumatol 2007; 23:26– 29.

11. Shabahang S, Torabinezad M. Treatment of teeth with open apices using mineral trioxide aggregate. Pract Periodontics Aesthet Dent 2000;12(3):315–320.
12. Cotti E, Mereu M, Lusso D. Regenerative treatment of an immature, traumatized tooth with apical periodontitis: report of a case. J Endod 2008;34(5):611–616. DOI: 10.1016/j.joen.2008.02.029
13. Ham JW, Patterson WW, Mitchell DF. Induced apical closure of immature pulpless teeth in monkeys. Oral Surg Oral Med Oral Pathol. 1972, 33
14. Iwaya S, Ikawa M. Revascularization of a tooth with apical periodontitis and a sinus tract Dent Traumatol, 2001; 17:185-187.
15. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol? J Endod. 2004; 30:196-200.
16. Cvek M, Cleaton-Jones P, Austin J, et al. Pulp revascularization in reimplanted immature monkey incisors: predictability and the effect of antibiotic systemic prophylaxis. Endod Dent Traumatol 1990;6:157–169.
17. Ostby BN. The role of the blood clot in endodontic therapy: an experimental histologic study. Acta Odontol Scand 1961; 19:324 –53.
18. Rule DC, Winter GB. Root growth and apical repair subsequent to pulpal necrosis in children. Br Dent J 1966; 120:586 –90.
19. Ham JW, Patterson SS, Mitchell DF. Induced apical closure of immature pulpless teeth in monkeys. Oral Surg Oral Med Oral Pathol 1972; 33:438–49.
20. Yousef Saad A. Calcium hydroxide and apexogenesis. Oral Surg Oral Med Oral Pathol. 1988; 66:499-501.
21. Gronthos S, Brahim J, Li W et al. Stem cell properties of human dental pulp stem cells. J Dent Res. 2002; 81:531-535.
22. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000; 97:13625-13630
23. Wang Q, Lin XJ, Lin ZY, Liu GX, Shan XL. Expression of vascular endothelial growth factor in dental pulp of immature and mature permanent teeth in human. Shanghai Kou Qiang Yi Zue. 2007; 16:285-289.
24. Andreasen JO. Pulp and periodontal tissue repair - regeneration or tissue metaplasia after dental trauma: a review. Dent Traumatol. 2012; 28(1):19-24.
25. Bansal R, Bansal R. Regenerative endodontics: A state of the art. Indian J Dent Res. 2011; 22:122-131.
26. Harrison JW, Jurosky KA. Wound healing in the tissues of the periodontium following periradicular surgery. I. The incisional wound. J Endod. 1991; 17:425-435.
27. Saber SE. Tissue engineering in endodontics. J Oral Sci. 2009; 51:495-507.
28. Guo W, He Y, Zhang X, Lu W, Wang C, Yu H et al. The use of dentin matrix scaffold and dental follicle cells for dentin regeneration. Biomaterials, 2009; 30:6708-6723
29. Begue-Kirn C, Smith AJ, Loriot M, Kupferle C, Ruch JV, Lesot H. Comparative analysis of TGF beta s, BMPs, IGF1, msxs, fibronectin, osteonectin and bone sialoprotein gene expression during normal and in vitro-induced odontoblast diff erentiation. Int J Dev Biol. 1994; 38:405-420.
30. Freymiller EG, Aghaloo TL. Platelet-rich plasma: Ready or not? J Oral Maxillofac Surg. 2004; 62:484-488
31. Torabinejad M, Turman M. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: A case report. J Endod. 2011; 37:265-268.
32. Zhu W, Zhu X, Huang GT, Cheung GS, Dissanayaka WL, Zhang C. Regeneration of dental pulp tissue in immature teeth with apical periodontitis using platelet-rich plasma and dental pulp cells. Int Endod J. 2013; 46:962-970.
33. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009; 108:707-713.
34. Vacanti JP, Morse MA, Saltzman WM, Domb AJ, Perez-Atayde A, Langer R. Selective cell transplantation using bioabsorbable artifi cial polymers as matrices. J Pediatr Surg. 1988; 23:3-9.
35. Mooney DJ, Powell C, Piana J, Rutherford B. Engineering dental pulp-like tissue in vitro. Biotechnol Prog. 1996; 12:865-868.
36. Nör JE, Peters MC, Christensen JB, Sutorik MM, Linn S, Khan MK et al. Engineering and characterization of functional human microvessels in immunodeficient mice. Lab Invest. 2001; 81:453-463.

37. Sakai VT, Zhang Z, Dong Z, Neiva KG, Machado MA, Shi S et al. SHED differentiate into functional odontoblasts and endothelium. J Dent Res. 2010; 89:791-796.

38. Young CS, Terada S, Vacanti JP, Honda M, Bartlett JD, Yelick PC. Tissue engineering of complex tooth structures on biodegradable polymer scaff olds. J Dent Res. 2002; 81:695-700.

39. Gotlieb EL, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F. An ultrastructural investigation of tissue-engineered pulp constructs implanted within endodontically treated teeth. J Am Dent Assoc. 2008; 139:457-465.

40. Zhang W, Walboomers XF, van Kuppevelt TH, Daamen WF, Bian Z, Jansen JA. The performance of human dental pulp stem cells on different three-dimensional scaffold materials. Biomaterials. 2006; 27:5658-5668.

41. Mastrangelo F, Nargi E, Carone L, Dolci M, Caciagli F, Ciccarelli R et al. Tridimensional response of human dental follicular stem cells onto a synthetic hydroxyapatite scaffold. J Health Sci Tokyo. 2008; 54:154.

42. Daculsi G, LeGeros R. Tricalcium phosphate / hydroxyapatite biophasic calcium phosphates. Handbook of Bioceramics and their Clinical Applications. London: Woodhead Publishing Ltd. 2008, 395.

43. Galler KM. Clinical procedures for revitalization: current knowledge and considerations. Int Endod J 2016; 49: 929–936.

44. Skoglund A, Tronstad L and Wallenius K. A microangiographic study of vascular changes in replanted and autotransplanted teeth of young dogs. Oral Surg Oral Med Oral Pathol 1978; 45: 17–28.

45. Cvek M. Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. A retrospective clinical study. Endod Dent Traumatol 1992; 8: 45–55.

46. Kontakiotis EG, Filippatos CG, Tzanetakis GN, et al. Regenerative endodontic therapy: a data analysis of clinical protocols. J Endod 2015; 41: 146–154.

47. Martin DE, De Almeida JF, Henry MA, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod 2014; 40: 51–55.

48. Chang YC, Huang FM, Tai KW, et al. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 92: 446–450.

49. Ring KC, Murray PE, Namerow KN, et al. The comparison of the effect of endodontic irrigation on cell adherence to root canal dentin. J Endod 2008; 34: 1474–1479.

50. Ruparel NB, Teixeira FB, Ferraz CC, et al. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod 2012; 38: 1372–1375.

51. Nosrat A, Homayounfar N and Oloomi K. Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic immature teeth: a literature review and report of a case. J Endod 2012; 38: 1428–1434.

52. Wigler R, Kaufman AY, Lin S, et al. Revascularization: a treatment for permanent teeth with necrotic pulp and incomplete root development. J Endod 2013; 39: 319–326.

53. Lovelace TW, Henry MA, Hargreaves KM, et al. Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. J Endod 2011; 37: 133–138.

54. Shah N, Logani A, Bhaskar U, et al. Efficacy of revascularization to induce apexification/apexogensis in infected, nonvital, immature teeth: a pilot clinical study. J Endod 2008; 34: 919–925; discussion 1157.

55. Galler KM, D'Souza RN, Federlin M, et al. Dentin conditioning codetermines cell fate in regenerative endodontics. J Endod 2011; 37: 1536–1541.

56. AAE clinical considerations for a regenerative procedure [https://www.aae.org/specialty/wp-content/uploads/sites/2/2017/06/currentregenerativeendodonticconsiderations.pdf]

57. Galler KM, Krastl G, Simon S, Van Gorp G, Meschi N, Vahedi B, et al. European Society of Endodontology position statement: revitalization procedures. Int Endod J. 2016;49(8):717–23. https://doi.org/10.1111/iej.12629.

58. Yuee Liang†, Rongyang Ma†, Lijuan Chen, Efficacy of i-PRF in regenerative endodontics therapy for mature permanent teeth with pulp necrosis: study protocol for a multicentre randomised controlled trial. Liang et al. Trials (2021) 22:436 https://doi.org/10.1186/s13063-021-05401-7

59. Dr. Udhya J, Dr. Varadharaja MM. Revascularization of dental pulp - contemporary review. IJRID. 2013; 3(6).