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Cell homing in regenerative endodontic procedures for adult mature permanent necrotic teeth: A narrative review

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Abstract
Regenerative Endodontic Procedure (REP) aims to restore damaged teeth and the vitality of the teeth by biological obturation [pulp like tissue], especially in immature permanent teeth with thin roots, and it has shown successful outcome in restoring the vitality in mature permanent teeth that are associated with periapical periodontitis or periapical lesion, unlike traditional root canal treatments that fill the canal with gutta-percha. This Review is supported by studies showing tissue healing in sterile conditions. REP is now favored over apexification, which stops root development and weakens teeth.

Keywords: Regenerative endodontics, immature permanent teeth, pulp regeneration

Introduction
The main goal of endodontic treatment is to remove the inflamed or infected pulp tissue while preserving the health of the periapical tissues, allowing the affected tooth to function normally without any signs or symptoms. To achieve this, a few steps should be followed for conventional endodontic treatment: removing the causes, such as caries or old restorations with recurrent caries, then completing the access cavity. After that, the tooth length is determined, and mechanical preparation and irrigation are performed to eliminate the remaining infected pulp tissues and bacterial biofilm from the root canal. The final step is obturation, which aims to create a hermetic seal to prevent any secondary infection.
Although the success rate of primary endodontic treatment has reached up to 98%, some procedural errors can affect the outcome, such as separated instruments, ledge formation, perforation, transportation, or inadequate or over-extended root canal fillings. These errors may impair proper disinfection of the root canal system, potentially allowing microorganisms to survive inside the canal. If an optimal apical seal is not achieved during the root filling step, these microorganisms and their byproducts can spread to the periapical tissue, initiating an inflammatory reaction [1].
Different research articles discussed the common procedural errors that may occur during endodontic treatment. It was shown that the two most common errors were overfilling [Most common in young patients], which could be due to over-instrumentation or inadequate length determination, and underfilling [most common in elderly patients] due to sclerotic canals, pulp stones, and inadequate length determination [2].

The rationale for using the regenerative endodontic procedure in mature teeth
In endodontically treated teeth, the main concern is the possibility of tooth fracture due to the loss of tooth structure, either because of decay or during instrumentation. A study by Lama *et al.*, compared the response of endodontically treated teeth and vital teeth to occlusal load to determine whether the dental pulp plays a role in the mechanoreceptive mechanism. They found that the endodontically treated teeth are highly sensitive to the occlusal load in comparison to the vital dental pulp. So it is suggested that the vital dental pulp has interdental receptors that provide non-pain-related function, which is detecting harmful pressure and

protecting the teeth during mastication. Endodontically treated teeth are more susceptible to fracture than vital teeth because of the changes in the mechanical properties of dentine after caries removal, access preparation, chemo-mechanical preparation, and obturation as well [3].

As a result, Loss of mechanoreceptors may contribute to the higher frequency of tooth damage after root canal treatment. This emphasizes the need for protective precautions and modifications in occlusal contact when restoring a root canal-treated tooth because they depend on periodontal ligaments to control the masticatory function of teeth [3].

The new era of endodontics is now concerned with the regenerative endodontic procedures. Different outcomes have been anticipated from successful regenerative procedures, such as maintaining the offended tooth in function, while preserving the arch length of the jaw. An outcome that could not be achieved with any other treatment modality.

According to this study, the endodontic treatment success had not improved, regardless of the high technology nowadays; the conventional root canal treatment depends on cleaning and shaping, and sealing the prepared pulp space by gutta-percha to minimize bacterial colorizations, which are found mainly in the apical ramifications, which are hard to disinfect. If the pulp is regenerated, the innate immunity [natural killer cells, lymphocytes, macrophages] will be restored. The innate immunity will reduce the possibility of reinfection [4].

The concept of regenerative endodontic procedure

The definition of regeneration is 'It is a biologically based procedure designed to physiologically replace damaged tooth structure, including dentin and root structures, as well as the pulp-dentin complex' [5].

According to the American Association of Endodontics [AAE], the primary goal of regeneration is the resolution of the signs and symptoms, which is similar to the goal of the conventional non-surgical endodontic treatment. As for the second goal, it is to allow thickening of the root canal walls and to complete the root maturation. So the difference between regenerative endodontic treatment and conventional non-surgical endodontic treatment is that in the former therapy that the canal will be filled with the host's vital pulp, while in the latter therapy, the canal will be filled with foreign biocompatible material [5].

In 1965, S. Kakehashi made a study that strongly supported the idea of tissue regeneration under aseptic conditions. The study was all about comparing the healing and repairing potential of opposite extremes of the microbial environments. In the first group, there was pulpal exposure and perforation with bacterial contamination. On the other hand, in the second group, there was pulpal exposure and perforation, but in an aseptic condition. The results had shown that in the first group, the pulp had become necrotic on the eighth experimental day, while in the second group, dentine bridges had started to form after fourteen days, and were completed in 21-28 days, regardless of the angle, severity of the exposure [6].

The regeneration concept was only used to treat the traumatized immature young permanent teeth. As they are susceptible to pulp necrosis, periapical periodontitis, and root resorption, and are liable to shedding or root fracture because the root walls are still thin and weak. Moreover, the dentinal tubules are larger, while the intertubular dentine and the peritubular dentine are not well mineralized yet; all of these factors contribute to a weak dentin root. As a result, they considered undergoing vital pulp therapy techniques to allow regeneration, starting with the most conservative technique, the indirect pulp capping, if the pulp shadow shows, and there is no direct exposure. Suppose the pulpal exposure is 0.5-1 with controlled bleeding. In that case, we go for direct pulp capping, if the diameter of exposure is larger or the bleeding cannot be controlled, we should go for partial pulpotomy or complete pulpotomy or even we can go for pulpectomy depending on the pulpal inflammation, size of the exposure, time, etc. These are the approaches used to maintain pulpal vitality and allow root maturation. The regeneration concept aims to remove the inflamed or necrotic part of the pulp, leaving the vital part of the pulp to allow repair and dentin pulp complex formation either by revascularization or tissue-engineered scaffolds and stem cells [7].

On the other hand, some studies have shifted focus toward regenerating immature non-vital cases instead of performing apexification. Apexification is a procedure that aims to induce the formation of an apical barrier in immature necrotic permanent teeth. This barrier allows for conventional root canal obturation. Although apexification using MTA [Mineral Trioxide Aggregate] or calcium hydroxide may promote limited mineralization, it also increases the risk of root fracture because the root is thin, short, and has an open apex. Additionally, apexification inhibits dentin-pulp complex and root development, thus arresting the completion of tooth development. These teeth become more susceptible to fracture and early loss. When the dental pulp becomes necrotic, it cannot be saved and must be amputated, pulpotomized, debrided, or completely removed, while the surrounding vital or mineralized tissues need to be disinfected to create a root canal system suitable for revascularization and the regeneration of new dental pulp tissue or apexification.

The regeneration concept aims to remove the inflamed or necrotic part of the pulp, to regenerate the damaged part of the pulp with new pulp-like tissues, and to maintain the vitality of the teeth [8].

The strategy of endodontic regeneration

The regeneration procedure could be done by two strategies: cell-based transplantation or cell homing. The first strategy involves the delivery of autogenic or allogenic stem cell transplants; the latter is defined as stem cells that have the potential to migrate into an environmental niche, whether endogenous or exogenous. The advantage of the cell-homing over the cell-based transplantation, that it allows the regeneration of the pulp-dentine complex in a simpler way, less expensively, and the clinicians will be able to perform it without special training [8].

Cell-homing strategy

The differences between cell homing and cell transplantation are; cell transplantation will require; isolation of cells from patients, *Exvivo* cell manipulation, while cell homing will not any of these steps which will make it an easier treatment option and less expensive than cell transplantation [9].

The cell-homing strategy depends on the regeneration triad: stem cells, growth factors, and a scaffold to obtain a successful outcome. This strategy could be initiated by bleeding induction into the empty canal space by over-instrumentation. The initiated blood clot is loaded with the needed stem cells and growth factors for successful tissue regeneration.

The stem cells and the factors that might affect their recruitment: The stem cells are responsible for tissue renewal, healing, and regeneration after any damage. Dental

stem cells originate from undifferentiated mesenchymal cells. Cell homing strategy depends mainly on the recruitment of the [SCAP] Stem Cells from the Apical Papilla because they are just adjacent to the apex of the root canal. They migrate from the periapical tissue to the pulp chamber by passing through the apical foramen. SCAP can produce dentine, blood vessels into the empty canal space, bone, cementum, and a functional periodontal ligament. Thus, it has high proliferative power and odontogenic capacity [10]. The SCAP may be a superior cell type for the regeneration process because of its ability to differentiate without exogenous growth factors [8].

A. The size of the apical foramen

In the regeneration procedure, the size of the apical foramen plays a significant role in the outcome of the regeneration process. There is an ongoing debate about the minimal width of the apical foramen necessary for successful regeneration. This could be one of the limiting factors in the regeneration process, as it may influence the migration of stem cells to the root canal system and their adhesion to the root canal walls [7].

In 1986, Kling *et al.*, stated that if the apical foramen was less than 1 mm, no revascularization would take place [11]. On the other hand, a study that was carried out on beagle dogs in 2004, [12] they used catheter tubes with [0.6- 1.5 mm] in diameter. The catheter tubes did not carry any growth factors, scaffolds to stimulate revascularization. They were fixed in the alveolus, fixed and covered with gingiva. After 9 weeks, new ingrown tissues were observed histologically in the catheter tube. This concept was supported by another study in 2013 using auto-transplantation. They found tissue grown in the pulp chamber. Based on the results of the animal study, it can be concluded that the size of the apical foramen before transplantation is not the decisive factor for successful revascularization and ingrowth of new tissue in the pulp chamber after transplantation in dogs. The minimum diameter of the apical foramen required for ingrowth is not defined, but a diameter significantly smaller than 1 mm does not hinder revascularization and ingrowth of new vital tissue [13].

The histological and pathological observation of the newly formed pulp from revascularization, it has a different histology than the previously formed pulp. It contains mineralized dentine-like structure, periodontal-like tissues, blood vessels, nerves, blood cells, lymphatic vessels, and immune cells. The de novo dental pulp revascularized tissues suggest that it includes stem cells sourced from remaining vital stem cells in the apical part of the root canal. Stem cells may home by migration toward growth factors released within the disinfected root canal space, from the apical papilla, periodontal ligament, and from bone [7].

B. The condition of the periapical tissues

There is an interesting case report that discusses the regeneration of immature teeth with an apical lesion. The patient presented with a complaint regarding a lower premolar that had a lingual sinus tract. The regeneration procedure was performed over two visits. The second visit occurred 26 days after the first and followed the resolution of the symptoms. This was achieved after proper canal disinfection with sodium hypochlorite to eliminate bacteria and necrotic tissue. Subsequently, a proper coronal seal was placed with MTA just below the cemento-enamel junction, followed by bonded resin to prevent the ingress of further microorganisms. After six months, the follow-up visit demonstrated that the primary goals of regeneration had been met, as indicated by the resolution of symptoms, resolution of apical periodontitis, and completion of root development. In this study, it was expected that some apical pulp tissues and Hertwig's epithelial cells would survive, despite the pulp being devitalized and heavily infected. As the inflammatory conditions were reversed through canal disinfection, these cells were able to proliferate.

There was a controversy about the vitality of the stem cells in the teeth with apical periodontitis and periapical lesions. In a study that was performed in 2014, [15] by Vanacker *et al*, they proved that the stem cells were still vital in the hypoxic conditions. The up-regulation of neuro- and osteo-specific genes and the proangiogenic factor in SCAP [Stem Cell of Apical Periodontium] cultured in basal medium supports the potential of SCAP to promote pulp-dentin regeneration. The hypoxia condition motivated the neuro-differentiation, which may support the concept of regenerating new pulp-like tissue that responded to vitality tests [15].

In 2016, another study was performed to support that the stem cells can withstand the periapical inflammation and pulpal infection for at least 90 days while at least being moderately inflamed. This was explained as the apical papilla previously was shown as a relatively vascular tissues, where the endothelial markers are present, which allow the angiogenesis process. The exposure of stem cells of the apical papilla to pro-inflammatory cytokines induced greater cell proliferation and mineralization potential. So the osteogenic differentiation had increased, as well as the induction of angiogenesis [16].

C. Irrigating solution and the intracanal medicaments

The newly formed pulp tissues were able to reestablish the innate immunity of the tooth, so the root canal reinfection is controlled. As a result, the level of disinfection should be maintained, as disinfection is the key factor to improve the prognosis and the outcome [17].

Sodium hypochlorite remains the irrigant of choice for maintaining a high level of disinfection in canals during and after the regenerative procedure. There is ongoing concern about the concentration of sodium hypochlorite (NaOCl) due to its cytotoxic effect on stem cells; therefore, a recommended concentration of 1%-2.5% is advised to protect the vitality of the remaining stem cells. The use of 17% EDTA [Ethylenediaminetetraacetic acid] as a final irrigation reverses the negative impact of NaOCl on stem cells. Research has shown that the survival rate of the stem cells increases by up to 35% when treated with 17% EDTA under these conditions. Thus, the cytotoxic effect of NaOCl is significantly reduced.

After finishing the mechanical preparation and canal disinfection with sodium hypochlorite 2.5%. We apply calcium hydroxide as an intracanal medication for its antimicrobial effect due to the dissociation of the calcium and the hydroxyl ions, which will create an alkaline medium that inhibits the growth of the bacteria [18].

In a study that was conducted in 2021, the results proved that calcium hydroxide has no significant effect on cell viability upon increasing the time of treatment or the concentration. That is very important to be considered during the regeneration procedure in the necrotic mature teeth [18].

The calcium hydroxide can also release transforming growth factor beta-1 from the dentine, which will have a proliferative effect on the mesenchymal cells [19]. So, the calcium hydroxide is still the gold standard as an intracanal medication.

The growth factors and the factors that affect its release

The growth factors are the second corner of the regeneration triad. It is defined as 'polypeptides or proteins that, when bound, give rise to a broad range of cellular activities such as migration, proliferation, differentiation, and maturation'.

They are released upon dentine conditioning with demineralizing agents. It includes vascular endothelial factor, transforming growth factor beta-1 [TGF-β1], bone morphogenic growth factor, nerve growth factor, and platelet-derived growth factors [20]. In the cell-homing strategy, the growth factors could naturally exist in the blood that migrated from the periapical tissues during the over-instrumentation, or trapped in the dentin. The TGF-β1 exists in high amounts at the peritubular dentin [toward the pulp], and there are no traces of TGF-β1peripheral non-tubular dentin [toward the cementum] [18]. Since the growth factors are important for the REP, it was found that the distribution of TGF-β1 in young is similar, which suggests that both could represent a successful outcome in regeneration.

A. Irrigating solutions

In addition to the role of calcium hydroxide, EDTA has an essential role in the process of regeneration. As the EDTA is used in the irrigation protocol, which allows the release of the growth factors that are trapped during dentinogenesis, where they preserve their biochemical signaling. Galler *et al.*, had shown that EDTA promotes migration, differentiation, and adhesion of the stem cells by the release of the growth factors. As well as its ability to neutralize the negative properties of the sodium hypochlorite, which was used in the 1st visit [17, 19].

The other role for the EDTA is the removal of the smear layer, which creates a physiological environment for the incoming cells by exposing the dentinal tubules and the collagen fibers. The use of EDTA, accompanied by ultrasonic activation, enhances the release of a higher amount of the growth factors. This is accomplished by using the EDTA for 10 minutes since it is a time-dependent irrigant and by placing the activating ultrasonic file 1 mm shorter than the anatomical apex [18].

There is a meta-analysis that compared the success rate of studies that have used EDTA, which was 97%, with the success rate of other studies that didn't use EDTA, which was 94% [22].

As a result, by the use of proper canal disinfection by using the irrigation as sodium hypochlorite with the use of intra-canal medication as calcium hydroxide to enhance the eradication of microorganisms and by using the EDTA as a final irrigation, the success rate of the regenerative endodontic procedure has significantly increased [22].

The scaffold:

The third part of the triad is the scaffold. It is defined as ''a complex three-dimensional material with mechanical and chemical properties that mimic the native extracellular matrix. Scaffold provides structural support for colonizing cells, enhance cell survival, proliferation, and differentiation, and enhance cell interactions as adhesion and deposition of extracellular matrix. The most widely used scaffold is the fibrin, as it naturally exists in the patient's blood, so there is a limited risk of foreign body interaction and infection [8].

The placement of the MTA

MTA is the gold standard material that is used in regeneration. Due to its biocompatibility, bioactivity, alkalinity, radio-opacity, induction of pulp cells, antibacterial effect, and calcium ion release. 'MTA is a Portland cement-based material with bismuth oxide added to enhance its radiopacity.' It is sold in the form of powder, and it is mixed with distilled water to start its hydration reaction to form calcium silicate hydrate gel on the calcium silicate particles and calcium hydroxide upon setting [23, 24].

Calcium hydroxide will allow the release of calcium ions, which have a direct relation to antimicrobial action. The metallic ions released upon MTA setting may allow the release of dentine matrix components that potentially influence cellular events for dentine repair and regeneration [23, 24].

After bleeding induction, a collagen plug is placed into the canals 3mm below the orifices for MTA [Mineral Trioxide Aggregate] retention, and it avoids the MTA displacement into the canals [23, 24].

The successful outcome of REP

In 2012, there was a study that supported the regeneration concept in the mature teeth with apical periodontitis after canal debridement, using the disinfecting protocol and inducing bleeding. Naseem Shah and Ajay Logani represented a new term, sealbio, in other words, the canals will be sealed with biological pulp-like tissues. As they suggested that there would be periapical healing only by proper canal debridement and disinfection, even without obturation, as the source of infection is removed. As they stated that they have undergone mechanical preparation up to size #30 k file, with the proper disinfection of the canals, after that, they induce bleeding into the canals by size #20 k-file near the apical foramen, 2-3 clockwise turns gently, and then pulled counterclockwise. Then the pulp chamber will be filled with MTA below the CEJ [25].

After 7 years, they made another follow-up study on their cases. Upon the long-term follow up there was a significant healing and no further progression of the apical lesion as there was a proper coronal seal [26].

In 2021, a systematic review and meta-analysis compared the conventional non-surgical endodontic treatment (NSET) to the regenerative endodontic treatment. Even though the success rate of NSET ranges between 72%-83% in the necrotic mature permanent teeth, depending on the size of the lesion. The regenerative endodontic treatment has shown very promising results, since it restores the innate immunity of the tooth, preserves the periapical proprioception, which is important to protect the tooth from fracture, to resolve the signs and symptoms, as well as [22]. It also stated that the success of the cell homing strategy was 96%, while cell transplantation strategy was 95% [22].

Another systematic review and meta-analysis in 2021, which reported the results of seven studies. Of 228 necrotic mature teeth were managed with REP, 217 are considered a success, in comparison to the predicted results for REP in young immature teeth. As for detecting the healing of the periapical lesion, five studies were included. 0f 100 necrotic mature teeth, 91 were considered successful [27].

In summary, the literature had already supported the conventional endodontic treatment, and started to support the regeneration in the mature teeth.

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