RGUHS
Journal of Dental Sciences

Vivek T, RJDS 2025;17(1):1-6

REVIEW ARTICLE

Clinical Challenges in Regenerative Endodontic Therapy: A Review

Vivek Thakur
Medical Officer (Dental), Department of Conservative Dentistry and Endodontics, Himachal Pradesh Government Dental College and Hospital, Shimla, Himachal Pradesh, India. E-mail: vtvivekthakur421@gmail.com

Received date: January 31, 2024; **Accepted date:** May 15, 2024; **Published date:** March 31, 2025

Abstract

Regenerative endodontic therapy (RET) has drawn a great deal of interest and is widely recognized because it preserves the function and endurance of teeth. Although it is routinely performed on immature teeth with periapical disease, the treatment outcomes remain unpredictable. Instead of producing fibrous connective tissue and amorphous mineral deposits, pulp-dentin regeneration should be the real benchmark of RET. Still, we have not yet achieved the benchmark of full innervation and vascularization of the generated tissue in the root canal. The main challenges faced with the current clinical protocol of RET are the complete disinfection of root canal space and enhancing pulplike tissue regeneration. This article will address the key challenges and strategies that focus on realizing the goal of regenerative endodontic therapy.

Keywords: Disinfection, Immature teeth, Regenerative endodontic therapy, Scaffolds, Stem cells

Introduction

Regenerative endodontic therapy (RET) is a relatively new treatment approach introduced only in the past two decades.[1] RETs are a feasible method for treating immature necrotic teeth. This technique has a good survival and success rate.[2] RETs have recently been effective in treating mature necrotic teeth with apical periodontitis because, an open apex allows for better blood flow, immature teeth with open apexes are thought to be better candidates for this form of therapy.[3]

According to a recent study, controlling the bacterial load in the intricate root canal system is the most critical stage in regenerative endodontic therapy.[4] However, different disinfection treatment techniques may be required for necrotic mature and immature teeth. Necrotic immature teeth are mainly disinfected by chemical methods such as antimicrobial irrigation and intracanal dressing of medicament without mechanical instrumentation.[5]

The primary challenge with disinfection in RET is that a lower dosage of different intracanal medications and irrigation solutions is advised to preserve the survival of stem cells from apical papillae.[6] Therefore, the goal of RET-related research has been to enhance disinfection strategies in infections to a level compatible with the growth of vital tissue to encourage mineralization and root development. Finally, the development of new connective tissue into the root canal to support revascularization/revitalization is severely hampered by the presence of the bacterial biofilm attached to the surface of dentin and penetrating the large tubules.[7]

The size of the apical foramen appears to be a key consideration in regenerative endodontic therapy. It is quite challenging to achieve an adequate blood supply in the root canal system due to the narrow apical aperture. However, a systematic review shows positive outcomes following RET may be attained if the apical foramen's size falls between 0.5 and 1 mm.[8]

Teeth with large and open apices are considered suitable for pulp rejuvenation. Mature teeth have several calcifications inside the pulp tissue, a narrower apical foramen, and a smaller-sized pulp chamber than immature teeth.[9] In mature teeth, the apical region undergoes an increase in secondary dentin and cementum deposition, which causes apical foramen constriction, hypercementosis, and stenosis. [10]

The migration of local stem cells from the surrounding tissue into the root canal may, therefore, be inhibited by these modifications. The blood arteries, lymphatics, and nerve supplies that typically reach the root canal through the apical foramen are diminished by apical constriction. All of this results in a decreased capacity for regeneration and complicates the utilization of regenerative therapies in mature teeth.

History

Revascularization procedures were initially proposed by Nygaard-Ostby more than 60 years ago.[11] Ten years later, Nygaard-Ostby and Hjortdal noticed that when bleeding is caused and the root canals are partially filled with blood, cellular cementum deposits occur in the disinfected canals.[12] A technique called revascularization that Iwaya *et al.,* described in 2001 thickens the walls of the root canal and promotes further root growth.[1] After a few years, Trope and Banchs proposed a clinical protocol for RET in infected necrotic immature teeth. RETs have now successfully been utilized to treat apical periodontitis and necrotic pulp in mature teeth.[13] The treatment resolved apical periodontitis and eliminated clinical signs and symptoms like immature teeth.[3]

Disinfection challenges in regenerative endodontic therapy

A recent comprehensive study shows that persistent infection accounted for 79% of failed regenerative clinical cases.[14] Because mature teeth have a more complex root canal system than immature teeth, adequate disinfection is more challenging in mature teeth than in young teeth. Since the introduction of RET, the goal of effectively disinfecting root canals while preserving stem cell viability and the dentin matrix's biofunctionality has been sought.[15]

The most potent disinfectant and root canal irrigation solution is sodium hypochlorite, which also has favorable properties such as tissue solubility, proteolytic activity, and bactericidal action against bacteria and bacterial endodontic biofilms.[16] High concentrations of biocides, such as chlorhexidine or sodium hypochlorite, are effective against microorganisms. Still, they adversely affect the survival and differentiation of stem cells of the apical papilla (SCAPs) and as a result, they are no longer advised to undergo RET.[6,17] In these situations, it has been found that a more biocompatible method of using irrigants is low-concentration sodium hypochlorite followed by EDTA. Furthermore, EDTA did not have noticeable toxic effects and released significantly higher growth factors into root canal space.[18]

Although calcium hydroxide [$Ca(OH)_2$] is a commonly used intracanal medicament, calcium hydroxide has low antimicrobial action and no persistent antimicrobial activity.[19] Because dentin and hydroxylapatite decrease the antibacterial action of $Ca(OH)_2$, a standard intracanal medicine used in root canal therapy, calcium hydroxide is not as effective at killing intracanal microbes.[20] Compared to $Ca(OH)_2$ and double antibiotic paste (DAP), triple antibiotic paste (TAP) has a broader antibacterial efficacy.[21] TAP can, however, discolor teeth at an amount higher than 1 mg/mL.[22] It has been suggested that clindamycin be used instead of minocycline to create modified TAP (mTAP). Without discoloring the dentin, it has been found to have an antimicrobial action similar to that of TAP. [23]

A randomized trial found that TAP at a concentration of 1 mg/mL was more effective than $Ca(OH)_2$ in 2% chlorhexidine gluconate about reducing the intracanal bacterial load in conventional endodontic infections, but $Ca(OH)_2$ still seems to be the most effective treatment in the majority of cases.[24]

Antibiotics can be potentially dangerous to stem cells even though they are more efficient against intracanal bacteria when taken at higher dosages.[25] Although the cells can tolerate them at lower concentrations, they still have lesser impact on intracanal microbial biofilms.[7] These residual microorganisms might affect The success of the regeneration process, tissue development, and stem cell differentiation.[26]

To attain a proper balance between the survival of the stem/progenitor cells and the disinfection of the root canal system, the RET disinfection regimen needs to be carefully reevaluated which could ultimately result in a positive outcome.

Stem/progenitor cells-based challenges in regenerative endodontic therapy

Three key components for pulp-dentin complex regeneration are stem cells, scaffolds, and growth factors. Five different types of human dental stem cells have been identified and isolated as possible cell sources for pulp-dentin complex regeneration. These include stem cells from the apical papilla (SCAP), tooth germ progenitor cells (TGPCs), dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (SHED), and periodontal ligament stem cells (PDLSCs).[27]

Because the quantity and quality of dental pulp tissue decrease significantly with age, these changes may impact the regeneration capability of stem cells. If the canal lacks the pulp tissue characterized by the presence of highly specialized odontoblasts, the biological activities of a revitalized or revascularized tooth, such as defense against caries invasion, will be affected.

According to earlier studies, mature and immature dental pulps contain various stem cells that can develop into odontoblast-like cells in response to suitable stimuli. Uncertainty exists regarding the biological activity of the odontoblast-like cells that evolved from several populations of stem cells. The mesenchymal stem cells derived from dental pulp currently possess the greatest capacity to develop into cells resembling odontoblasts. Mesenchymal stem cells are considered as dental pulp stem cells.[28]

A notable accumulation of undifferentiated stem cells expressing CD105, CD73, and STRO-1 into the canal space was caused by the generation of bleeding during revitalization treatments. They postulated that rather than originating from the systemic circulation, these stem cells were most likely derived from local tissues, such as the apical papilla near the root apex.[29] Thus, it is possible that these stem cells will play a role in the regeneration of pulp tissue. The periapex, including the periodontal ligament, may provide stem cells introduced into the canal through induced intracanal bleeding. These stem cells can develop into cells that resemble cementoblasts and osteoblasts. However, clinically, it might be difficult to tell if the apical papilla is still there, especially when apical periodontitis is present.[30] Even though mesenchymal stem cells can be administered into the canals during revitalization procedures, they still need the appropriate environmental cues in the canal to guide them toward differentiating into odontoblast-like cells. After revitalization techniques, the type of microenvironment in the canals remains unknown. To regulate stem/progenitor cells to differentiate into odontoblast-like cells in the root canal space *in vivo*, revitalization processes make it difficult to recreate an ideal microenvironment during embryonic pulp development. The objective of regenerative treatment is to replicate the pulpal microenvironment in the canals of young teeth with necrotic pulps. A particular anatomical site known as a niche (microenvironmental cues) controls how stem cells contribute to tissue homeostasis, regeneration, and repair. The new microenvironment will affect stem cells' biological behavior and fate if they leave from their specified niches.[31]

According to a study, rather than being origin-associated, the destiny of transplanted stem/progenitor cells may be site-associated.[32] As an example, stem cells will probably differentiate into cells that resemble cementoblasts or osteoblasts if they are introduced into an environment that supports the formation of cementum or bone. Aged stem/progenitor cells can be made to function again by a young microenvironment, while young stem/progenitor cells can be affected by an old microenvironment.[33] Thus, the ideal conditions for tissue regeneration are young stem cells and a young microenvironment.

Scaffolds based challenges in regenerative endodontic therapy

Scaffolds direct and promote tissue regeneration, serve as carriers for particular cell types and give stem cells and growth factors a suitable environment. A biodegradable scaffold that delivers cells carrying growth factors is appropriate for regenerative endodontics. Different scaffolds, like those made of synthetic or natural polymers, are being studied and reported on with various degrees of success.[34] Recent attempts have been made at using blood clots, platelet-rich plasma and platelet-rich fibrin as scaffolds in regenerative endodontics.[35]

These days, regenerative endodontics creates a scaffold for pulp-dentin regeneration by causing bleeding and forming an intracanal blood clot. The BC may act as a cross-linked scaffolding on which fibroblasts, macrophages and stem cells migrate.[36] Due to bleeding induction, SCAPs are delivered into the root canal space through the apical foramen of young teeth with open apices. With this method, injecting foreign stem cells is not necessary. Along with the low cost, clinical ease of use, quick setting time, and cervical sealability of materials based on tricalcium silicate, these attributes make this a treatment option that appeals to patients and dental professionals.

During RETs, the induction of bleeding into the root canal is a crucial yet uncertain step. Indeed, even after using a vasoconstrictor-free anesthetic, there have been multiple instances when it was difficult to induce bleeding.[37] The failure to cause bleeding into the canal after unsuccessful regenerative treatments was linked to the resolution of the inflammatory response following intracanal medication dressing, which possibly made it more challenging to cause bleeding. Additionally, periapical tissue laceration may harm cells necessary for epithelial-mesenchymal interactions. A fibrin blood clot is also unstable and prone to disintegration.[38] Furthermore, compared to other platelet concentrates, the concentration of growth factors BC is limited.[39]

Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) concentrates are better alternatives due to their higher concentration of stem cells and growth factors and their predictable introduction into the canal system, which overcomes the drawbacks of a blood clot. [40]

PRP is an autologous matrix, rich in growth factors found in platelet alpha (α) granules. It also satisfies many requirements for an appropriate matrix, suggesting it may be used as a suitable matrix in regenerative endodontic procedures. The simplicity of administration and rapid induction of vital tissues into the root canal are benefits of using PRP as scaffold. However, the cost of treatment could go up because PRP preparation requires specific tools and chemicals.[41]

The drawbacks of using PRP have led to developing PRF, a second-generation platelet concentrate. Choukroun's platelet-rich fibrin has gained more attention due to its low production costs, ease of preparation and entirely autologous composition without chemical additions. Moreover, an extremely resilient and flexible fibrin membrane is produced, which breaks down slowly after application.[42]

PRF, the second generation of platelet concentrates, is superior to PRP. First, exogenous agents like thrombin are excluded during PRF preparation. Second, PRF is a structured fibrin gel that entails leukocytes and platelets and eventually releases various growth factors. Third, cytokines and immune cells within a PRF clot may also fight against infection. Nevertheless, a study has also shown that PRP, when used in RETs, performed better than blood clots and PRF in periapical wound healing.[43]

As an autologous platelet and leukocyte mesh, PRF serves as a reservoir for the gradual, continuous release of growth factors for 7 to 14 days, as opposed to PRP, which only has an effect for the first 24 hours. Platelets, leukocytes and a number of crucial healing proteins are all incorporated into PRF's dense fibrin matrix. Additionally, PRF attracts nearby populations of stem cells with its chemoattractant properties, which induces mineralization and regeneration.[44] According to recent studies, platelet-rich fibrin may be more capable of regenerating than conventional platelet-rich plasma.[45]

Conclusion

Regenerative endodontic therapy uses tissue engineering technologies to repair the dentin-pulp complex in the canal space of immature permanent teeth compromised by trauma or caries, thereby resuming the formation of the tooth root that has been stopped. The major objective of endodontic therapy is to resolve apical periodontitis and eliminate the patient's signs and symptoms, which RET can accomplish. Revascularization treatment has replaced apexification therapy as an alternate option for immature permanent necrotic teeth with or without infected pulp since it can promote canal wall thickening and/or continuous root growth.

Furthermore, RET is a biologically based treatment promising for treating mature teeth with infected necrotic pulp and apical periodontitis. It can eliminate clinical signs/symptoms and resolve apical periodontitis, with a favorable outcome comparable with traditional root canal therapy. Following RET, pulp-like tissues rather than true pulp tissue form in the canal space. After RET, the biological function of the tooth pulp is lost, but the vitality of the damaged tissue in the canal space is preserved.

Additionally, this review highlighted the drawbacks, including the inability of bleeding induction and the imbalance between disinfection and stem/progenitor cell viability. However, it is clear that pulp-dentin regeneration offers both clinical benefits, such as encouraging root development and biological benefits, such as improved immune system function and tooth homeostasis. Instead of allowing for complete regeneration, the current techniques allow repair; however, proper regeneration is expected to be possible with further investigations in stem cell-based pulp engineering.

Conflict of Interest

Nil

References

1. Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol 2001;17:185-7.
2. Li L, Pan Y, Mei L, Li J. Clinical and radiographic outcomes in immature permanent necrotic evaginated teeth treated with regenerative endodontic procedures. J Endod 2017;43:246-51.
3. Saoud TMA, Sigurdsson A, Rosenberg PA, *et al*. Treatment of a large cystlike inflammatory periapical lesion associated with mature necrotic teeth using 76 regenerative endodontic therapy. J Endod 2014;40:2081-6.
4. Diogenes A, Hargreaves KM. Microbial modulation of stem cells and future directions in regenerative endodontics. J Endod 2017;43:S95-101.
5. Diogenes A, Ruparel NB, Shiloah Y, *et al*. Regenerative endodontics: a way forward. J Am Dent Assoc 2016;147:372-80.
6. Trevino EG, Patwardhan AN, Henry MA, *et al*. Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. J Endod 2011 37(8):1109-15.
7. Verma P, Nosrat A, Kim JR, *et al*. Effect of residual bacteria on the outcome of pulp regeneration *in vivo*. J Dent Res 2017;96:100-6.
8. Fang Y, Wang X, Zhu J, *et al*. Influence of apical diameter on the outcome of regenerative endodontic treatment in teeth with pulp necrosis: a review. J Endod 2018;44:414-31.
9. Carvalho TS, Lussi A. Age-related morphological, histological and functional changes in teeth. J Oral Rehabil 2017;44:291-8.
10. Pinchi V, Forestieri AL, Calvitti M. Thickness of the dental (radicular) cementum: a parameter for estimating age. J Forensic Odontostomatol 2007;25:1-6.
11. Ostby BN. The role of the blood clot in endodontic therapy. An experimental histologic study. Acta Odontol Scand 1961;19:324-53.
12. Nygaard-Ostby B, Hjortdal O. Tissue formation in the root canal following pulp removal. Scand J Dent Res1971;79:333-49.
13. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol? J Endod 2004;30:196-200.
14. Almutairi W, Yassen GH, Aminoshariae A, *et al*. Regenerative endodontics: a systematic analysis of the failed cases. J Endod 2019;45:567-77.
15. Fouad AF. Microbial factors and antimicrobial strategies in dental pulp regeneration. J Endod 2017;43:S46-50.
16. Mohammadi Z. Sodium hypochlorite in endodontics: an update review. Int Dent J 2008;58(6):329-41.
17. Galler KM, Buchalla W, Hiller KA, *et al*. Influence of root canal disinfectants on growth factor release from dentin. J Endod 2015;41:363-8.
18. Galler KM, Widbiller M, Buchalla W, *et al*. EDTA conditioning of dentine promotes adhesion, migration and differentiation of dental pulp stem cells. Int Endod J 2016;49:581-90.
19. Kim SG, Malek M, Sigurdsson A*, et al*. Regenerative endodontics: a comprehensive review. Int Endod J 2018;51:1367-88.
20. Portenier I, Haapasalo H, Rye A, *et al*. Inactivation of root canal medicaments by dentine, hydroxylapatite, and bovine serum albumin. Int Endod J 2001;34:184-8.
21. Diogenes AR, Ruparel NB, Teixeira FB, *et al*. Translational science in disinfection for regenerative endodontics. J Endod 2014;40:S52-7.
22. 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(10):1372-5.
23. Karczewski A, Feitosa SA, Hamer EI, *et al*. Clindamycin-modified triple antibiotic nanofibers: a stain-free antimicrobial intracanal drug delivery system. J Endod 2018;44:155-62.
24. Arruda MEF, Neves MAS, Diogenes A, *et al*. Infection control in teeth with apical periodontitis using a triple antibiotic solution or calcium hydroxide with chlorhexidine: a randomized clinical trial. J Endod 2018;44:1474-9.

25. Phumpatrakom P, Srisuwan T. Regenerative capacity of human dental pulp and apical papilla cells after treatment with a 3-antibiotic mixture. J Endod 2014;40:399-405.

26. Vishwanat L, Duong R, Takimoto K, *et al*. Effect of bacterial biofilm on the osteogenic differentiation of stem cells of apical papilla. J Endod 2017;43:916-2.

27. Ikeda E, Tsuji T. Growing bioengineered teeth from single cells: Potential for dental regenerative medicine. Expert Opin Biol Ther 2008;8:735-44.

28. Huang GT-J, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources. Their biology and role in regenerative medicine. J Dent Res 2009; 88(9):792-806.

29. 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(2):133-8.

30. Gay IC, Chen S, MacDougall M. Isolation and characterization of multipotent human periodontal ligament stem cells. Orthod Craniofac Res 2007; 10(3):149-60.

31. Scadden DT. The stem-cell niche as an entity of action. Nature 2006;441(7097):1075-9.

32. Chmilewsky F, Jeanneau C, Dejou J, *et al*. Sources of dentin-pulp regeneration signals and their modulation by the local microenvironment. J Endod 2014;40(4):S19-25.

33. Conboy IM, Conboy MJ, Wagers AJ, *et al*. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 2005; 433(7027):760-4.

34. Galler KM, D'Souza RN, Hartgerink JD, *et al*. Scaffolds for dental pulp tissue engineering. Adv Dent Res 2011;23(3):333-339.

35. Ulusoy AT, Turedi I, Cimen M, *et al*. Evaluation of Blood Clot, Platelet-rich Plasma, Platelet-rich Fibrin, and Platelet Pellet as Scaffolds in Regenerative Endodontic Treatment: A Prospective Randomized Trial. J Endod 2019;45(5):560-566.

36. Mehta S, Watson JT. Platelet rich concentrate: basic science and current clinical applications. J Orthop Trauma 2008;22(6):432-8.

37. Petrino JA, Boda KK, Shambarger S, *et al.* Challenges in regenerative endodontics: a case series. J Endod 2010;36(3):536-41.

38. Lenzi R, Trope M. Revitalization procedures in two traumatized incisors with different biological outcomes. J Endod 2012;38:411-4.

39. Hargreaves KM, Giesler T, Henry M, *et al*. Regeneration potential of the young permanent tooth: what does the future hold? J Endod 2008;34(7 Suppl):S51-6.

40. Keswani D, Pandey RK. Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin. Int Endod J 2013;46:1096-104.

41. Torabinejad M and Turman M. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: a case report. J Endod 2011;37(2):265-268.

42. Dohan Ehrenfest DM, Del Corso M, Diss A *et al*. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol 2010;81(4): 546-55.

43. Shivashankar VY, Johns DA, Maroli RK, *et al.* Comparison of the effect of PRP, PRF and induced bleeding in the revascularization of teeth with necrotic pulp and open apex: a triple blind randomized clinical trial. J Clin Diagn Res 2017;11(6):ZC34-ZC39.

44. Murray PE. Platelet-rich plasma and platelet-rich fibrin can induce apical closure more frequently than blood-clot revascularization for the regeneration of immature permanent teeth: a metaanalysis of clinical efficacy. Front Bioeng Biotechnol 2018;6:139.

45. Chai J, Jin R, Yuan G, *et al*. Effect of liquid platelet-rich fibrin and platelet-rich plasma on the regenerative potential of dental pulp cells cultured under inflammatory conditions: a comparative analysis. J Endod 2019;45(8):1000-8.