REVIEW ARTICLE

Revascularization: A New Hope for Necrotic Permanent Teeth with Immature Apex - A Review

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ABSTRACT

Root development commences after the completion of enamel formation. Irritation of pulp tissue results in major changes in pulp microcirculation that can lead to pulp necrosis and arrest root formation. The treatment of necrotic young permanent tooth with an immature open apex still presents multiple challenges in pediatric and endodontic dentistry. Conventionally, tooth that has lost its vitality is being treated by apexification. Through this technique, the formation of an apical barrier to close the open apex is promoted so that the filling materials can be confined to the root canal but it involves long-term periodic exchanges of calcium hydroxide paste into canal which may lead to weakening of the canal and tooth fracture. Furthermore, tissue regeneration cannot be achieved with apexification. Recently, revascularization is introduced as a new treatment modality for immature non-vital teeth. Revascularization not only provides apical closure but also increases the dentine wall thickness. As there is diversity in the treatment protocol for revascularization, it is pivotal to describe and discuss these protocols guiding researches in this field and thereby providing the clinicians to succeed in the treatment of non-vital tooth with immature apex. Hence, the present review aims to provide different protocol on revascularization.

Keywords: Revascularization, Non–vital immature teeth, Triple antibiotic paste

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INTRODUCTION

Root development commences after the completion of enamel formation. The cells of inner and outer enamel epithelia unite at point forming cervical loop, begin to proliferate, and form a structure known as Hertwig epithelial sheath. This sheath determines the size and shape of root/s of the tooth.[1] Apical root closure is completed approximately 2–3 years after tooth eruption.[2] Irritation of pulp tissue results in major changes in pulp microcirculation that can lead to pulp necrosis and arrest root formation. The major irritants to pulp tissue include various bacteria, trauma, dental procedure generating thermal stimulation, and chemical agents.[3]

The treatment of young necrotic permanent tooth with an immature open apex still presents multiple challenges in pediatric and endodontic dentistry. The traditional endodontic treatment using standard chemomechanical radicular instrumentation and sodium hypochlorite irrigant has been proven ineffective to achieve proper cleaning and disinfection of the overall dentin wall, particularly at the diverged apex.[4] Furthermore, an inadequate apical seal in such cases is a major problem during a conventional nonsurgical endodontic approach. The presence of thin apical root thickness creates a significant risk of root fracture.[5] The traditional management of such cases includes an apexification technique using calcium hydroxide or mineral trioxide aggregate (MTA) and biodentine.[6-9] This technique is successful in including apical closure; however, there is no expectation of root lengthening.[10]

The path of endodontic treatment took a sharp turn almost a decade ago when Iwaya *et al*.[11] showed continued root development and apical closure in a necrotic immature tooth were possible when successful disinfection of the root canal was achieved. The thickness of the dentinal wall of the tooth had increased; apical closure and repair of periapical lesion after monitoring for 5 months were seen radiographically.

Banchs and Trope *et al*.[12] published a case report of a new treatment procedure for management of open apex called "revascularization." The procedure had a different protocol from traditional apexification techniques in that disinfection of canal is done using both hypochlorite and chlorhexidine and the combination of triple antibiotic paste.

The regenerative endodontics is a treatment revolution in dentistry era in which root canal therapy brings diseased teeth back to life, rather than leaving a "non-vital" or dead tooth in the mouth.[13]

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PULP REVASCULARIZATION CONCEPTS

Revascularization can be defined as the invagination of undifferentiated periodontal cells from the apical region in immature teeth.[14,15] Tissue ingrowth is directed toward the root canal space after passive decontamination that removes, partially or totally, pulp tissue and/or its necrotic remnants. Root canal space filled with blood clots from periapical tissues, which can contribute to transporting periodontal stem cells inside the root canal space. Periodontal/periapical cells have been related to the desired outcomes of pulp revascularization (root-end development and apical closure).[16]

The stem cells from apical papilla (SCAP) are capable of differentiating into odontoblast-like cell-forming root dentin. Another type of mesenchymal cells, which are called dental pulp stem cells (DPSCs), were discovered, to have the ability to differentiate into odontoblast-like cells and form pulp/dentine-like complex.[17] Thus, the concept of revascularization has been used to form the vital tissue inside the root canal.

HOW REVASCULARIZATION DOES HAPPEN?

There are a number of theories that explain the revascularization mechanism. The periapical region of immature teeth presents multipotent periodontal cells with great potential for differentiating into new fibroblast and cementoblasts.[18] Hence, it has been suggested that differentiated cementoblasts and fibroblast are responsible for increasing dentinal walls and apical closure.[19] 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.[18] A third possibility involves the ingrowth of SCAP that could proliferate inside root canals through the blood induction of periapical tissues, since these cells have high proliferative capacity, probably being transported inside root canals in association with bleeding induced from the periapical tissue.[20]

In addition to the above-mentioned hypothesis, various growth factors incorporated in the blood clot and/or dentine may play an important role in the cell proliferation inside the root canal space.[21,22] Finally, the root anatomy of immature teeth (e.g., presenting open apex, wide root canal, and thin radicular dentine walls) may favor the communication of the canal space and periodontal tissue to achieve apical healing with periodontal tissue. With regard to the apical opening, revascularization seems to be more predictable when the apical diameter is >1 mm and is unlikely to occur in apical openings narrower than 0.3 mm.[23]

PROTOCOLS FOR REVASCULARIZATION ENDODONTIC TREATMENT PROCEDURES

Case Selection

Indications for treatment of the pulp revascularization are the presence of deep dental caries or trauma including a stop in the development of root canal of an immature non-vital tooth. It is important to keep in the mind that an endodontic treatment on an immature tooth, often necessary up to now, involves a root canal treatment on an apex tooth with thin and fragile walls.[24]

Currently, there is no evidence-based guideline to help clinicians determine which condition of cases can be treated with this conservative approach. The presence of radiolucency at periradicular region and vitality test can no longer be used as determining factor for conservative treatment. In both condition vital pulp tissue may be present in the canal which may helps in development of the apex. Logically, any remnant of visible soft tissue that can be visualized under the dental microscope should give the clinician an incentive to take the conservative approach, even though the soft tissue may be purely granulation tissues. However, one can also not rule out the possibility that there are not any remaining pulp tissues in the very apical part of the canal only because it cannot be detected clinically.[25] This pulp is used for revascularization of necrotic immature permanent teeth. Even if pulp has lost its vitality, residual pulp stem cells are able to survive. Apical papilla stem cells can also survive to an apical lesion thanks to an abundant blood supply which assist or may assist in revascularization.[14,24,26]

Operative Protocol

Two pulp revascularization techniques are found in the literature: One using calcium dihydroxide and another using a triple antibiotic paste for disinfection of pulp necrosis. Both are two-step procedure.[27]

Second step takes place 2 or 3 weeks after the first one, only if the tooth is asymptomatic and if there is a visual reduction of the apical lesion.[26]

In pulp revascularization, at 3 months' post-operative, the tooth is normally asymptomatic, and about 9 months later, X-ray radiography shows an increasing thickness of dentinal walls and an apical closure. Root development and apical closure may be visible after 3 months.[26]

Instrumentation

Using root canal instrument could not only increase the fragility of dentin walls but also injure stem cells present

in the apical area of these dentin walls. These also contain growth factor imprisoned during dentinogenesis. Growth factor and other cells essential for the regeneration process could also eliminated by instrumentation. Most of authors agree to advocate no instrumentation procedure.[14] Two types of cells are required to achieve a normal root development: Odontoblasts and epithelial cells of Hertwig's sheath. These two cell types are present in abundance in the apical area of immature teeth and are able to resist inflammation phenomena.[14,24,26,28] These cells will be able to differentiate into secondary odontoblasts that will generate dentin on root canal walls and thus allow root maturation.[14] No instrumentation procedure remains consistent with vital stem cells preservation and avoids weakening of already thin root canal walls.[29-31]

According to the study of Cehreli *et al*.,[32] even if the number of cases is not sufficient to be statistically significant, it can be noticed that some patients have regained tooth sensitivity (vitality) after treatment. This was observed in the case which was done without instrumentation. Thus, elements mentioned so far in favor of no instrumentation protocol seem to be more suggested.

Irrigation

Irrigators play a role of primary disinfection. They should have a maximal bactericidal and bacteriostatic effect while having a minimal cytotoxic effect on stem cells and fibroblasts to allow their survival and ability to proliferate.[33]

Pulp infection can usually spread to the apical region and create an acidic environment inside the canal. This one is not conducive to the creation of tissue regeneration. Bacterial invasion of root canal system causes the formation of bacterial biofilms. Those hang on root canal walls, entrance of dentinal tubules, and in the apical area containing more complex anatomical crevices. At these locations, bacterial biofilms are more resistant to disinfection procedures. Bacteria existing in depth and within the biofilm are in lag phase and therefore refractory to action of antibiotics and irrigators. To ensure optimal root canal disinfection for tissue regeneration, it is necessary to disrupt or eliminate biofilms. Using a tool such as fine "interdental brush" could probably be useful to disrupt biofilms without injuring hard dental tissue. However, the disadvantage of this kind of tool is the potential risk of leaving non-biocompatible residues (hairbrush) into root canal.[33]

Activating the irrigation solution within the root canal system is the only possibility to realize disintegration of the bacterial biofilm in non-instrumented areas. It justifies the use of endosonics means of irrigation. They generate a process of cavitation that induces a temperature increase of the irrigator and currents propelling the irrigator in all crevices. These whole systems have the effect of potentiating the efficacy of irrigator to disintegrate bacterial biofilm.[33] However, during this activation, it is essential to avoid touching the canal walls with endosonic tool to respect the decision to avoid any contact between dentinal walls and instruments.[26]

Hydrogen peroxide

Solvent properties of hydrogen peroxide are almost nonexistent, but it has an interesting hemostatic action. Hydrogen peroxide is antiseptic by the release of oxygen radical. Unfortunately, its action is too short and quickly neutralized by organic debris. Moreover, it requires a rinse to reduce pain and possible post-operative gaseous emphysema.

Chlorhexidine

Chlorhexidine 2% gel was proposed as a temporary medication. It has good action on candida and Gram-positive bacteria by the carryover effect. Indeed, its positively charged molecules confer the property of being absorbed by the dentin walls and thus allow the release of chlorhexidine for at least 2–12 weeks, preventing reinfection of the root canal during this period.[34] Despite this advantage, chlorhexidine does not have an effective dissolving action.

Sodium hypochlorite

So far, sodium hypochlorite remains irrigator reference in endodontic. It has a solvent action on necrotic tissue and an antiseptic effect widely demonstrated.[35] However, it must be supplemented by a desalting. Recommended concentrations vary between 0.5% and 5.25%.[36-39] Cytotoxicity of sodium hypochlorite is proportional to its concentration. The concentration of 2.5% seems to be the best compromise between efficiency and lack of toxicity.[40] Furthermore, Cunningham and Joseph[41] showed that elevation of the temperature at 37°C of the 2.5% sodium hypochlorite solution potentiates its solvent effect and its efficiency becomes comparable to that of the solution to 5.25%.

Iodine

Iodine is bactericide, antifungal, antiviral, sporicidal, and sedative. Purulent secretions and blood do not inactivate it.[42] Its disadvantage is that it colors dental tissues in brown.[43]

Ethylenediaminetetraacetic acid (EDTA) + irrigators

Chelators are weak acids, which react with the mineral portion of dentinal walls. They replace calcium ions

with sodium ions, which combine with the dentin to give soluble salts. EDTA-type chelating agents allow better wettability of the irrigator and a removal of the smear layer.[44,45]

According to Trevino *et al*.[46] who studied the effects of irrigants on the survival of human stem cells of the apical papilla, the use of EDTA before irrigators would allow maximum survival of these cells. 17% of EDTA is often used in cases of bacterial infection to remove the smear layer and allow access to the entrance of dentin tubules (allowing a better chance of joining tissue of regeneration) and induce a better penetration of the irrigator (increases wettability of the irrigator) and root canal medications.[44,45] EDTA is also a "sealer" that maximizes bacteriostatic and bactericidal effects of different agents. Its chelating effect would allow the release of growth factors imprisoned in the dentin during dentinogenesis that would stimulate the proliferation of stem cells.[47,48] Since EDTA appears to have many advantages, it is important to know how to combine the irrigators. Ring *et al*.[49] compared the effects of chlorhexidine and hypochlorite after treatment with EDTA. They had shown that there was no survival stem cell after using a combination of EDTA and 2% chlorhexidine. Moreover, precipitate chlorhexidine salts are formed and maintained in root canal. These precipitates can be toxic and prevent cell adhesion to the canal wall. The combination of EDTA and 6% of hypochlorite seems to moderately reduce the vitality of stem cells. It is also recommended to rinse with saline after irrigating to minimize the risk of possible precipitates and to remove residual debris and remain of irrigant.[50]

Disinfection Protocol

A successful vital pulp treatment requires a good seal against bacteria, no severe inflammatory reactions, and stable hemodynamics within the pulp. The use of intracanal irrigants with the placement of antibiotics for several weeks as a means of disinfection of the canal is a very important step to achieve revascularization.

Calcium hydroxide ($Ca(OH)_2$) has been advocated as a root canal disinfectant and for stimulation of hard tissue repair at the apex of infected immature teeth. Several favorable biological properties have been attributed to it when used clinically. It is antimicrobial, it has the ability to dissolve necrotic tissue in the root canal, and it can induce apical closure by hard tissue formation. It also acts as a physiochemical barrier, which precludes the proliferation of residual microorganisms and prevents the reinfection of the root canal from the oral cavity.[50]

However, a freshly mixed paste of $Ca(OH)_2$ has a pH of approximately 12.5 and is potentially toxic to not only bacterial cells but also human cells too.[50] The use of $Ca(OH)_2$ in revascularization is, therefore, not without criticism, which is as follows:

1. $Ca(OH)_2$ may destroy the ability to induce the nearby undifferentiated cells to become odontoblasts and damage the remaining pulp tissue, apical papilla, and Hertwig's epithelial root sheath (HERS).
2. Direct contact of $Ca(OH)_2$ paste with the tissue which will induce the formation of a layer of calcific tissue which may occlude the pulp space therefore prevents pulp tissue from regeneration.
3. Even if apexification with $Ca(OH)_2$ is rendered successful owing to its antibacterial properties, the procedure will leave behind a short root with thin dentinal walls with a high risk of root fracture.[51]

The gentle treatment regimen is, therefore, an attempt to conserve any viable tissues that may remain in the canal system which harbor stem cells, i.e., SCAP in the apical papilla and DPSCs in the pulp. Avoidance of trauma to the tissue around the apex is advised. After proper disinfection, the remnants of the survived HERS at the apices of immature teeth may organize the apical mesodermal tissue into root components.[52]

In the first appointment, continuous irrigation with NaOCl should be carried out for 30 min at every 5 min. This continuous procedure completely disinfects the root canal, as the survival of microorganisms or its toxins will prevent the revascularization procedure.[52]

In the next appointment, after 1 week, irrigation of the canal is again carried out for 15 min. If vital tissue is present in the canal, the concentration of NaOCl used is 5.25%, and if it is not present and revascularization is carried out by blood clot, then the concentration used by most authors is 1.25–2.5%.[52]

Revascularization procedure has also been carried out only with the copious irrigation of the canal without instrumentation. The non-instrumentation procedure using 6% NaOCl and 2% chlorhexidine coronal irrigation has shown to preserve the remaining vital DPSCs in single step revascularization procedure. Hence, it is believed to be critical for pulp revascularization.[26]

However, Rossi-Fedele *et al*.[52] have shown that the formation of precipitate when chlorhexidine and NaOCl are mixed leading to discoloration and other side effects. Combination of 17% EDTA with 6% NaOCl is safe till now and has shown to be effective for the regeneration of pulpal stem cells. EDTA very effectively releases growth factors from human dentin as well as helps in the survival of stem cells of apical papilla.[53,54]

The Use of Antimicrobial Paste

As calcium hydroxide has its own disadvantages in revascularization process, combination of antibiotic paste has been used as an intracanal medicament. Using

the antibiotic paste, the pulp tissue is able to fill in the remaining canal space. There is a particular combination of antibiotics which effectively disinfects root canal systems and increases revascularization of avulsed and necrotic teeth. This combination includes metronidazole, minocycline, and ciprofloxacin, which is known as triple antibiotic paste. The triple-antibiotic regimen was first tested by Sato *et al*.[55] and found to be effective against the *Escherichia coli*-infected dentin *in vitro*. The same research group also tested their bactericidal efficacy against microbes from carious dentin and infected pulp. They found that the mixture of antibiotics is sufficiently potent to eradicate the bacteria.

The application of antibacterial drugs may represent one method of eradicating bacteria in root canal treatment. This concept is also known as lesion sterilization and tissue repair therapy. This technique has been developed by Cariology Research Unit of Nigata University School of Dentistry, Japan.[56] Composition and mixing instructions for the tri-antibiotic paste are adapted from Hoshino *et al*.[57]

Antibiotics (3M mix-MP) combines ciprofloxacin 200 mg, metronidazole 500 mg, minocycline 100 mg, and a carrier (MP): Macrogol ointment or propylene glycol is also used. The combination of drugs has been shown to penetrate efficiently through dentine from prepared root canals, suggesting that topical application of the drug combination may be potent in sterilizing lesions in root canal treatment.[57]

Disadvantages of using Antimicrobial Paste

The concern of the antibiotic paste is that it may cause bacterial resistance. Furthermore, the paste contains both bactericidal (metronidazole and ciprofloxacin) and bacteriostatic (minocycline) antibiotics. In addition, minocycline may cause tooth discoloration. The discoloring effect of the minocycline can be minimized by occluding the dentinal tubules in the pulp chamber with a bonding agent, then placing a root canal projector into the chamber, and filling the space between the projector and the dentin with a flowable composite resin. After the resin sets, the projector can be removed and the triple mix antibiotics paste can be placed into the canal in a backfill manner to the level of CEJ.

When discoloration occurs after using the triple antibiotic paste, internal bleaching can be performed during the follow-up examinations when evidence of maturation of the tooth has been observed. Cefaclor instead of minocycline can also be substituted in the paste to avoid discoloration.[58] In addition, the use of white MTA instead of gray MTA should also be considered.[51]

Blood Clot Formation in Canal Space

Revascularization can be carried out with or without the formation of blood clot. As until now no guideline has been proposed for revascularization, it is completely on the clinician to decide whether the canal should be instrumented for inducing blood clot or not depending on the visual or tactile perception of soft tissue remaining within the root canal system. Lack of responsiveness to cold or electric testing is not considered to be an indication of loss of vitality, as most of the revascularization procedures are carried out in immature tooth with open apex. Despite the pre-operative irresponsiveness of vitality testing of the tooth, if some vitality is noted during treatment either by sensitivity to instrumentation within the root canal system or by the visual or tactile perception of soft tissue remaining within the root canal system, then blood clot is not induced within the canal space, as the remaining vital pulp supplies the stem cells and growth factor responsible for revascularization.[26]

However, if there is a lack of evidence of residual vital pulp tissue within the root canal system either by tactile or visual perception, then treatment is administered with the addition of evoking an intracanal blood clot. Induction of bleeding to facilitate healing is a common surgical procedure. It was first proposed by Ostby in 1961[59] to induce hemorrhage and form blood clot in the canal space of mature teeth in the hope to guide the tissue repair in the canal. Later in 1974, Myers[60] attempted to regenerate dental pulp with blood clot filled in the canal. The mechanism of how a blood clot benefits the root canal revascularization is not entirely clear, although the possible reasons could be that blood clot may act as a natural fibrin scaffold for cell attachment, proliferation, and differentiation to facilitate the regeneration and repair of tissues into the canal. SCAP cells from the apical papilla may migrate into the root canal, produce dentin-pulp complex-like tissue, and deliver abundant growth factors within the blood clot, such as platelet-derived growth factor which will aid in revascularization.

Hemorrhage is induced by over instrumentation with either endodontic files or an endodontic explorer penetrating slightly into the remaining pulp tissue or periapical tissue. This procedure induces bleeding into the canal, and the bleeding is left for 15 min so that the blood would clot in the canal and stopped at a level 3 mm below CEJ. MTA is then placed over the blood clot.[26]

At this point, it is unsure of which factors in the blood clot are important. When these factors are isolated, they can be incorporated into a synthetic scaffold that will be easier for clinicians to manipulate compared

with a blood clot. However, platelet-rich plasma has been tried as a successful clinical alternative. Ding *et al*.[28] discussed the value of the use of PRP in whom it is difficult to produce bleeding in the canal with a file. A recent case report has suggested the possibility of PRP as a potentially ideal scaffold for pulp revitalization in the tooth with necrotic pulp and a periapical lesion.[58]

MTA Barrier, or Equivalent, Placed Over Blood Clot, Final Restoration, and Follow-up

Once the intracanal infection is controlled and a physical scaffold to promote cell growth and differentiation has been achieved, the next important step is coronal seal to prevent reinfection. In revascularization after inducing blood clot, MTA is placed over the clot. If revascularization is carried out without the use of blood clot then after drying the canal with paper point, MTA is placed carefully over the tissues in the root canal. A small piece of Collacote may be placed at the pulp chamber to support the MTA cement which is to be placed over it. This is followed by the placement of a wet cotton pellet and temporary filling material. The patient is then recalled after 2–3 weeks, and if the tooth is asymptomatic, then the temporary filling material and the cotton pellet are replaced with a bonded resin restoration or glass ionomer cement.[26]

The use of MTA is known for its excellent microleakage-proof property and biocompatibility. Additional placement with glass ionomer/resin provides a double seal, further securing the sealing ability and the integrity of the filled access.

The tooth should be followed up periodically to observe the maturation of the root. If after several rounds of intracanal irrigation and medication, the clinical symptoms show no sign of improvement, i.e., persistent presence of sinus tract, swelling and/or pain, and apexification procedure should then be carried out. If no signs of regeneration are present after 3 months, then more traditional treatment methods can be initiated.

FACTORS THAT AFFECT THE RESULTS OF REGENERATIVE ENDODONTIC TREATMENT

There are some factors that affect the results of regenerative endodontic treatment. To achieve successful results of the treatment procedure, a thorough understanding of these factors is very important.

The first factor is the disinfection of the canal. The absence of bacteria is critical for successful revascularization because the new tissue will stop at the level it meets bacteria in the canal space. In necrotic cases with apical periodontitis, it must be recognized that the vital tissue might not be normal pulp tissue, although root development continues and dentine maturation occurs. In teeth with open apices and necrotic pulps, it is possible that some vital pulp tissue and Hertwig's Epithelial Root Sheath remain. When the canal is properly disinfected, the inflammatory process reverses and these tissues may proliferate.[51]

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 the 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%–34% of teeth with immature roots.[51]

The third factor is the patient age. Several case reports of regenerative endodontic treatment procedures have generally been limited to patients who are reaching adolescence, mostly aged from 8 to 16 years. [12,15,61,62] Based on these case reports, it would not be advisable to perform regenerative endodontic treatment procedures in children younger than 8 years or older than 16 years.

CONCLUSION

Pulp revascularization represents a recent and promising therapy for immature teeth, recommended as an alternative to apexification in cases of endodontic treatment of irreversible pulpitis and pulp necrosis, whether or not associated with the periapical lesion. It is a technically simple treatment with advantageous outcomes because, unlike apexification, it promotes thickness of the dentin wall width and apical closure, avoiding weakening of the tooth. However, considering that it has only recently began to be applied, little is known about long-term side effects of revascularization. Further clinical studies with long-term follow-up may contribute to an understanding of the composition and mechanical properties of the mineralization developed in the inner dentinal walls. In addition, the need for endodontic retreatment and intracanal post-rehabilitation in revascularized teeth must be planned to extend immature tooth longevity and improve future prognosis.

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