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Grado en Odontología

ADVANCES IN REVASCULARIZATON OF

YOUNG PERMANENT TEETH

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Resumen:

Introducción: El tratamiento de los dientes permanentes inmaduros con una pulpa necrótica o una inflamación pulpar irreversible se ha limitado durante mucho tiempo a la creación de una barrera apical calcificada para permitir un correcto sellado apical deteniendo así el diente en su fase de desarrollo. La revascularización tiene el potencial de estimular la continuación del desarrollo radicular. **Objetivos**: Comparar los diferentes protocolos disponibles mediante el análisis de los resultados a corto y largo plazo. **Materiales y métodos**: Se realizó una investigación de artículos científicos utilizando PubMed para el período 2001 a 2021 utilizando las siguientes palabras clave: "revascularización pulpar", "revitalización pulpar". Se seleccionaron estudios de casos (realizados en humanos) de esos artículos, se analizaron y se organizaron en diferentes tablas. **Resultados**: Sobre los 142 dientes analizados, se observó apexogenesis en el 84,5% de los casos, aumento de la longitud radicular en el 50%, aumento de la anchura radicular en el 63,4% y cierre apical en el 61,3%. **Discusión**: La etiología de la patología fue principalmente traumática y casi todos los dientes presentaron necrosis y afectación periapical. Los protocolos utilizados fueron muy diferentes al igual que los resultados. Parece que los resultados satisfactorios de la revascularización no se vieron influidos por los protocolos. **Conclusión**: La revisión sistemática realizada estableció el potencial de la revascularización para promover el aumento de la longitud radicular, el aumento del grosor radicular y el cierre apical. La comparación de varios protocolos de revascularización y la observación de los resultados no permitieron deducir el mejor. El resultado reveló no ser predecible y no depender del protocolo utilizado.

Abstract:

Introduction: Treatment of immature permanent teeth with a necrotic pulp or an irreversible pulp inflammation have for long been limited to the creation of an apical calcified barrier to permit a correct apical seal thus arresting the tooth in its development stage. Revascularization has the potential to stimulate root development continuation. **Objectives**: Compare the different protocols available through the analysis of short and long term outcomes. **Materials and methods**: Research of scientific articles was conducted using PubMed for the period 2001 to 2021 using the following keywords: "pulp revascularization", "pulp revitalization". Case Studies (conducted on humans) from those articles were selected, analysed and organized in different tables. **Results**: Over the 142 analysed teeth, apexogenesis was observed in 84.5% of the cases, increase in root length in 50%, increase in root width in 63.4% and apical closure in 61.3%. **Discussion**: The etiology for the pathology was mainly trauma and nearly all the teeth presented with necrosis and periapical affectation. The protocols used were very different as were the results. It appeared that the successful outcomes of revascularization were not influenced by the protocols. **Conclusion**: The conducted systematic review established the potential of revascularization to promote increase in root length, increase in root thickness and apical closure. Comparing several protocols for revascularization and looking at the results did not allow to deduct the best one. The outcome revealed not to be predictable and not to depend on the protocol used.

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1. Introduction

This systematic review first includes a presentation of the concepts of revascularization, how it was developed, what it consists of, which factors influence it and what are its future possibilities of use. Case analysis will be conducted and presented in result tables which will then be analysed to attempt uncovering the most efficient protocol of revascularization.

1.1. Immature Permanent Teeth

Immature permanent teeth are defined as newly formed teeth that have not completed their apex/root formation and thus possess an open apex (1). The normal growth cycle for the root of a permanent teeth to finish its formation is three years after it has emerged in the dental arch (1). Previous studies have shown that among children aged for school, 25% will experience trauma (in most cases before 19 years old (2)) and 25% to 65% of them exhibit untreated dental caries (3). Those traumas and pathologies, together with developmental anomalies such as dens evaginatus (prevalence of 0,5% to 4,3%(4)) and dens invaginatus (prevalence of 0,25% to 10% (4)), usually provoke pulpar pathologies due to the infiltration of bacteria in the pulpar microenvironment (1). This bacterial filtration will thus trigger an inflammatory reaction that can cause irreversible pulpitis and end up as necrosis. If left untreated, it will lead to subsequent periapical pathologies (1).

The filtration of bacteria will impact not only the pulp and its functions (inductive, formative, nutritive, sensory and protective purposes (5)) but also the periapical tissues involved in root development consisting of root lengthening, lateral wall thickening and apical closure. Root development is due to the extension of the cervical loop of the enamel organ in apical direction (6). From this cervical loop, an epithelial bilayer will extend and form the Hertwig's epithelial root sheath (HERS) (6). HERS is a double layer cell of cells consisting of the inner and outer enamel epithelium (7). It is responsible for the differentiation of odontoblast from the pool of mesenchymal stem cells (known as apical papilla) as well as determining the final shape of the root (5). Apical papilla is in short a new terminology for the dental papilla located at the apex of growing immature permanent tooth (8). It was recently discovered that it contains new types of stem cells apart from the dental pulp stem cells (DPSCs) named stem cells from the apical papilla (SCAP) (8). DPSC were considered to be the origin of odontoblast although it seems that SCAP are the ones that differentiate into the primary odontoblast responsible for root dentin formation while DPSC differentiate into replacement odontoblast (8). Although it was shown that HERS was able to induce continuous root formation in teeth that exhibited irreversible pulpitis and necrosis (7), without appropriate treatment there will be an aggravation of the signs and symptoms and the subsequent destruction of the HERS ending the process of normal root development (lengthening, lateral wall thickening and apex closure(7)). Thereby in the light of these considerations, and once the HERS is destroyed, continuation of root development can only be achieved through new therapeutic approaches centred on the potential of apical stem cells and dentine matrix-derived growth factors entrapped in mineralized dentin (9).

1.2. Treatment available for young permanent teeth

Proper establishment of the pulp vitality is critical in order to select the proper treatment for to the tooth. If the pulp is neither irreversibly inflamed nor necrotic, partial pulpotomy or conventional pulpotomy could be undertaken (10). This procedure could be successful thanks to the high cellular content in young permanent teeth (10). If the pulp is diagnosed as irreversibly inflamed or necrotic, the treatment options will be apexification and revascularization (3).

1.2.1. Apexogenesis

A brief summary of apexogenesis procedure is relevant even though it is part of the vital pulp therapy. It was the only therapeutic approach, until revascularization procedures were introduced, to achieve an increase in root length, root thickness and root-end closure of immature teeth (11). This treatment is used in vital immature permanent teeth with an incomplete root formation and a reversible pulpitis (10). It consists of the removal of the inflamed pulp followed by the placement of a biocompatible intracanal medication (Calcium hydroxide or mineral trioxide aggregate) (7). The clinical relevance of this procedure lies in the fact that pulp tissue remains vital and, in comparison with necrotic pulp, HERS did not suffer lack of blood supply; thus, leaving the HERS viable for the physiological continuation of root formation (7). In respects to correct case selection, the correct establishment that the pulp is vital (even though assessment is difficult) is crucial : the outcome is predictable and the success of the procedure goes up to 93,5% whereas it is not predictable for revascularization (9).

Relying on the fact that HERS has not been damaged, stem cells of the apical papilla are still present and that pulp vitality is maintained : the formation of the root up to its final length, thickness and the subsequent root-end closure is reached (7).

1.2.2. Apexification

Apexification procedures were introduced in order to treat immature permanent teeth (necrotic or irreversibly inflamed) to achieve formation of an apical calcified barrier or to provide continued apical development (5). Until its introduction in the 60s, various approaches were used to treat necrotic teeth with an open apex. One example is custom fitting of the filling material, such as gutta-percha cone. However this technique has the disadvantage, due to the specificity of immature teeth (wider diameter in apical portion than in the coronal one) to offer a poor apical seal (7). Surgery procedures have also been used to get a proper sealing, but, because immature permanent teeth present thin, irregular, fragile walls, they may fracture during instrumentation or during the obturation of the retrocavity (7). Because of the poor outcomes due to lack of apical sealing of these procedures, the need for an apical barrier formation was established. Calcium hydroxide was used as a conventional material in apexification procedure (6). Despite its favourable potential to attract calcium ions from the bloodstream to initiate hard tissue formation (that could be made of cementum, dentin, bone or “osteodentin”), and its antimicrobial property (through the release of hydroxyl ions and its alkalinity (11)), several disadvantages have been found(7).

Firstly, Calcium hydroxide dressing should be changed multiple times during the treatment which would increase the risk for bacterial contamination due to an improper coronal sealing (7).

Secondly, it could possibly weaken the mechanical properties of dentin (6). Even though calcified apical barrier formation occurs in 74% to 100% of the case with calcium hydroxide (7), 25% fracture rate have been reported in teeth following a long term calcium hydroxide treatment (6) with majority of the fractures occurring at the cervical part of the root (10).

Thirdly, the discovery of mineral trioxide aggregate (MTA) in 1993, allowed to partially overcome some of the problems previously mentioned, and to create an apical barrier in a single step technique (one visit apexification) (7). Due to its low solubility, MTA does not need to be replaced, allowing to fill the root canal instantly all the while decreasing the risk of bacterial contamination (7). In addition, it potentially increases fracture resistance (6) through the immediate placement of an intracanal bonded core (7) such as fiber post or composite core (6).

As stated before, immature permanent teeth treatments following necrosis and/or periapical pathologies is challenging for each clinician. Until now, the desire treatment outcomes of the conventional apexification techniques using calcium hydroxide or MTA as intracanal dressing materials (7) were: the resolution of the periapical pathologies and the subsequent healing of bone as well as the formation of a calcified barrier at the root end which allowed for a proper sealing at the apex during condensation (12). However, this technique (apexification) does not intend to complete the vertical and horizontal growth of the tooth (13) thus leaving the tooth with thin dentinal wall and shorter crown to root ratio (14). A higher risk of tooth fracture and bone loss is then detected (14). Even though fracture rate following MTA apexifications is lower than calcium hydroxide apexifications, because of its still thin dentinal wall, immature teeth treated with MTA remained prone to fracture (15). This fracture, when happening at the root portion, usually leaves the tooth with a poor prognosis and will most likely result in tooth extraction (3). Therefore, the fracture decreases the success rate outcome in prosthetic treatment and, in patient still experiencing cranioskeletal development, consequences are even more damaging (12). They include growing alterations in maxillary and/or mandibular bones, along with masticating, breathing and pronunciations problems leading to pernicious psychological effects (12). At the light of the possible complications of the traditional apexification techniques and due to their limitations (impossibility to achieve further root maturation and increase in root length and/or thickness), a new therapeutical approach was needed.

1.3. History of revascularization

The first steps into the field of regenerative endodontic procedure were made by Nygaard Östby in 1961 on dogs and humans (16). He started with the postulate that in general pathology blood clots play a key role in healing and that it should also be the case in endodontic (16). Therefore, he conducted an histological study on dog and humans teeth. In dogs, the tooth was extracted and the apical part of the apex was cut before immediate re-implantation. Once inside the socket, the pulp was removed through the crown. On humans, the pulp diagnosis range from normal pulp to necrotic pulp. The pulp teeth were extracted, and an apical bleeding was created by a laceration of the periapical tissue and a partial obturation of the canal was done by gutta-percha coated with Kloroperka N-Ö powder (made of chloroform). Histological examination exhibited ingrowth of granulation tissue from the periapical space which is progressively replaced by fibrous connective tissue and cellular cementum.

The term revascularization was first used in 2001 by Iwaya et al. They attempted to treat a mandibular molar with incomplete root formation, apical periodontitis and sinus tract by using antibiotics intracanal dressing capped by calcium hydroxide. The results showed: resolution of the symptoms, increase in root length and wall thickening together with formation of an apical barrier in addition to positive response to electrical stimulus (13).

The first protocol was introduced by Branchs & Trope in 2004 together with the introduction of the triple antibiotics paste by addition of minocycline (17). Initial clinical situation was similar as the one encountered by Iwaya *et al.* After the complete removal of the pulp tissue and copious irrigation with sodium hypochlorite, the triple antibiotics paste was used as intracanal dressing until inflammation has diminished. Then, irritation of the periapical tissues was performed in order to create bleeding and generate a blood clot that was covered with MTA. Results were also promising with the resolution of apical pathology and the subsequently achieving root formation (17).

1.4. What is revascularization

The tooth is organized around and controlled by the pulp. The pulp is a complex network of loose connective tissues performing many fundamentals roles such as the formation of primary dentin (until root completion) and secondary dentin (throughout life), perception of noxious stimulus and transmission of sensory input like pressure or temperature (6). Pulp tissues also provide defence mechanisms through an immunological complex and the clearance of pathogens, as well as the production of tertiary or reparative dentin in response to external aggressions (13). Its cellular content is varied, including odontoblast (responsible for dentin formation), fibroblasts, undifferentiated mesenchymal stem cells, nerve plexus and capillaries (6). As previously established, vascularity is essential in cell survival and thus for root completion (18). Therapeutic approaches for immature permanent teeth now focus on the regeneration of the pulp tissue. This field named regenerative endodontic is defined as a “biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex” (19). It is now widely recognized as part of the therapeutic arsenal of clinicians, as 96% of the endodontists are willing to use regenerative endodontic (3).

Revascularization is a procedure aimed to promote physiological root completion in immature permanent teeth relying on the potential that mesenchymal stem cells located at the apical papilla will differentiate into odontoblast upon induction from growth factors once they invade the root canal after induced bleeding at the apex (12). This procedure is mainly performed in young patients with immature permanent teeth, and while it was mostly performed in incisors and premolars (20), it has also recently been used in molars (21). (However it has only been performed in a canine once (22)). The AAE (American Association of Endodontists) has defined three objectives for this procedure to be successful (14):

- Primary: Elimination of symptomology and evidence of bone healing.
- Secondary: Increase in root wall thickness and/or in increase root length.
- Tertiary: Positive response to vitality test.

While the primary objective is common to every endodontic treatment and indispensable (being fulfilled in more than 90% of cases in regenerative endodontic procedures (REP)) (12); the second one, being the real added value of REP, is a desired outcome which shows variabilities in distinctive clinical cases. In a review of clinical cases from 2015, increased root length was observed in 76,2%, increased root width in 79,2% and apical closure in 55,4% of cases (23). The third objective being the ultimate goal and the leading objective in the current research, has only been witnessed a very few times (24).

While revascularization is an easily duplicated procedure, it remains unpredictable as it is subjected to many factors : operator variability, duration and extension of the pathology, stage of the root development, size of apical diameter, and finally the type and concentration of irrigant, antibiotics or disinfectants being used.

1.5. Stage of root development & size of apical diameter.

As dentists, we might wonder to which degree of root development, it is acceptable to use REP over traditional apexification technique. Different degrees of root maturation (5 stages) have been established by Cvek in 1992 (9) defined as follows : 1St stage is defined as being under 1/2 of root length development; 2nd stage corresponds to ½ of root length formation; 3rd stage to 2/3 of root length; 4th stage to a wide open apical foramen and a nearly completed root length and finally 5th stage equalling a complete root development and closed apical foramen (9). While all immature permanent teeth with a necrotic pulp are suitable for REP, the clinical management of a tooth at stage 4 (nearly complete root formation with open apex) could also be done with traditional apexification due the presence of the physiological length & thickness, the closure of the apex being the key point in this case (9). While during apexification, the only possible drawback was that a large apex size which could lead to material extrusion (negative outcome), in revascularization a bigger apex size favours the vascularization of the tooth. Previous studies on reimplantation of avulsed permanent incisors showed that natural revascularization occurred in 18% to 34% of teeth with an apical foramen larger than 1.1 mm (3). Based on this information, it was supposed that apex with a size smaller than 1 mm would impede revascularization and some studies excluded teeth with an apex smaller than 1 mm (24). But further research demonstrated that even with an apex of 0.5 mm, successful revascularization could be achieved (even if the subgroup with apex larger than 1mm obtained greater increase in root maturation) (25).

1.6. Protocol

First appointment:
Local anesthesia, dental dam isolation and access.
Copious, gentle irrigation with 20ml NaOCL using an irrigation system that minimizes the possibility of extrusion of irrigants into the periapical space (e.g., needle with closed end and side-vents, or EndoVac™). Lower concentrations of NaOCL are advised [1.5% NaOCL(20mL/canal, 5 min) and then irrigated with saline or EDTA (20 mL/canal, 5 min), with irrigating needle positioned about 1 mm from root end, to minimize cytotoxicity to stem cells in the apical tissues.
Dry canals with paper points.

Place calcium hydroxide or low concentration of triple antibiotic paste. If the triple antibiotic paste is used: 1) consider sealing pulp chamber with a dentin bonding agent (to minimize risk of staining) and 2) mix 1:1:1 ciprofloxacin: metronidazole: minocycline to a final concentration of 0,1 mg/ml.
Deliver into canal system via syringe • If triple antibiotic is used, ensure that it remains below CEJ (minimize crown staining).
Seal with 3-4mm of a temporary restorative material such as Cavit™, IRM™, glass-ionomer or another temporary material. Dismiss patient for 1-4 week
Second appointment: 1-4 weeks after 1st visit
Assess response to initial treatment. If there are signs/symptoms of persistent infection, consider additional treatment time with antimicrobial, or alternative antimicrobial.
Anesthesia with 3% mepivacaine without vasoconstrictor, dental dam isolation.
Copious, gentle irrigation with 20ml of 17% EDTA.
Dry with paper points.
Create bleeding into canal system by over-instrumenting (endo file, endo explorer) (induce by rotating a pre-curved K-file at 2 mm past the apical foramen with the goal of having the entire canal filled with blood to the level of the cemento–enamel junction).
An alternative to creating of a blood clot is the use of platelet-rich plasma (PRP), platelet rich fibrin (PRF)or autologous fibrin matrix (AFM). Stop bleeding at a level that allows for 3-4 mm of restorative material.
Place a resorbable matrix such as CollaPlug™, Collacote™, CollaTape™ over the blood clot if necessary and white MTA as capping material.
A 3–4 mm layer of glass ionomer (e.g. Fuji IX™, GC America, Alsip, IL) is flowed gently over the capping material and light-cured for 40 s
MTA has been associated with discoloration. Alternatives to MTA (such as bioceramics or tricalcium silicate cements (e.g., Biodentine®, Septodont, Lancasted, PA, USA, EndoSequence® BC RRM-Fast Set Putty, Brasseler, USA) should be considered in teeth where there is an esthetic concern
Anterior and Premolar teeth - Consider use of Collatape/Collaplug and restoring with 3mm of a non-staining restorative material followed by bonding a filled composite to the beveled enamel margin.
Molar teeth or teeth with PFM crown - Consider use of Collatape/Collaplug and restoring with 3mm of MTA, followed by RMGI, composite or alloy.
Follow-up: (6, 12, 24 months)
Clinical and Radiographic exam:
• No pain, soft tissue swelling or sinus tract (often observed between first and second appointments).
• Resolution of apical radiolucency (often observed 6-12 months after treatment)
• Increased width of root walls (this is generally observed before apparent increase in root length and often occurs 12-24 months after treatment).
• Increased root length.
• Vitality test

Recommended yearly follow-up after the first 2 years
CBCT is highly recommended for initial evaluation and follow-up visits

Table 1: AAE Clinical consideration for a regenerative procedure (26)

1.7. Mechanical debridement

Mechanical debridement has always been one of the key to success of conventional endodontic treatment. Its role is to disrupt the bacterial biofilm in order to facilitate its elimination together with irrigation (27). Although efficient, during instrumentation, 35% or more of the surface of the canals remains untouched (27) and that way a smear layer is created (which could contain bacteria and obstruct the dentinal tubule that potentially enclose bacteria too) (28). It is advisable to perform little to no mechanical debridement in order to preserve the already thin dentinal wall and impede any fracture risks (14). Although many clinicians and the AAE advise against mechanical debridement, it is still used in many cases. Subsequent to histobacteriological analysis in failed revascularization, some authors still advocate for mechanical debridement after the discovery that it leads to a treatment failure mainly because of the presence of bacteria at the apical part of the root (14). It is important to remember that until now, the efficiency of removing bacteria during instrumentation in immature permanent teeth hasn’t been investigated. Considering the previous points, a chemical debridement is then essential in order to get a proper bacterial control as mechanical one on itself is insufficient and could cause a fracture of the thin dentinal wall (29).

1.8. Chemical debridement

Irrigation with sodium hypochlorite (NaOCL) is used in endodontic since 1920 (28). Since then, it is the most widely used irrigant (29) as it holds effective bactericidal capacity, lubrication capacity and also a tissue dissolution capacity (property that does not share chlorhexidine (27)). The main roles of chemical irrigants are : the control of bacteria inside the canals (being the most essential one), as well as the impediment of debris compaction and extrusion of bacteria at the apex (27). It was established that organic and inorganic compound of the dentin in suspension in a solution could alter the bactericidal efficiency of the irrigant (27); thus, the AAE in the protocol for REP recommended a copious irrigation of 20ml of sodium hypochlorite of the canal in order to renew the irrigant and remove the organic and inorganic compound present in the canal (14). As in regenerative endodontic the treated tooth is already weakened due to its thin dentinal walls, maintenance of adequate structural characteristics should be a requirement. Sodium hypochlorite has proteolytic activity over dentin collagen matrix when used in high concentration (28), and has shown to affect those structural characteristic by decreasing elastic modulus and flexural strength significantly when used in high concentration (concentration of 5.25% compared to one of 0.5%) (30). On the other end, previous studies exposed the fact that reduction of intracanal bacteria was not greater at a 5% concentration in comparison to one of 0.5% (28). In addition to the previously established key factors in successful endodontic outcome, namely, bacterial control and structural strength maintenance; new key factors in the achievement of REP have emerged : maintenance of viable stem cells together with growth factors availability. Following the discovery made in 2011, it was detected that subsequent to evoked bleeding, numerous mesenchymal stem cells were delivered into the root canal (29).

Taking into account the central role that occupied stem cells play in the success of revascularization, irrigation should not only be guided by the need of bacterial control but also by its possible deleterious effects on stem cells. It has been demonstrated that NaOCL, at a concentration of 0.5%, 1.5% and 3%, decreased the availability of stem cells from the apical papilla (SCAP) by around 37% whereas at a concentration of 6%, the reduction was very significant around 85% (31). This deleterious effect of NaOCL was partially reversed after using 17% EDTA (Ethylenediaminetetraacetic acid) (31). Another mineralization precursor of odontoblast; dentin sialophosphoprotein (DSPP) is affected by NaOCL depending on its concentration. At a concentration of 1.5% NaOCL does not affected DSPP gene expression, whereas at 3%, it reduces DSPP expression by half and completely erases its expression when NaOCL is used at 6% (31). Those effects were reversed, one completely and the other by half (respectively) after the use of 17% EDTA. NaOCL has demonstrated to have long lasting effect on dentin that couldn't be attributed to the presence of remaining irrigant in the dentinal tubule; after its deactivation with 5% sodium thiosulfate , the negative effects of NaOCL were not reversed (29). Those effects include denaturation of dentin-derived growth factors together with a decrease in dental pulp stem cells (DPSCs) attachment on dentin (31). DPSC exhibited (*in vivo*), higher proliferation rate and higher number of odontoblast-like marker when placed in a tooth-sliced model in comparison to DPSCs placed in a scaffold only (32). It could be established that morphogens and more precisely growth factors, play a key role in dental stem cells survival, proliferation and differentiation (32). EDTA has shown to solubilize growth factors from dentin and thus to increasing their bioavailability (31). It also promoted stem cells differentiation and attachment (29). A balance should be found between the leading concepts around bacterial control and the emerging ones around stem cells and growth factor survival. Therefore, the actual recommendations for chemical irrigation are : the use of 1.5% NaOCL followed by 17% EDTA.

1.9. Intracanal medication

The first intracanal medicament used in REP was the double antibiotic paste (DAP) by Iwaya *et al*. in 2001 composed of metronidazole and ciprofloxacin (14). Later Branchs & Trope in 2004 added minocycline to what would soon become the triple antibiotic paste (TAP) (33). This modification was created because of previous studies showing the efficacy of the TAP to completely eliminate bacteria *in vitro* with a concentration of 100 µg/ml (29). *In vivo* study on dog teeth also revealed the efficacy of the TAP, being effective in eliminating cultivable bacteria in 70% of the treated teeth and where the remaining bacteria were only present in a very small amount (29). It was then modified again by Thibodeau & Trope in 2007; minocycline, which is associated with discoloration (34) has been replaced by cefaclor (33). In many case reports, clinicians focused on the consistency of the TAP and not on the concentration (29). They wanted to achieved a creamy texture by mixing it with water, saline or propylene glycol; favouring its handling and delivery into the canal (29). But, at this described consistency, the concentration of antibiotic is around 1,000 mg/ml which is known to have detrimental effect over survival of SCAPs (35). In a study from 2012, the effect of different concentrations of antibiotics commonly used in revascularization was analysed; it was found that all the antibiotics studied (DAP, TAP, modified TAP with cefaclor and Augmentin) had a detrimental effect over SCAP survival when used at a concentration of 1 mg/ml, 10 mg/ml, 100 mg/ml and 1,000 mg/ml (35). This detrimental effect followed a concentration dependent pattern (35). The use of antibiotics at a concentration of 0.1 mg/ml and 0.01 mg/ml is safe over SCAP survivals (35) and effective against endodontics bacteria (29). In their comprehensive review from 2018, Kim et al. remind that Augmentin, despite being effective in bacterial destruction, only affects bacteria's cells wall (which is absent in human cells) thus it is not supposed to be harmful on human cells (9), but according to the study of Ruparel from 2012, at a certain concentration, Augmentin decreases survival rate of SCAP (35). Antibiotics exhibited several problems : their potential to induce bacterial resistance, the possible systemic allergic reaction, the cytotoxic effects on cells and the problem of removability (13). Following different common irrigation methods, it was revealed that more than 80% of TAP was still present in the canals whereas more than 85% of calcium hydroxide was removed (29). Despite its effect on SCAP, TAP have a negative impact on growth factors released from dentin, while calcium hydroxide positively influence their release (13). Calcium hydroxide on the other hand, doesn't affect survival of SCAPs (35) and exhibits bactericidal properties (11). It also promotes the release of bio-active molecules from the dentine matrix which possess the capacity to support reparative dentinogenesis (36). However Yassen et al. in 2013, showed that the use of calcium hydroxide, DAP and TAP for a long period of time (3 months) had a significant impact on fracture toughness and reduced it by 30%, 21% and 19% respectively (37). TAP and DAP additionally, significantly decrease the root microhardness; thus, the additional effect of EDTA irrigation on demineralization should be taken into account when selecting the intracanal medication (37). At the time of intracanal medication election, the clinician should consider : its efficiency to create a bacteria-free environment, its possible effect on dentine matrix (structural strength, growth factors release), its consequence on SCAP and the potential for adverse reactions.

1.10. Stem cells and growth factors

Regenerative endodontic procedures rely on stem cells' potential (9). They have the ability to differentiate into different phenotypes (odontoblasts, neural progenitors, osteoblasts, endothelial cells) upon induction from environmental stimuli such as, hypoxia, extracellular matrix, growths factors or other conditions (6). To achieve the desired result in revascularization, namely the recovery of a functional dentinal-pulp complex, three major principles of tissues engineering are applied (33). They enclose an adequate source of progenitor/stem cells, viable growth factors capable of inducing differentiation of stem cells and a 3D scaffold that regulate stem cells differentiation through local release of growth factors (6). The main source of stem cells in REP is the periapical tissue (33). Following laceration of the apical papilla, the influx of blood inside the root canal contained a concentration of markers specifics to undifferentiated mesenchymal stem cells 400 to 600 times superior (6). Following this influx, stem cells should be guided by growth factors which regulate survival, migration, proliferation and differentiation of cells (9). Mineralized dentin contains several biological molecules such as glycosaminoglycans, non-collagenous proteins and growth factors which are solubilized during dentin conditioning by EDTA (9). They could orientate the behaviours of stem cells (9). Different essential families of growth factors are needed to achieve a functional dentinal-pulp complex. Transforming growth factors β (TGF- β) are associated with cell proliferation, extracellular matrix synthesis, odontoblastic differentiation and dentinogenesis (38). They also have a chemotactic effect on dental pulp stem cells (*in vitro*) and play a role in immune response of the pulp (38). Platelet derived growth factors (PDGF) act on angiogenesis and promote cell differentiation, dentin matrix synthesis, odontoblastic differentiation and dentinogenesis (38). Bone morphogenic protein (BMP) act on odontoblastic differentiation and dentinogenesis (38). Vascular endothelial growth factors (VEGF) promote odontoblastic differentiation and cell proliferation and act on angiogenesis(38). Fibroblast Growth factors (FGF2) promote chemotaxis, angiogenesis but also cell proliferation and dentinogenesis(38). Insulin-like growth factors (IGF) act on cell proliferation and odontoblastic differentiation (38). Nerve growth factors (NGF) encourage cell differentiation and angiogenesis (38). Through the use of those growth factors it was shown that the functional pulp of a permanent teeth could be recovered (*in vivo*) by a cell homing approach (39). Even though it was an ectopic transplantation, it exhibited the complete pulp revascularization without the need for cell transplantation (39).

The role of SCAP in the root development hasn't been clearly established yet, but still it has been enlightened by previous studies (8). It was observed (*in vivo*) that in teeth where the pulp tissue was removed and the apical papilla retained (following trauma and pulp exposure); a continuation of root tip formation could be appreciated (8). Further histological examinations are needed to confirm if the formed tissue is dentin or cementum. Another interesting study conducted in minipigs, exhibited the discontinuation of root formation when apical papilla was removed at early stage of tooth development (8). Even though the discontinuation in formation could be due to a damaged HERS during surgical removal of SCAP, those results are promising in showing the role of SCAP.

1.11. Scaffold

A scaffold is one of the main element of REP; it is a three dimensional structure that allows cell binding, cell localisation and which provides local release of growth factors (24) through platelet activation (40). It should be biodegradable and simulate the extracellular matrix (9). Blood clot (BC) is the most widely used scaffold. Besides being cheap, it does not require manipulation by the clinicians and provides the entry of numerous cells into the root canal (41). However the types of cells and their concentration couldn't be selected, making the outcome unpredictable (41). Another issue that could appear would be the impossibility of inducing bleeding into the root canals, maybe because of an extended periapical destruction. This concern might be solved either by postponing the procedure until recovery from the peri-apical tissue (9) or by going through an apexification procedure in single rooted teeth (as for multi-rooted teeth, bleeding can be harvested from the others canals) (41). Autologous platelet concentrates have been used as scaffolds (either alone or together with induced bleeding) because of their high concentration of platelets and their possible improvement of revascularization outcomes (42). Platelet rich plasma (PRP), platelet rich fibrin (PRF) are the most commonly used. Platelet pelet (PP) have been tried for the first time in REP in 2019 (42). Even though their use was associated with positive clinical and radiological outcomes, following histological analysis, no pulp like-tissue or odontoblastic cells were found (41). Additionally no differences were found in terms of root maturation between PRP, PRF, PP and BC (42). Thus because of the high cost of autologous platelet concentrate and their small benefice over BC, their use isn't recommended (41). The development of an artificial scaffold that would contain a cocktail of different growth factors at precise concentrations and with angiogenic, proliferative, differentiative and chemotactic properties specific of the dentinal pulp complex should be investigated.

1.12. Coronal seal

Once the scaffold is set, bacterial contamination remains an issue and should be prevented (41). This is achieved through the placement of coronal plug of biocompatible material capable of an effective bacterial seal (14). The capping materials advised by the AAE are white MTA or calcium hydroxide (14). Calcium hydroxide has been associated with discoloration problems (14). White MTA has been developed due to the discoloration problems of grey MTA but white MTA remain associated with discoloration problems (41). New types of bioceramics cements could be used such as BioAggregate or Biodentine to overcome those aesthetic problems in anterior sector (41). It is recommended to place the capping material over a sponge of collagen to prevent its overextension (41). The capping material is then covered by a layer of 3-4mm of glass ionomer cement (29). This will be reinforced by a final layer of bonded reinforced composite restoration (29).

1.13. Adverse effects

Discoloration is one of the most known adverse effects of revascularization. It affects around 40% of teeth following REP (9). It is usually associated with TAP that contained minocycline (14). An alternative to minocycline involves the use of DAP or modified TAP (with cefaclor) (34). Capping material such as MTA (grey or white) and calcium hydroxide are known to produce discoloration as well (9). Therefore, when working on aesthetic anterior sectors, the choice of material is of particular importance. Bacterial reinfection, because of improper canal disinfection or bacterial leakage may also happened and lead to failure of the procedure (9). Another major issue are fractures, which have been observed in some cases (9). Ultimately, root canal calcification following revascularization was found in 62.1% of the cases (9). Its prevalence might be increased when using blood clot as a scaffold, as blood clots have been associated with a progressive root canal obliteration independently of the intracanal medication used (TAP or Calcium hydroxide) (42).

1.14. Histology of the formed tissues

Repair is defined as "the development of replacement tissue, such as scar tissue, without restoration of function" (19). Numerous histological examinations following revascularization exhibited tissue formation resembling cementum, bone and fibrous connective tissue (14). Two of them found dentin-like and pulp like tissues inside the root canal (43,44). It is important to note that in these two cases there was irreversible pulpitis. Stem cells from the dental papilla, stem cells from apical papilla and stem cell from inflamed pulp tissues have the potential, upon appropriate induction to differentiate into odontoblast-like cells (9). Thanks to the tissues found in many animals and humans histological study, it is supposed that the stem cells recruited during evoked bleeding were mainly from the periodontal ligament and bone marrow (9). Even though positive response to pulp vitality was found in 50% to 60% of the cases following revascularization, histologic examination discard the possibility that this is due to a mature pulp tissue (9). The positive response is mainly due to the vital tissue that were formed which are vascularized and innervated (9). Hence, revascularization could be defined as repair instead of regeneration (9). The mechanical behaviour and the junction (attachment or adhesion) of this newly formed mineralized tissue with the tooth has not been studied yet (41).

1.15. Future perspectives

An exciting perspective in the field of revascularization is to be able to achieve a true regeneration. It would bring through the pulp organ, new "inductive, formative, nutritive, sensory and protective functions" (5) to the tooth. Currently the revascularization is achieved through two approaches that are both based on tissue engineering principles: endogenous stem cells, growth factors with inductive capacities and a biomimetic scaffold which provide a three dimensional support for the growth factors and with the capacity to attract stem cells (13). Cell-free or cell homing is the technique used nowadays; it relies on local stem cells and does not need any ex-vivo manipulation and expansion (14). This procedure, despite being cheap, does not allow to choose the stem cells that will enter the root canals. They could come from the apical papilla, periodontal ligament or bone marrow (14). Cell-base method is the other option to achieve revascularization, it consists of the sampling of autologous stem cells of pulp tissue followed by their expansion in the adequate culture medium for their future reimplantation (13). It has proven, in several animals studies, the ability to regrow a functional pulp complex (9). But it faces problems such as handling and the availability of autologous stem cells (14). Stem cells could be dental pulp stem cells, cells from exfoliated deciduous teeth and stem cells from apical papilla (14). Another interesting aspect of the procedure is its potential use in mature permanent teeth. In a 60 years-old subject, Inflamed periapical progenitor cells of periapical tissue have been able to differentiate into mineralized phenotype (6) and revascularization were successful in subject with an apex of 0.5mm (25). More randomized clinical trial are needed to establish the feasibility and possible benefit of the procedure in mature teeth.

2. Objective:

At the light of the several possible approaches of revascularization, through different protocols and the observation that they all possibly could be successful, the main objectives of this work is to compare the different protocols available. To support this work and answer to this objective, two secondary objectives have been established : compare the short term and long term outcomes of each protocol through the review of clinical cases and the determination of the advantages and disadvantages of each protocols.

3. Methodology:

To conduct the research of scientific articles for the systematic review, articles ranging from 2001 to 2021 on the database PubMed, clinical research on revascularization using the following keywords: “pulp revascularization” or “pulp revitalization” was conducted.

Inclusion criteria : Studies conducted on humans, patients with immature permanent teeth suffering from necrosis or irreversible pulpitis, a follow-up of at least 12 months in each case, clearly described protocols and in English language.

Exclusion criteria: Study on animals, patient with permanent mature teeth, a follow-up of less than 12 months, unclear protocols, studies in languages other than English, insufficient data. The search produced 418 results. 43 were eliminated as they were not within the date range established, 23 were removed because the text was not full. 11 were not in English, 120 were not conducted on humans, 141 were discarded because there was no case report, clinical trials or randomized controlled trials. Thus 80 articles were selected. From those 37 were discard as they did not have a sufficient follow-up or the clinical protocol was unclear or the subject was not about

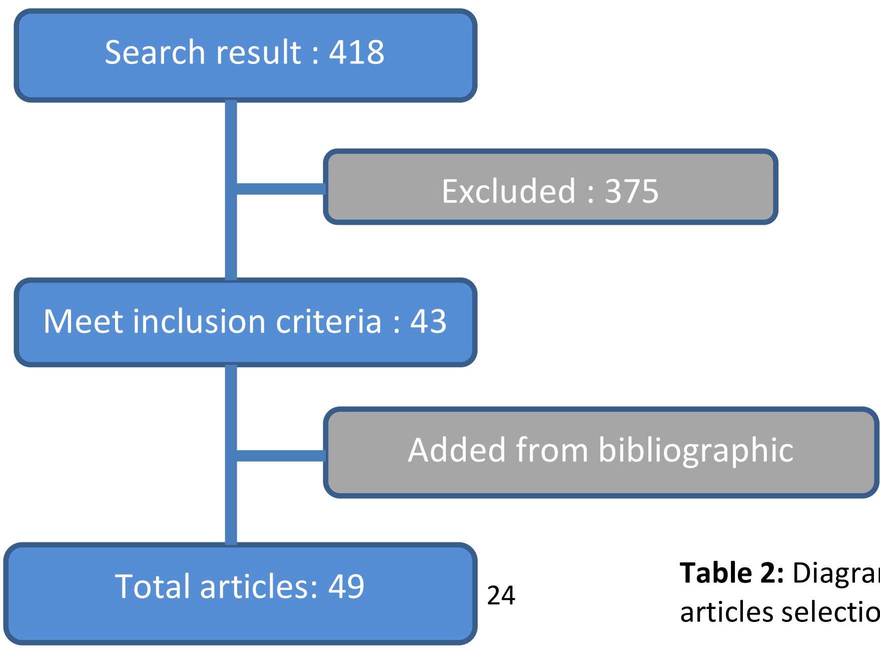

Table 2: Diagram showing the articles selection process

revascularization. 6 articles were added following a bibliographic review of the selected articles. A total of 49 cases were included.

The following information was collected : sample size, patient age, pathology of the patient (pulp state, periapical state, aetiology), mechanical debridement, irrigation (type of irrigant and concentration), intracanal medication (type and concentration), final irrigation (type and concentration), evoked bleeding, additional scaffolds, follow-up, outcomes (resolution of the periapical pathology, increase in root length, increase in root width, apical closure, failure), adverse effects (discoloration or others), response to vitality testing.

4. Results:

References	No. Of teeth	Tooth type	Avg. age	Sex	Aetiology	Pulp state	Periapical affectation	Instrumentation	Irrigation	Intracanal medication	Final irrigation	Evoked bleeding	Additional scaffold
Iwaya et al. (45)	1	PM	13	F	*	N	Y	Minimal	5% NaOCL 3% H_2O_2	DAP	*	*	*
Branchs & Trope (17)	1	PM	11	M	*	N	Y	N	5.25% NaOCL 0.12% CHX	TAP	*	*	*
Thibodeau & Trope (46)	1	I	9	M	T	N	Y	N	1.25% NaOCL	TAPm	*	Y	*
Cotti et al. (47)	1	I	9	F	T	N	Y	N	5% NaOCL 3% H2O2	Ca(OH)2	*	Y	*
Jung et al. (48)	4	4PM	10.75	4F	4*	4N	4Y	4*	2.5% NaOCL	3TAP 1Ca(OH)2	4*	4Y	4*
Thibodeau (49)	1	I	9	M	T	N	Y	N	1.25% NaOCL	TAPm	*	Y	*
Reynolds et al. (50)	2	2PM	11	F	2DE	2N	2Y	2*	6% NaOCL 2% CHX	2TAP	2*	2Y	2*
Ding et al. (51)	3	1I 2PM	8.5	2F 1M	1DE 2T	3*	3Y	3*	5.25% NaOCL	3TAP	3*	3Y	3*
Shin et al. (52)	1	PM	12	F	DI	N	Y	*	6% NaOCL 2% CHX	*	*	*	*
Iwaya et al. (53)	1	I	8	M	T	N	Y	N	5% NaOCL 3% H_2O_2	Others	*	*	*
Jung et al. (54)	2	2PM	11	2F	2DE	2N	2Y	2*	2.5% NaOCL	Ca(OH)2 TAP	2*	1Y 1*	2*
Nosrat et al. (55)	2	2M	8,5	1F 1M	2C	2N	2Y	2N	5.25% NaOCL	2TAP	2*	2Y	2*
Miller et al. (56)	1	I	9	M	T	IR	Y	Y	2% CHX 17% EDTA	TAP	EDTA 17%	Y	*
Aggarwal et al. (57)	1	I	24	F	T	N	Y	Minimal	5.25% NaOCL 2% CHX	TAP	*	Y	*
Shivashankar et al. (58)	1	I	9	M	T	N	Y	Y	5.25% NaOCL 0.2% CHX	TAP	*	*	PRF
Dabbagh et al. (59)	16	13I 1PM 2M	9.4	12*	12T 2C 1DE 1DI	16N	9Y 7*	16*	5% NaOCL	2TAP 14TAPm	16*	16Y	16*
Cehreli et al. (60)	1	I	8.5	M	T	*	Y	N	2.5% NaOCL	Ca(OH)2	*	Y	*
Lenzi et al. (61)	2	2I	8	M	2T	2N	1Y 1*	2*	2.5% NaOCL	2TAP	2*	2Y	2*
Kim et al. (62)	3	3PM	11.5	2M	3*	3N	3Y	3*	3% NaOCL	3TAPm	3*	3Y	3*
Narayana et al. (63)	1	I	11	M	DI	N	Y	*	5.25% NaOCL	TAP	*	Y	*

Shimizu et al. (43)	1	I	9	M	T	IP	N	Minimal	5.25% NaOCL	Ca(OH)2	*	Y	*
Kottoor et al. (64)	1	I	12	M	T	N	Y	Partial	5.25% NaOCL	TAP	*	Y	*
Martin et al. (65)	1	M	9	M	C	N	Y	Y	5.25% NaOCL	TAP	*	Y	PRP
Soares et al. (66)	1	I	9	F	T	N	Y	Partial	2% CHX	Ca(OH)2 & 2% CHX	EDTA 17%	Y	*
Yang et al. (67)	1	I	11	M	DI	N	Y	N	5.25% NaOCL	TAP	*	F	*
Sönmez et al. (68)	3	3M	9	2F 1M	3C	3N	3Y	3*	5.25 NaOCL	3TAP	3*	3Y	3*
Keswani et al. (69)	1	I	7	M	T	N	Y	N	5.25% NaOCL	TAP	3*	3N	3*
Mishra et al. (70)	1	I	11	M	T	N	Y	Minimal	2.5% NaOCL	TAP	*	*	PRF
Jadhav et al. (71)	6	6I	15.3	2M 1F	4T 2*	6N	6Y	6*	2.5% NaOCL	6TAP	6*	6Y	3PRP
Forghani et al. (72)	1	I	9	M	T	N	Y	*	5.25% NaOCL	TAP	*	Y	*
Shimizu et al. (73)	1	I	9	M	T	N	Y	*	2.6 NaOCL	Ca(OH)2	*	Y	*
Bezgin et al. (74)	2	2PM	12	2M	1C 1*	2N	2Y	2N	2.5% NaOCL 0.12% CHX	2TAPm	2EDTA 5%	2*	2PRP
Becerra et al. (75)	1	PM	11	F	1DE	N	T	N	5.25% NaOCL 2% CHX	TAP	*	Y	*
Lin et al. (76)	1	I	6	M	T	PIT	Y	N	5.25% NaOCL	TAP	*	Y	*
Nagy et al. (77)	20	20I	10.85	9M 11F	20*	20N	20*	20Minimal	2.6% NaOCL	20TAPd	20*	20Y	10 aHS + bFGF
Mccabe et al. (78)	1	I	7	F	T	N	Y	N	5% NaOCL	N	EDTA 17%	Y	*
Sachdeva et al. (79)	1	I	16	M	T	N	Y	N	5.25% NaOCL	TAP	*	*	PRP
Pini et al. (80)	1	I	7	M	T	N	Y	*	6% NaOCL 2% CHX	TAP	*	Y	*
Wang et al. (81)	2	2PM	39	F	2DE	2N	Y	*	2.5% NaOCL	2TAP	2*	2*	2PRP
Ray et al. (82)	1	I	11	M	T	N	Y	*	0.5% NaOCL 17% EDTA	DAP	EDTA 17%	Y	PRF
Santiago et al. (40)	5	5I	8.6	3M	5T	5N	5Y	5*	5.25% NaOCL	5TAP	5*	5*	5*
Zhujiang et al. (83)	1	M	20	M	*	N	Y	Y	6% NaOCL 2% CHX	Ca(OH)2	EDTA 17%	Y	rhPDGF-BB
Cho et al. (22)	1	C	11	M	DI	N	Y	Y	3% NaOCL	DAP	*	Y	*
Asgary et al. (84)	1	I	12	F	T	N	Y	Y	5.25% NaOCL	TAP	*	Y	*
Schmoeckel et al. (85)	1	M	8	M	C	N	Y	Minimal	0.6% NaOCL	TAP	EDTA 17%	Y	*
Bakhtiar et al. (86)	5	5I	11	3F 1M	3PIT 1DI 1T	5N	4Y 1N	5Minimal	1.5% NaOCL	5TAPm	EDTA 17%	Y	PRF
Peng et al. (44)	1	PM	11	F	DE	IP	Y	*	5.25% NaOCL	TAP	*	F	*
Kahler et al. (87)	2	2PM	11	F	2*	2N	2Y	2*	1% NaOCL	2TAPa	2*	2Y	2*
Elsheshtawy et al. (88)	31	31I	12.65	15M 11F	30T 1DI	31N	18Y 13*	31Minimal	5.25% NaOCL	31TAP	31 EDTA 17%	17Y 14*	14 PRP

No, number; Avg, average; *, not specified in the article; I, incisor; C, canine; PM, premolar; M, molar; F, female; M, male; N, necrosis; IP, irreversible pulpitis; PIT, previously initiated therapy; Y, yes; N, no; F, failure; NaOCL, sodium hypochlorite; CHX, chlorhexidine; H_2O_2 , hydrogen peroxide: EDTA, ethylenediaminetetraacetic acid; DAP, double antibiotic paste – metronidazole, ciprofloxacin; TAP, triple antibiotic paste –

metronidazole, ciprofloxacin, minocycline ; TAPm, triple antibiotic paste modified – metronidazole, ciprofloxacin, cefaclor; TAPd, triple antibiotic paste with doxycycline - metronidazole, ciprofloxacin, doxycycline; TAPa, triple antibiotic paste with amoxicillin – metronidazole, ciprofloxacin, amoxicillin; PRP, platelet rich plasma; PRF, platelet rich fibrin; aHS, artificial hydrogel scaffold; bFGF, basic fibroblast growth factor; rhPDGF-BB, recombinant human platelet derived growth factors.

Table 3: Pre-treatment

References	Average follow-up (in months)	Resolution of symptoms and periapical healing	Apexogenesis	Root length increase	Root width increase	Apical closure	Pulp vitality	Discoloration	Results
Iwaya et al. (45)	**30**	**Y**	**Y**	*****	**Y**	**Y**	**Y**	*****	**S**
Branchs & Trope (17)	**24**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	**S**
Thibodeau & Trope (46)	**12.5**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	*****	**S**
Cotti et al. (47)	**30**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	*****	**S**
Jung et al. (48)	**19.25**	**4Y**	**3Y 1N**	**1Y 1N 2***	**3Y 1N**	**3Y 1N**	**4***	**4***	**3S 1F**
Thibodeau (49)	**41**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	*****	**S**
Reynolds et al. (50)	**18**	**2Y**	**2Y**	**2Y**	**2Y**	**2Y**	**2Y**	**1Y 1N**	**2S**
Ding et al. (51)	**17**	**3Y**	**3Y**	**3Y**	**3Y**	**3Y**	**3Y**	**3***	**3S**
Shin et al. (52)	**19**	**Y**	**Y**	*****	**Y**	**Y**	**N**	*****	**S**
Iwaya et al. (53)	**156**	**Y**	**Y**	*****	**Y**	**Y**	**Y**	**N**	**S**
Jung et al. (54)	**33.5**	**2Y**	**2***	**1N 1***	**1N 1***	**2***	**2***	**2***	**2F**
Nosrat et al. (55)	**16.5**	**2Y**	**2Y**	**1Y 1N**	**2Y**	**1Y 1N**	**2N**	**2***	**2S**
Miller et al. (56)	**18**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**S**
Aggarwal et al. (57)	**24**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	*****	**S**
Shivashankar et al. (58)	**12**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	**S**
Dabbagh et al. (59)	**24**	**16Y**	**16Y**	**16Y**	**16Y**	**16Y**	**16***	**2Y 14***	**16S**
Cehreli et al. (60)	**18**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	**S**

Lenzi et al. (61)	**21**	**2Y**	**1Y 1N**	**1Y 1***	**1Y 1***	**1Y 1***	**2***	**2***	**1S 1F**
Kim et al. (62)	**38**	**3Y**	**1Y 2***	**1Y 2***	**3Y**	**1Y 2***	**3***	**3***	**3S**
Narayana et al. (63)	**12**	**Y**	*****	**N**	**N**	*****	*****	*****	**F**
Shimizu et al. (43)	**1,5**	*****	*****	*****	*****	*****	*****	*****	**F**
Kottoor et al. (64)	**60**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	*****	**S**
Martin et al. (65)	**25**	**Y**	**Y**	*****	**Y**	*****	*****	*****	**S**
Soares et al. (66)	**24**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	*****	**S**
Yang et al. (67)	**24**	**Y**	**Y**	*****	**Y**	**Y**	**N**	**Y**	**S**
Sönmez et al. (68)	**24**	**3Y**	**3Y**	**3***	**3Y**	**3Y**	**3***	**3***	**3S**
Keswani et al. (69)	**15**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	**S**
Mishra et al. (70)	**12**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	**S**
Jadhav et al. (71)	**12**	**6Y**	**6Y**	**6Y**	**6Y**	**6Y**	**6***	**6***	**6S**
Forghani et al. (72)	**18**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	*****	**S**
Shimizu et al. (73)	**26**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	*****	**S**
Bezgin et al. (74)	**12**	**2Y**	**2Y**	**2***	**2***	**2Y**	**2N**	**2***	**2S**
Becerra et al. (75)	**24**	**Y**	**Y**	**N**	**Y**	**Y**	*****	*****	**S**
Lin et al. (76)	**16**	**N**	*****	*****	*****	*****	*****	**Y**	**F**
Nagy et al. (77)	**18**	**17Y 3***	**17Y 3***	**17Y 3***	**17Y 3***	**17Y 3***	**20***	**20***	**17S 3F**
Mccabe et al. (78)	**18**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	*****	**S**
Sachdeva et al. (79)	**36**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	*****	**S**
Pini et al. (80)	**18**	**Y**	**Y**	**Y**	**N**	**Y**	**N**	*****	**S**
Wang et al. (81)	**30**	**2Y**	**2N**	**2N**	**2N**	**2N**	**2N**	**2***	**2F**
Ray et al. (82)	**36**	**Y**	**Y**	**Y**	**N**	**N**	**N**	**Y**	**S**
Santiago et al. (40)	**63**	**1Y 4N**	**1Y 4N**	**1Y 4N**	**1Y 4N**	**1Y 4N**	**5***	**1Y 4N**	**5F**
Zhujiang et al. (83)	**15**	**Y**	**Y**	*****	**Y**	**Y**	**Y**	*****	**S**
Cho et al. (22)	**24**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	**Y**	**S**
Asgary et al. (84)	**36**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	*****	**S**
Schmoeckel et al. (85)	**24**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	*****	**S**
Bakhtiar et al. (86)	**18**	**5Y**	**5Y**	**5***	**5Y**	**4Y 1***	**5***	**3Y 2***	**5S**
Peng et al. (44)	**12**	**Y**	**Y**	**Y**	**Y**	**Y**	*****	*****	**S**
Kahler et al. (87)	**96**	**2Y**	**2Y**	**2N**	**2Y**	**2***	**2Y**	**2Y**	**2S**
Elsheshtawy et al. (88)	**12**	**31Y**	**27Y 4N**	**31***	**31***	**31***	**31***	**25Y 6***	**27S 4F**

Table 4: Post-treatment

Variable	%
Sex	
Male	50%
Female	40.2%
Not specified	9.8%
Tooth type	
Incisor	74.7%
Canine	0.7%
Premolar	17.6%
Molar	7%
Aetiology	
Trauma	54,2%
Dens invaginatus	4.9%
Dens evaginatus	7%
Caries	7%
Others	2%
Not specified	24.7%
Pulp state	
Necrosis	94.4%
Irreversible pulpitis	2%
Others	0.7%
Not specified	2.8%
Periapical state	
Apical periodontitis	28.2%
Apical abscess	15.5%
Normal periapical tissue	1.4%
Not precise enough	38.7%
Not specified	16%
Instrumentation	
No	11.3%
Yes	4.2%
Partial	1.4%
Minimal	43.7%
Not specified	39.4%
Irrigant	
NaOCL	64.4%
NaOCL with other irrigants	26.5%
Others combinations	4.1%
Intracanal medication	
TAP	56.3%
TAPm	18.3%
DAP	2.1%
Ca(OH)2	4.9%
Others combinations	17%
None	0.7%
Not specified	0.7%

Table 5: Demographic and study

Variable	Yes (%)	No(%)	Not specified (%)
Periapical healing	**93.4%**	**2.8%**	**3.5%**
Apexogenesis	**84.5%**	**8.6%**	**7%**
Increase root length	**50%**	**9.15%**	**40.9%**
Increase root thickness	**63.4%**	**7.8%**	**27.5%**
Apical closure	**61.3%**	**6.4%**	**32.4%**
Pulp vitality	**11.3%**	**12%**	**76.8%**
Discoloration	**27.5%**	**4.2%**	**68.3%**

Table 6: Follow-up variables

5. Discussion:

According to this systematic review, 142 teeth were studied. The success rate outcome for revascularization was of 85.2%. This means that in those cases, some increased root length, thickness and/or apical closure were detected. But care must be taken at the time of interpreting the results as the majority of the data was extracted from case reports and/or case series and not only from randomized clinical trials. Thus, this data might be subject to bias publication as authors are more prone to report cases with positive outcomes rather than cases with negative outcomes. Another important point at the time of interpreting the results is the great diversity in protocols and the lack of standardized analysing methods. Although no differences were found between 2-dimensional radiograph and CBCT at the time of assessment in root development (88), the use of standardized analysing methods for each patients and at each appointment is mandatory in order to reduce the risk of distortion due to angulation which could alter the results.

The tooth was considered as whole, thus for multirooted teeth if a canal presents a characteristic and/or pathology, it was recorded as if all the canals were subject to it. In the articles, An indicated “continued root formation and/or physiological root formation” which informed of the outcome was recorded as an increase in length and width. Minimal instrumentation referred to a light instrumentation up to the apical third whether partial instrumentation referred to an instrumentation of the coronal and/or middle third. The case was considered a failure if the tooth did not achieve the primary objective (Elimination of the symptoms and the evidence of bone healing) but also if it did not achieve one of the secondary objectives (Increase root wall thickness and/or increase root length and/or apical closure) or if it was recorded by the author as failure.

5.1. Revascularization protocols:

5.1.1. Mechanical debridement

Regarding instrumentation, nearly half of the cases were found to have some kind of root canals mechanical debridement which is not in accordance with the clinical consideration for regenerative procedure proposed by the AAE. It is supposed that authors had chosen to use it following the discovery that failure in REP were mainly due to remaining bacteria in the apical third (14). As the procedure is performed on teeth with thin dentinal wall, the potential benefit on bacterial control might not exceed the risk of weakening the already fragile, fracture prone immature teeth (3). The instrumentation created a smear layer that will occlude the dentinal tubule and alter the cell migration, proliferation and adhesion (25). However, in the ten cases with a failed outcome (40,43,48,54,61,63,76,77,81,88), only two used instrumentation (77,88). Among the failed cases, EDTA was used in only one study (88). EDTA use (a chelator agent) as final irrigation solution for chemical debridement, has the potential to remove the smear layer created during the mechanical debridement; EDTA also promotes the release of growth-factors entrapped into dentin that will enhance differentiation of stem cells into odontoblast-like cells (31). This property of EDTA is known by many clinicians but it was only used in 9 out of 49 studies which represent 28% of the treated teeth. It was used with a concentration of 17% except one study which used it at 5% (74).

5.1.2. Chemical debridement

During irrigation, NaOCL was used in the majority of the case, either alone (64.4%) or together with another irrigant (26.5%). Its concentration varied from 0.5% to 6% but in many studies, it was used at concentration higher than 5%. The rationale for using this high concentration is unidentified especially because it was demonstrated that 5% NaOCL did not eliminate more bacteria inside the canal compared to 0.5% NaOCL (28) and also that 5.25% NaOCL significantly decreased the elastic modulus of dentin compared to a 0.5% concentration (28). It was also stated that it is the TAP and not the irrigant that have the greater bactericidal effect (23). Five of the failed cases used a concentration of 2.5% or 2.6% (44,48,54,61,77) while the rest use a concentration of 5.25% (40,43,63,76,88). Although it was demonstrated that NaOCL had deleterious effects on stem cells survival, especially when used a 6% (31) and if EDTA (that could reverse this deleterious effect) use was sparse; many more cases should have failed as stem cells are believed to be the corner stone in successful revascularization and that theoretically stem cells should have been damaged. In some cases chlorhexidine was used, either alone or together with NaOCL at a concentration that varied from 0.12% to 2%. While the cytotoxicity of chlorhexidine over stem cells have been verified (89), 11 studies used it in there protocols and all were all successful while among them 4 used EDTA as a final irrigation. Three studies used H_2O_2 together with NaOCL and all were also successful.

5.1.3. Intracanal medication

Regarding intracanal medication, TAP has been the most used (56%). It is plebiscite for its powerful antibacterial properties (studied *in-vitro*) (9), but it also raises concern for its potential systemic allergic effect, for the development of bacterial resistant strains (9). TAP moreover has a harmful effect over stem cell when used at high concentration (29) which is often the case as many author are concerned about the consistency of the paste and not about the concentration of antibiotics (13). It also appears that the elimination of the paste is complicated and that antibiotics have a profound effect on dentin (29). TAP has been associated with discoloration problems due to minocycline (14) which explained that some authors removed it from the formula or that it was replaced by cefaclor, doxycycline or amoxicillin. Five cases that used TAP exhibited discoloration (50,56,59,67,88), while three others case that used substitutes of minocycline such as DAP (22), TAPm (86) and TAPa (87) also suffered discoloration. $Ca(OH)_2$ is also recommended by the AAE as intracanal medication due to its antibacterial properties. It appears to have a positive impact over stem cells from the apical papilla (29). One of its adverse effects which is also common to the antibiotic paste, is its effect on dentin structure (29) but this deleterious effect only appears 2 months after the medication has been placed inside the canal (13).

5.1.4. Evoked bleeding

There is one successful case of revascularization in which there was not any evoked bleeding. The pulp was necrotic but the author used an additional scaffold of PRF (69). This scaffold rich in growth factor attracted apical stem cells and replaced the need for evoked bleeding (41). Two other cases failed to induced bleeding inside the canal. One from Yang (67) : the pulp was necrotic but apically there was a periapical periodontitis meaning that HERS could still be viable and capable of physiological root formation once bacterial control was established. The last one from Peng (44) is about a premolar with irreversible pulpitis and periapical periodontitis. It is observed by the author that the tooth was previously treated with partial pulpectomy which again should have favoured the survival of HERS. This could explain the success of the procedure. Also upon histologic analysis, pulp-like and dentin-like tissue were found apically. It was not specified if the tooth was instrumented. Thus the growing of those physiological tissues could be explained by remanent of pulp tissue.

5.2. Revascularization outcomes:

5.2.1. Primary and secondary objectives

Periapical healing and resolution of the symptoms which is essential were observed in 93,4%. Apexogenesis, that here encompass either an increase in root length, thickness and/or signs of apical closure, was observed in 84,5%. Increase in root length reach 50%, root thickness reach 63,4% and apical closure 61,3%. Those results are coherent with the procedure as the 3-dimensional scaffold inside the canal produce a progressive obliteration of the canal following formation of mineralized tissue, which means that the increase in root thickness is higher than the increase in root length. The high percentage of apical closure could also be explained as the increase in thickness of the root canal could lead to a closure of the apex by mineralized tissue without necessarily involving the increase in length.

5.2.2. Tertiary objective

Pulp vitality was found in 11,3% of the cases. This result, which is the tertiary goal of revascularization, could be misleading : the tissue growing inside the canal could be composed of periodontal ligament, cementum and bone, thus be vascularized and elicit a positive response upon vitality test (41) without being proper pulp tissue. Another factor that could alter the vitality testing is the capping layer of MTA of usually 3 to 4mm which could give a false negative response (41). Thus without a proper histological confirmation the positive response to vitality test might not be considered as a true regeneration (13).

5.2.3. Discoloration

Discoloration represent 27.5% of the cases. While it was mainly due to minocycline present in the TAP (13), the capping material, whether it is grey MTA or white MTA, could also cause discoloration (20). In this review, 7 cases reported discoloration subsequent to the use of TAP (40,50,56,67,76,88) and 1 changed its protocols to TAPm after two teeth exhibited discoloration (59). Following this modification in the protocols, no more teeth experienced discoloration. Three others cases experienced discoloration even though they didn't use TAP. No explanations were given about the aetiology, but each of them used MTA (22,82,87). One case suffered minimal discoloration (86). The author used TAPm and attributed the discoloration to the capping of Biodentine.

6. Conclusion:

Regarding the present study, revascularization showed signs of apexogenesis in the majority of cases, and a pronounced increase in root thickness, root length together with the closure of the apex in a great number of teeth. Those results demonstrate the great variability in procedures and outcomes regardless of the applied protocols. Due to a high number of protocols and their heterogenicity, the superiority of one over the others cannot be established. More randomized clinical trials are needed to determine the most effective protocol. This study encloses a majority of case reports and thus they can be subject to publication bias, which is an issue. Finally, All the studies were not analysed with the same rigour by their author due to the lack of standardized analysing methods.

A guideline which lists the different information that the dentist must follow should be established for a better understanding and analysis of each case. The precise role of stem cells in revascularization should be studied more in depth to guide the clinicians to the need of bacterial-free microenvironment while preserving stem cells.

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8. Annexes

1. **Interventions for managing immature permanent teeth with necrotic pulps**

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ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

The objective of this review will be to assess the effects of different interventions to manage immature permanent teeth with necrotic pulps.

BACKGROUND

Description of the condition

Dental caries and traumatic injuries are common problems in young children with immature permanent teeth, often leading to pulp necrosis, which is defined as a clinical diagnostic category indicating the death of the dental pulp (Glossary of endodontic terms 2015). Dental pulp is a richly vascularized and innervated specialized connective tissue of ecto-mesenchymal origin; contained in the central space of a tooth, surrounded by the dentine, with inductive, formative, nutritive, sensory and protective functions (Glossary of endodontic terms 2015).

An immature permanent tooth is a young/newly erupted permanent tooth with incomplete root apex formation. After a permanent tooth emerges in the mouth, it usually takes three more years for the root development to complete (Fouad 2009). The shape and form of these developing roots are determined by a two-layered cellular structure called the Hertwig's epithelial root sheath (HERS) (Bhasker 1991). Development of roots in the postemergent years consists of the increase in root length, increase in root wall thickness and narrowing of root canals in the apical region (root apex). Any disruption in the blood supply to HERS as a result of pulp necrosis can disrupt cell proliferation and differentiation causing cessation of root development. Abnormal hard tissue deposition may also occur in this situation (Torneck 1982).

2.

Dental Traumatology

Dental Traumatology 2008; **24**: 603–611; doi: 10.1111/j.1600-9657.2008.00696.x

Epidemiology of traumatic dental injuries – a 12 year review of the literature

Ulf Glendor

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Abstract – *Background/Aim*: A traumatic dental injury (TDI) is a public dental health problem because of its frequency, occurrence at a young age, costs and that treatment may continue for the rest of the patient's life. The aim of this paper is to present a12-year, international review of the prevalence and incidence of TDIs including some background factors and a quick, easy method in registering TDIs to receive a primary understanding of the extent and severity of dental trauma. *Material and method*: The databases of Medline, Cochrane, SSCI, SCI and CINAHL from 1995 to the present were used. *Result*: The results indicate a high prevalence of TDIs in primary and permanent teeth and that TDIs exists throughout the world. The prevalence show that one third of all preschool children have suffered a TDI involving the primary dentition, one fourth of all school children and almost one third of adults have suffered a trauma to the permanent dentition, but variations exist both between and within countries. Activities of a person and the environment are probably more determining factors of TDIs than gender and age. A risk profile why some patients sustain multiple dental trauma episodes (MDTE) is necessary to present. All dental clinics should have a prospective ongoing registration of TDIs. The NUC method (N = no TDI, U = uncomplicated TDI, C = complicated TDI) presents if there has been any TDI and the severity of that trauma. *Conclusion*: The trend of TDIs seems to be stable on a high level with variations largely reflecting local differences. Because of the complexity of TDIs, every dental clinic should have a prospective ongoing registration of number and severity of TDIs.

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Is a traumatic dental injury (TDI) a public dental health problem today? The answer is an overwhelming 'yes' and the reasons are fourfold. First, trauma to the oral region occurs frequently and makes up 5% of all injuries for which people seek treatment in all dental clinics and hospitals in a county(1). Second, TDIs tend to occur at a young age during which growth and development take place (2). In preschool children, for example, the figure is as high as 18% of all injuries (1). Third, treating a TDI can often be complicated and expensive (3), frequently involving participation of specialists in several disciplines. Fourth, in contrast to many other traumatic injuries treated on an outpatient basis, a TDI is mostly irreversible and thus treatment will likely continue for the rest of the patient's life (2, 3).

Other questions about TDIs are of interest. Will TDIs in the future look the same as they do today? Will it still be 10 to 12-year-old boys that are the most likely victims of dental trauma (4)? Or, will it be an increase among girls because of their increasing interest in sports? Or, are we going to see elderly people who still have their own teeth, with an increased risk of a TDI because of falling (5)? Another new category could be individuals who have lost an anterior tooth because of a TDI and, as an elegant solution, have received an implant. What happens to the implant and the bone when they encounter another TDI? Recent case reports have focused on this type of problem (6–8).

Evidence suggests that there is also an impact of treatment of dental trauma on the quality of life (QoL) of the individual. Recent studies of adolescents have indicated that treatment of permanent incisors with enamel-dentin fractures does not eliminate the impact of trauma on daily life (9, 10). On average, children with an untreated TDI were 20 times more likely to report an impact on QoL because of the injury when compared with children without any TDI (9). More adolescents with a history of treatment of an enamel-dentin fracture suffered from reduced QoL than adolescents with no history of dental trauma. Until now, only a few studies have presented findings on this subject matter (10).

The aim of this 12-year review is to present a broad international review of the prevalence and incidence of TDIs including back ground variables. A quick, easy method of registering TDIs to receive a primary understanding of the extent and seriousness of dental trauma in every dental clinic will also be presented.

3.

Dental Traumatology

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Recommendations for using regenerative endodontic procedures in permanent immature traumatized teeth

INVITED REVIEW

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Abstract – The regeneration of immature permanent teeth following trauma could be beneficial to reduce the risk of fracture and loss of millions of teeth each year. Regenerative endodontic procedures include revascularization, partial pulpotomy, and apexogenesis. Several case reports give these procedures a good prognosis as an alternative to apexification. Care is needed to deliver regenerative endodontic procedures that maintain or restore the vitality of teeth, but which also disinfect and remove necrotic tissues. Regeneration can be accomplished through the activity of the cells from the pulp, periodontium, vascular, and immune system. Most therapies use the host's own pulp or vascular cells for regeneration, but other types of dental stem cell therapies are under development. There are no standardized treatment protocols for endodontic regeneration. The purpose of this article is to review the recent literature and suggest guidelines for using regenerative endodontic procedures for the treatment of permanent immature traumatized teeth. Recommendations for the selection of regenerative and conventional procedures based on the type of tooth injury, fracture type, presence of necrosis or infection, periodontal status, presence of periapical lesions, stage of tooth development, vitality status, patient age, and patient health status will be reviewed. Because of the lack of long-term evidence to support the use of regenerative endodontic procedures in traumatized teeth with open apices, revascularization regeneration procedures should only be attempted if the tooth is not suitable for root canal obturation, and after apexogenesis, apexification, or partial pulpotomy treatments have already been attempted and have a poor prognosis.

Studies have shown that 25% of school-aged children will experience some kind of dental trauma (1) and 25% to 65% of school-aged children have untreated dental caries (2). The dental treatment for avulsed or traumatized teeth with an exposed pulp can vary considerably. Some dentists debride the pulp tissues and obturate the root canal with gutta-percha (3–6), composite resin, calcium hydroxide (7), or mineral trioxide aggregate (MTA) (8). Meanwhile, other dentists will attempt regenerative endodontic procedures to maintain or restore the vitality of a tooth. Regenerative endodontic procedures involving pulp capping and partial pulpotomy have been in use since the late 1970s (9). Some root canal revascularization procedures have also been used since the early 1970s (10). Practitioner interest in delivering regenerative endodontic procedures has been growing, with 96% of endodontists being willing to incorporate regenerative therapies into treatments (11). Regenerative endodontic procedures are diverse and can include direct pulp capping, revascularization, apexogenesis, apexification, and even stem cell therapy and tissue engineering (12). Regenerative endodontic procedures can be defined as biologically based procedures designed to predictably replace damaged, diseased, or missing structures, including dentin and root structures as well as cells of the pulp–dentin complex, with live viable tissues, preferably of the same origin, that restore the normal physiologic functions of the pulp–dentin complex (12). Over the past few years, progress in this field has been rapid. Several case reports give revascularization (10, 13–23), apexogenesis (24–29), and partial pulpotomy (9, 30–34), a good prognosis as an alternative to apexification but few or none describe any limiting factors to their success. Regenerative endodontic procedures can be beneficial to patients if they can prolong the functionality and vitality of traumatized teeth and help avoid fracture.

Apexification

Closure of the open apex of an immature tooth has traditionally been accomplished through an apexification procedure. Apexification is a method of inducing a calcified apical barrier or continued apical development of an incompletely formed root in which the pulp is necrotic (35, 36). Apexification can involve a single (37)

4.

A Novel Combination of *dens evaginatus* and *dens invaginatus* in a Single Tooth – Review of the Literature and a Case Report

Robert P Anthonappa * / Cynthia KY Yiu ** / Nigel M King ***

Dens evaginatus (DE) and dens invaginatus (DI) are rare developmental dental anomalies affecting both the primary and permanent dentitions. Concurrence of DE and DI within the same tooth is extremely rare. We report a case of DE and DI in a maxillary right lateral incisor tooth. Comprehensive clinical and radiographic examinations are essential to identify such defects; the early diagnosis can then result in the appropriate prophylactic treatment being performed, thus preventing undesirable pulpal complications.

Keywords: *case report, dens evaginatus, dens invaginatus*

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INTRODUCTION

Dens evaginatus (DE) is a rare developmental anomaly of a tooth resulting in the formation of an accessory cusp[1] comprising of enamel, dentin and varying amounts of pulp tissue.[2] This uncommon anomaly was first reported by Windel[3] where he mentioned two cases of supernumerary cusps on the palatal surface of permanent maxillary incisors and canines. Later, Mitchell[4] in her letter described a curved horn-like process extending from the palatal surface to the incisal edge of a permanent maxillary central incisor tooth. Since then numerous case reports have been published in the English literature,[1] and it is usually reported to be found on the occlusal surface of premolars or projecting from the lingual surface of anterior teeth in both the primary and permanent dentitions. Talon cusp occurs at the morphodifferentiation stage of tooth development, as a result of an out-folding of the enamel organ, or hyperactivity of the dental lamina.[5] It has a strong predilection for permanent maxillary incisors and may present unilaterally or bilaterally.

Dens invaginatus (DI), which is a rare malformation of the tooth, probably arises from an infolding of the dental papilla during tooth development,[6] it has a broad spectrum of morphological variations. It has been reported that DI was first described in a human tooth by a dentist named 'Socrates' in 1856.[7] It frequently affects the permanent maxillary lateral incisors, usually bilaterally[8, 9] and the morphology varies from a short pit confined to the crown of the tooth to a deep invagination into the root. Radiographically, it presents with an infolding of enamel and dentin (enamel located in the center which is covered by dentin peripherally due to the invagination), which may extend into the pulp cavity, the root, and sometimes even to the root apex.[6] Several classifications of invaginated teeth have been reported in the literature[6]; the most commonly used being the one proposed by Oehlers.[8]

The numerous reports of DE in the literature was recently reviewed by Levitan and Himel.[1] DE predominantly occurs in people of Asian descent (including Chinese, Malay, Thai, Japanese, Filipino, and Indian populations) with varying estimates reported at 0.5% to 4.3%, depending upon the population group studied.[10] Conversely, the prevalence of DI varies between 0.25% and 10% in different studies.[11] This variation is probably due to geographical differences and variations in diagnostic criteria and/or investigation methods. Talon cusp, which is a variation of DE, is found in the primary and permanent maxillary incisors with variations in size, shape, length and mode of attachment to the crown.[12] DE has been reported to be associated with numerous developmental dental anomalies like odontome, double tooth, supernumerary and impacted teeth, as well as several syndromes such as Mohr syndrome, Rubinstein–Taybi syndrome and Sturge–Weber syndrome.[12-16] The concurrence of DE and DI within the same tooth is a rare finding and has only been reported twice in the literature.[17, 18] Here we report a case of DE and DI affecting a maxillary right lateral incisor.

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5.

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6.

Stem Cell Biology and Tissue Engineering in Dental Sciences

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DENTAL TRAUMATOLOGY

Apexification: a review

Rafter M. Apexification: a review. Dent Traumatol 2005; 21: 1–8.

Abstract – This paper reviews the rationale and techniques for treatment of the non-vital immature tooth. The importance of careful case assessment and accurate pulpal diagnosis in the treatment of immature teeth with pulpal injury cannot be overemphasized. The treatment of choice for necrotic teeth is apexification, which is induction of apical closure to produce more favorable conditions for conventional root canal filling. The most commonly advocated medicament is calcium hydroxide, although recently considerable interest has been expressed in the use of mineral trioxide aggregate. Introduction of techniques for one-visit apexification provide an alternative treatment option in these cases. Success rates for calcium hydroxide apexification are high although risks such as reinfection and tooth fracture exist. Prospective clinical trials comparing this and one-visit apexification techniques are required.

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Key words: apexification; apexogenesis; calcium hydroxide; mineral trioxide aggregate; one-visit apexification

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The completion of root development and closure of the apex occurs up to 3 years after eruption of the tooth (1). The treatment of pulpal injury during this period provides a significant challenge for the clinician. Depending upon the vitality of the affected pulp, two approaches are possible – apexogenesis or apexification. Apexogenesis is 'a vital pulp therapy procedure performed to encourage continued physiological development and formation of the root end' (2). Apexification is defined as 'a method to induce a calcified barrier in a root with an open apex or the continued apical development of an incomplete root in teeth with necrotic pulp' (2). As always, success is related to accurate diagnosis and a full understanding of the biological processes to be facilitated by the treatment.

Root development

Root development begins when enamel and dentin formation has reached the future cementoenamel junction. At this stage the inner and outer enamel epithelium are no longer separated by the stratum intermedium and stellate reticulum, but develop as a two layered epithelial wall to form Hertwig's epithelial root sheath. When the differentiation of radicular cells into odontoblasts has been induced and the first layer of dentin has been laid down, Hertwig's epithelial root sheath begins to disintegrate and lose its continuity and close relationship to the root surface. Its remnants persist as an epithelial network of strands or tubules near the external surface of the root (1).

Hertwig's epithelial root sheath is responsible for determining the shape of the root or roots. The epithelial diaphragm surrounds the apical opening to the pulp and eventually becomes the apical foramen. An open apex is found in the developing roots of immature teeth until apical closure occurs approximately 3 years after eruption (1).

Pulpal injury in teeth with developing roots

Unfortunately traumatic injuries to young permanent teeth are not uncommon and are said to affect 30% of children (3). The majority of these incidents occur before root formation is complete (4) and may result in pulpal inflammation or necrosis. The root sheath of Hertwig is usually sensitive to trauma but because of the degree of vascularity and cellularity in the apical region, root formation can continue even in the presence of pulpal inflammation and necrosis (5, 6). Because of the important role of Hertwig's epithelial root sheath in continued root development after pulpal injury, every effort should be made to maintain its viability. It is thought to

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The Hidden Treasure in Apical Papilla: The Potential Role in Pulp/Dentin Regeneration and BioRoot Engineering

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Abstract

Some clinical case reports have shown that immature permanent teeth with periradicular periodontitis or abscess can undergo apexogenesis after conservative endodontic treatment. A call for a paradigm shift and new protocol for the clinical management of these cases has been brought to attention. Concomitantly, a new population of mesenchymal stem cells residing in the apical papilla of permanent immature teeth recently has been discovered and was termed stem cells from the apical papilla (SCAP). These stem cells appear to be the source of odontoblasts that are responsible for the formation of root dentin. Conservation of these stem cells when treating immature teeth may allow continuous formation of the root to completion. This article reviews current findings on the isolation and characterization of these stem cells. The potential role of these stem cells in the following respects will be discussed: (1) their contribution in continued root maturation in endodontically treated immature teeth with periradicular periodontitis or abscess and (2) their potential utilization for pulp/dentin regeneration and bioroot engineering.

Keywords

Apexogenesis; apical papilla; bioroot engineering; dental pulp stem cells; immature teeth; periodontal ligament stem cells; pulp regeneration; stem cells from human exfoliated deciduous teeth; stem cells from the apical papilla

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A number of recent clinical case reports have revealed the possibilities that many teeth that traditionally would receive apexification may be treated for apexogenesis. A call for a paradigm shift and new protocol for the clinical management of these cases has been made by the authors (1-3). A recent scientific finding, which may explain in part why apexogenesis can occur in these infected immature permanent teeth, is the discovery and isolation of a new population of mesenchymal stem cells (MSCs) residing in the apical papilla of incompletely developed teeth (4,5). These cells are termed stem cells from the apical papilla (SCAP), and they differentiate

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9.

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REVIEW

Regenerative endodontics: a comprehensive review

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Abstract

Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B. Regenerative endodontics: a comprehensive review. *International Endodontic Journal*, **51**, 1367–1388, 2018.

The European Society of Endodontology and the American Association for Endodontists have released position statements and clinical considerations for regenerative endodontics. There is increasing literature on this field since the initial reports of Iwaya *et al.* (Dental Traumatology, 17, 2001, 185) and Banchs & Trope (Journal of Endodontics, 30, 2004, 196). Endogenous stem cells from an induced periapical bleeding and scaffolds using blood clot, platelet rich plasma or platelet-rich fibrin have been utilized in regenerative endodontics. This approach has been described as a 'paradigm shift' and considered the first treatment option for immature teeth with pulp necrosis. There are three treatment outcomes of regenerative endodontics; (i) resolution of clinical signs and symptoms; (ii) further root maturation; and (iii) return of neurogenesis. It is known that results are variable for these objectives, and true regeneration of the pulp/dentine complex is not achieved. Repair derived primarily from the periodontal and osseous tissues has been shown histologically. It is hoped that with the concept of tissue engineering, namely stem cells, scaffolds and signalling molecules, that true pulp regeneration is an achievable goal. This review discusses current knowledge as well as future directions for regenerative endodontics. Patient-centred outcomes such as tooth discolouration and possibly more appointments with the potential for adverse effects needs to be discussed with patients and parents. Based on the classification of Cvek (Endodontics and Dental Traumatology, 8, 1992, 45), it is proposed that regenerative endodontics should be considered for teeth with incomplete root formation although teeth with near or complete root formation may be more suited for conventional endodontic therapy or MTA barrier techniques. However, much is still not known about clinical and biological aspects of regenerative endodontics.

Keywords: current perspectives, future prospectives, immature permanent teeth, regenerative endodontics, treatment outcomes.

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Introduction

What is regenerative endodontics?

Regenerative endodontics has not only many burning (Smith & Cooper 2017) but also unanswered questions. The treatments and outcomes of regenerative endodontics are so different from traditional endodontic therapy; therefore, it has attracted enormous interest and attention in the field of endodontics in recent years.

Immature permanent teeth with necrotic pulp/apical periodontitis are traditionally treated with apexification procedures using calcium hydroxide to induce apical hard tissue barrier formation or apical MTA plugs before root canal filling (Frank 1966, Heithersay 1975, Rafter 2005). The calcium hydroxide

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10.

Treatment Options: Apexogenesis and Apexification

Shahrokh Shabahang, DDS, MS, PhD

Abstract

This article will describe requirements for case selection and review the procedures for apexogenesis and apexification in immature permanent teeth. Nonclinical and clinical data will be presented to support the recommendations, and outcomes will be presented from clinical studies. The dental pulp is an ectomesenchymally derived connective tissue with certain unique properties such as being encased in hard tissues, which limits its collateral circulation. The pulp provides a matrix for binding of its cells and provides support allowing communication between the cells. In addition to immune cells, the dental pulp contains odontoblasts, which are specialized cells capable of producing dentin. In the absence of a vital pulp, dentin deposition is arrested. When an immature tooth is affected by caries or trauma, the pulp requires proper management according to the degree of inflammation and its vitality. Maintenance of pulp vitality will allow continued root development along the entire root length. If the pulp is irreversibly inflamed or necrotic, root-end closure procedures are required when the apex has not fully formed. *(J Endod 2013;39:S26–S29)*

Key Words

Apexification, apexogenesis, immature permanent teeth, treatment

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Role of the Dental Pulp in Tooth Development

The dental pulp contains immune cells that allow it to mount a response against offending irritants. The pulp also contains odontoblasts, which are specialized to form dentin. In the absence of a vital pulp, the tooth structure is susceptible to infection, and dentin deposition is arrested. Maintenance of pulp vitality is imperative in an immature permanent tooth to allow continued root development. The pulp tissue is removed when pathologically inflamed or necrotic.

Although studies are underway to develop materials and techniques for pulp regeneration procedures, the purpose of this review is to provide an overview of apexogenesis and apexification in immature permanent teeth with pulpal pathosis.

Apexogenesis or Apexification

Proper assessment of the affected tooth is critical in determining an accurate diagnosis and prescribing the appropriate treatment plan. Assessment of pulp vitality will aid in determining the proper treatment option. If vital and not irreversibly inflamed, maintenance of its vitality will allow natural continued root development. Figure 1 presents a flow chart to facilitate the decision-making process when treating permanent teeth with incomplete root development.

Assessment of the tooth in question is made by using radiographic evaluation to determine the maturity of the developing root and clinical evaluation that is based on history and clinical testing. Obviously immature teeth are frequently associated with child patients. Pulp testing in children is a complex procedure and subjective in nature.

Maintenance of pulp vitality by using apexogenesis will allow continued root development along the entire root length. Depending on the extent of inflammation, pulp capping, shallow pulpotomy, or conventional pulpotomy may be indicated. The dental pulp in young patients is more cellular and able to recover from injuries. Cvek et al (1) demonstrated that in teeth with complex crown fractures, the exposed pulp maintained its vitality for up to 7 days. In these teeth, only the most superficial 2 mm of the pulp is inflamed and requires removal.

If pulpal necrosis occurs in immature teeth, an alternative treatment approach must be used because of the presence of an open apex (2). The young pulpless tooth frequently has thin, fragile walls, which makes it difficult to adequately clean and to obtain the necessary apical seal (2). Traditionally, the approach has been to use calcium hydroxide (CH) to induce apexification after disinfection of the root canals in the conventional manner (3). Completion of endodontic therapy was typically delayed until completion of root-end closure through apexification. Apexification is defined as “a method of inducing a calcified barrier in a root with an open apex or the continued apical development of an incompletely formed root in teeth with necrotic pulp” (4).

The disadvantages of traditional, long-term CH therapy include variability in treatment time, unpredictability of formation of an apical seal, difficulty in following up patients, and delayed treatment. An alternative to CH therapy is placement of an apical plug. Several investigators have demonstrated the use of dentin apical plugs in nonsurgical root canal therapy of mature teeth (5–9). Mineral trioxide aggregate (MTA) is a material that may be best suited as an apical plug. In 1999, Shabahang et al (10) showed consistent barrier formation when MTA was used as an apical plug in an *in vivo* dog model with open apices. Several studies have confirmed successful clinical outcomes including healing of existing periapical lesions in majority of immature teeth that were treated with an MTA apical plug (11, 12).

11.

Mineral trioxide aggregate pulpotomies

A case series outcomes assessment

David E. Witherspoon, BDSc, MS; Joel C. Small, DDS; Gary Z. Harris, DDS

ABSTRACT

Background. The greatest threats to developing teeth are dental caries and traumatic injury. A primary goal of all restorative treatment is to maintain pulp vitality so that normal root development or apexogenesis can occur. If pulpal exposure occurs, then a pulpotomy procedure aims to preserve pulp vitality to allow for normal root development. Historically, calcium hydroxide has been the material of choice for pulpotomy procedures. Recently, an alternative material called mineral trioxide aggregate (MTA) has demonstrated the ability to induce hard-tissue formation in pulpal tissue. The authors describe the clinical and radiographic outcome of a series of cases involving the use of MTA in pulpotomy procedures.

Methods. Twenty-three cases in 18 patients were treated with MTA pulpotomy procedures in an endodontic private practice. All of the patients had been referred to the practice for diagnosis and treatment of a symptomatic tooth. All of the authors provided treatment. Pulpal exposures were either due to caries or complicated enamel dentin fractures.

Results. Nineteen teeth in 14 patients were available for recall. The mean time of recall was 19.7 months. Of the 19 cases, 15 involved healed teeth, and three involved teeth that were healing. One of 19 cases involved a tooth with persistent disease.

Conclusions. MTA may be useful as a substitute for calcium hydroxide in pulpotomy procedures. Further research, however, is required to clarify this conclusion.

Clinical Implications. MTA conceivably could replace calcium hydroxide as the material of choice for pulpotomy procedures, if future research continues to show promising results.

Key Words. Pulpotomy; mineral trioxide aggregate; apexogenesis.
JADA 2006;137:610-8.

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Traumatic injuries and dental caries are the greatest challenges to the integrity of the developing tooth. Both can cause the dental pulp to undergo irreversible damage, causing necrosis of the pulpal tissues, which can result in the arrest of normal root development. Abnormal root development will have an impact on the long-term prognosis for tooth retention.[1-4] The primary goal of treatment, therefore, should be to maintain pulp vitality so that normal root development can occur. By maintaining pulp vitality, apexogenesis can occur.[5-8] This is the preferred treatment because it promotes healing by regeneration rather than repair. Pulpotomy involves the surgical removal of a portion of an affected vital coronal pulp tissue, while the clinician leaves the radicular tissue in situ to allow for normal root development.

PULPOTOMIES: A REVIEW THE LITERATURE

Several materials have been advocated to induce normal root development.[9-11] To date, the material of choice has been calcium hydroxide.[12-18] In the past decade, an alternative material called mineral trioxide aggregate (MTA) became available for use in pulpal procedures. Clinicians should evaluate

12.

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ENDODONTICS

Regenerative endodontics

A way forward

Anibal Diogenes, DDS, MS, PhD; Nikita B. Ruparel, DDS, MS, PhD; Yoav Shiloah, DDS, MBA; Kenneth M. Hargreaves, DDS, PhD

ABSTRACT

Background and Overview. Immature teeth are susceptible to infections due to trauma, anatomic anomalies, and caries. Traditional endodontic therapies for immature teeth, such as apexification procedures, promote resolution of the disease and prevent future infections. However, these procedures fail to promote continued root development, leaving teeth susceptible to fractures. Regenerative endodontic procedures (REPs) have evolved in the past decade, being incorporated into endodontic practice and becoming a viable treatment alternative for immature teeth. The authors have summarized the status of regenerative endodontics on the basis of the available published studies and provide insight into the different levels of clinical outcomes expected from these procedures.

Conclusions. Substantial advances in regenerative endodontics are allowing a better understanding of a multitude of factors that govern stem cell–mediated regeneration and repair of the damaged pulp-dentin complex. REPs promote healing of apical periodontitis, continued radiographic root development, and, in certain cases, vitality responses. Despite the clinical success of these procedures, they appear to promote a guided endodontic repair process rather than a true regeneration of physiological-like tissue.

Practical Implications. Immature teeth with pulpal necrosis with otherwise poor prognosis can be treated with REPs. These procedures do not preclude the possibility of apexification procedures if attempts are unsuccessful. Therefore, REPs may be considered first treatment options for immature teeth with pulpal necrosis.

Key Words. Guided tissue regeneration; revascularization; endodontic therapy; stem cells; outcome assessment; regenerative endodontics.

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Immature teeth are at risk of developing pulpal necrosis due to trauma, caries, and anatomic variations such as dens evaginatus and dens invaginatus.[1-4] Dental trauma occurs with an incidence that varies from 2.6% to 35% in patients undergoing cranioskeletal development.[5-7] Up to one-half of these traumatized teeth may undergo pulpal necrosis, but only 8.5% will exhibit signs and symptoms of disease.[8] Dental anomalies also represent a common etiology leading to pulpal necrosis in immature permanent teeth.[4] Dens evaginatus and dens invaginatus are the most common anomalies associated with this clinical manifestation.[4] Full radicular maturation occurs up to 3 years after the eruption of a tooth in the oral cavity,[9] and the loss of pulp vitality during this period arrests further root development. These teeth traditionally have been treated with apexification procedures by using either long-term calcium hydroxide treatment[10,11] or immediate placement of a mineral trioxide aggregate (MTA) apical plug.[12] Although these treatments often result in the resolution of signs and symptoms of disease, they provide little to no benefit in restoring normal pulpal defenses and nociception and, more importantly, continued root development.[13] Thus, immature teeth remain with thin fragile dentinal walls, increasing susceptibility to fractures and lower survival rates.[14,15]

Tooth loss in patients still undergoing cranioskeletal development has devastating consequences that include altered maxillary and mandibular bone development;

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REVIEW

Clinical procedures for revitalization: current knowledge and considerations

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Abstract

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Revitalization or regenerative treatment approaches in teeth with incomplete root formation and pulp necrosis have become part of the therapeutic endodontic spectrum and should be considered as an alternative to conventional apexification. Ideally, regenerative endodontic procedures allow not only for a resolution of pain, inflammation and periapical lesions, but also for the formation of an immunocompetent tissue inside the root canal which can reconstitute the original biological structure and function of dental pulp and thus lead to an increase in root length, and thickness and strength of previously thin, fracture-prone dentine walls. Common features of regenerative procedures performed in immature teeth with pulp necrosis include (i) minimal or no instrumentation of the dentinal walls, (ii) disinfection with irrigants, (iii) application of an intracanal medicament, (iv) provocation of bleeding into the canal and creation of a blood clot, (v) capping with calcium silicate, and (vi) coronal seal. Although case reports and case series provide promising results, the protocol for regenerative endodontic treatment is not fully established; questions remain regarding the terminology, patient selection and informed consent as well as procedural details, especially on the choice of irrigants, intracanal medicaments and materials for cavity sealing. Animal studies document repair rather than regeneration, which opens the discussion on prognosis and outcome, especially the biological versus the patient-based outcome. This review will provide an overview of the current state of regenerative endodontic therapies, discuss open questions and provide recommendations based on the recent literature.

Keywords: dental pulp stem cells, pulp regeneration, regenerative endodontics, revascularization, revitalization.

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Introduction

The pulp–dentine complex and conventional root canal treatment

Although rather small, teeth are complex organs comprising several mineralized matrices, which enclose a soft tissue termed the dental pulp. A healthy dental pulp fulfils a number of different tasks, namely formation of dentine, perception of pain and transmission of sensory stimuli from the pulp–dentine complex (Magloire *et al.* 2010), immunoresponse and cellular clearance of pathogens as well as formation of dentine as active defence mechanisms against invading toxins and bacteria (Bjørndal 2008, Staquet *et al.* 2011). In young patients, a functional pulp tissue is essential for the completion of root formation (Mohammadi 2011). Irritation caused by caries or trauma induces an inflammatory tissue response termed pulpitis. Initially, this inflammatory reaction may be fully

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http://dx.doi.org/10.17096/jiufd.53911 INVITED REVIEW

A REVIEW OF REGENERATIVE ENDODONTICS: CURRENT PROTOCOLS AND FUTURE DIRECTIONS

Rejeneratif Endodonti Üzerine Bir Derleme: Güncel Protokoller ve Geleceğe Yönelik Öneriler

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ABSTRACT

This review outlines the biological basis and clinical protocols currently used in regenerative endodontic procedures (REPs) and discuss future directions in pulp regeneration approaches. The treatment of immature teeth with REPs has been described as a 'paradigm shift' as there is the potential for further root maturation. Clinically, REPs involve disinfection of the root canal system without damaging the endogenous stem cell potential present in the apical papilla and other tissues. These stems cells are introduced into the root canal space by inducing a blood clot followed by placement of an intracanal barrier to prevent microleakage. The biological concept of REPs involves the triad of stem cells, scaffold and signalling molecules. Currently, repair rather than true regeneration of the 'pulp-dentine complex' is achieved and further root maturation is variable. However, may clinicians consider the treatment of teeth with REPs as the optimal treatment approach for immature teeth with pulp necrosis.

Keywords: *Regenerative endodontic protocols; immature teeth; pulp necrosis; root maturation; triple antibiotic paste; calcium hydroxide*

ÖZ

Bu derlemede rejeneratif endodontik tedavinin biyolojik temelleri ve güncel klinik protokolleri anlatılmakta ve pulpa rejenerasyonu yaklaşımlarının geleceği tartışılmaktadır. Kök ucu kapanmamış dişlerin rejeneratif endodontik tedavi ile kök gelişiminin devam etme potansiyeli köklü bir değişim yaratmıştır. Klinik olarak rejeneratif endodontik tedavi apikal papilla ve diğer dokularda bulunan endojen kök hücrelere zarar vermeden kök kanalı sisteminin dezenfeksiyonunu içerir. Bu kök hücrelerin kan pıhtısı oluşturulması ile kök kanalı içine taşınmasını takiben mikrosızıntıyı önleme amacıyla kanal içine bariyer malzemesi yerleştirilir. Rejeneratif endodontik tedavinin biyolojik prensibi kök hücreler, doku iskeleleri ve sinyal molekülleri triadından oluşur. Günümüzde, "pulpa-dentin kompleksi" nin gerçek rejenerasyonundan ziyade tamir gerçekleşmektedir ve kök gelişiminin tamamlanması ise değişkenlik göstermektedir. Ancak, birçok klinisyen pulpası nekroze kök gelişimi tamamlanmamış dişlerde en uygun tedavinin rejeneratif endodontik yaklaşımlarla sağlanabileceğini düşünmektedir.

Anahtar kelimeler: *Rejeneratif endodontik tedavi protokolleri; kök gelişimi tamamlanmamış dişler; pulpa nekrozu; kök gelişimi; üçlü antibiyotik pat; kalsiyum hidroksit*

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Fracture resistance and histological findings of immature teeth treated with mineral trioxide aggregate

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Abstract – The objective of the present study was to test the hypothesis that the fracture strength of calcium hydroxide and mineral trioxide aggregate (MTA)-filled immature teeth decreased over time. Immature mandibular incisors from sheep were extracted and the pulps were extirpated using an apical approach with a barbed broach, and the teeth were divided into three experimental groups. Group 1: untreated teeth. Group 2: the root canals were filled with calcium hydroxide paste. Group 3: the root canals were filled with MTA. All specimens were kept in saline with 1% antibiotics at 4°C for certain periods of time: 2 weeks, 2 months, and 1 year. Then they were tested for fracture strength in an Instron testing machine. The results were subjected to statistical analysis by the Tukey–Kramer tests. A *P*-value (<0.05) was considered statistically significant. One tooth from each group was selected randomly for a histological study, examining matrix metalloproteinases (MMP2 and MMP14) and tissue inhibitor of metalloproteinase (TIMP). The results showed the mean fracture strengths decreased over time for all the three groups. Although the untreated teeth showed the highest value (45.5 MPa) at 2 weeks, the fracture strengths decreased significantly after 2 months ($P < 0.05$). On the other hand, the teeth treated with calcium hydroxide or MTA decreased, but not significantly over time ($P > 0.05$). For the MTA-treated teeth, the fracture strengths were not found significantly different from the untreated or calcium hydroxide-treated teeth at 2 weeks or 2 months ($P > 0.05$). However, the strength was significantly higher in the MTA group compared with the other two groups after 1 year ($P < 0.05$). Immunofluorescence images revealed expression of collagen type 1, MMP-2 and MMP-14 in both untreated and endodontically treated teeth. However, TIMP-2 was only observed in the MTA-treated teeth. In conclusion, the teeth with root treatment with MTA showed the highest fracture resistance at 1 year ($P < 0.05$). An explanation could be that MTA induced the expression of TIMP-2 in the dentin matrix and thereby possibly prevented destruction of the collagen matrix.

One of the challenges in dentistry is the treatment of immature, pulpless, permanent teeth that are candidates for endodontics. The introduction of apexification by the use of calcium hydroxide was pioneered by Heithersay (1) and Frank (2). This treatment gave adequate apical healing because of its antibacterial capability caused by a high pH and the ability to induce remineralization in periapical tissues (3, 4). However, it was reported that the fracture strength of calcium hydroxide-filled immature teeth decreased over time presumably because of changes in the organic matrix of dentin (5).

Mineral trioxide aggregate (MTA), a relatively new material currently being used in pulp therapy (6), is made primarily of fine hydrophilic particles of tricalcium aluminate, tricalcium silicate, silicate oxide, and tricalcium oxide (6–8). The initial pH of MTA when hydrated is 10.2 and the set pH is 12.5, which is comparable with that of calcium hydroxide. The material has been shown to have excellent biocompatibility (6, 9), antimicrobial properties (8), low cytotoxicity (7, 10), and low microleakage (11). However, recent study also indicated that root dentin was weakened after exposure to calcium hydroxide and MTA in 5 weeks (12).

The mechanical properties of dentin are fundamentally determined by dentin matrix, which is mostly composed of collagen type I (13, 14). Matrix metalloproteinase (MMP)-2, 14 and membrane type 1 (MT1) are found to play an important role during the degradation of collagen matrix of dentin (15–17). On the other hand, the tissue inhibitor of metalloproteinase (TIMP) inhibit active forms of MMPs, especially TIMP-2 inhibits MMP-2 (18). It is speculated that both calcium hydroxide and MTA in the root canal of dentin may affect the activities of MMPs or TIMP-2, thus influence the mechanical properties of dentin. As there is limited information in the dental literature concerning the long-term effect of either calcium hydroxide or MTA as root end-filling materials on the fracture strength of immature

THE ROLE OF THE BLOOD CLOT IN ENDODONTIC THERAPY

AN EXPERIMENTAL HISTOLOGIC STUDY

by

B. Nygaard Östby

INTRODUCTION

In general pathology and in surgery the significance of blood and the blood clot has been recognized (*Lorin-Epstein* 1927, *Fränkel* 1929, 1931, and 1932, *Carrel* 1930, and *Allgöwer* 1949). In the healing of bone fractures the blood clot is considered an extremely important factor (*Weinmann & Sicher* 1955, and *Ackerman* 1959). Therefore, it seems strange that in endodontic treatment bleeding is more or less looked upon as a complication to be feared. The writer, for one, has earlier (1958) maintained that a root filling should never be carried out if there are signs of even a slight bleeding in the canal.

However, in an experimental study on the effect of EDTA (*Nygaard Östby* 1957) a case was observed, which suggested that this concept needed re-evaluation.

It was decided to study how the periodontal tissue would react if the entire pulp was removed from the main canal and the apical part subsequently allowed to fill with blood. The aim was primarily to see if the results would have any significance in clinical endodontics. At the same time one might expect that an experimental series planned in this way would reveal details of general interest with regard to the organization of a blood clot. When the latter has connection with live tissue at a small well defined border only, it should offer possibilities for a histologic study of the dynamics of the organization processes. Finally, the purpose of the investigations was to test the effect of EDTAC on the periapical tissues.

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CLINICAL RESEARCH

Revascularization of Immature Permanent Teeth With Apical Periodontitis: New Treatment Protocol?

Francisco Banchs, DDS, MS, and Martin Trope, DMD

A new technique is presented to revascularize immature permanent teeth with apical periodontitis. The canal is disinfected with copious irrigation and a combination of three antibiotics. After the disinfection protocol is complete, the apex is mechanically irritated to initiate bleeding into the canal to produce a blood clot to the level of the cementoenamel junction. The double seal of the coronal access is then made. In this case, the combination of a disinfected canal, a matrix into which new tissue could grow, and an effective coronal seal appears to have produced the environment necessary for successful revascularization.

Regeneration of a necrotic pulp is considered possible only after avulsion of an immature permanent tooth. The advantages of pulp revascularization lie in the possibility of further root development and reinforcement of dentinal walls by deposition of hard tissue, thus strengthening the root against fracture. After reimplantation of an avulsed immature tooth, a unique set of circumstances exists that allows regeneration to take place. The young tooth has an open apex and is short, which allows new tissue to grow into the pulp space relatively quickly. The pulp is necrotic but usually not infected, so it will act as a matrix into which the tissue can grow. It has been experimentally shown that the apical part of a pulp may remain vital and, after reimplantation, may proliferate coronally, replacing the necrotized portion of the pulp (1–3). In addition, the fact that, in most cases, the crown of the tooth is intact ensures that bacterial penetration into the pulp space through cracks (4) and defects will be a slow process. Thus, the race between the new tissue and infection of the pulp space favors the new tissue.

The notion that successful regeneration depends on a race between the new tissue and bacteria populating the pulp space is strengthened by the fact that the incidence of revascularization is enhanced if the apex shows radiographic opening of more than 1.1 mm (5) and the tooth is replanted within 45 min (thus increasing the chances for revascularization by 18%) (5). The incidence can be significantly improved if the tooth is soaked in doxycycline (6, 7) or minocycline (8) before replantation, therefore ensuring that bacteria on the root surface and apical foramen are killed and no new bacteria are able to move through the blood clot in the socket.

Regeneration of pulp tissue in a necrotic infected tooth with apical periodontitis has been thought impossible. However, if it were possible to create a similar environment as described here for the avulsed tooth, regeneration should occur. Thus, if the canal were effectively disinfected, a matrix into which new tissue could grow were created, and the coronal access were effectively sealed, regeneration should occur as in an avulsed immature tooth.

This case report describes the treatment of an immature second lower right premolar with radiographic and clinical signs of apical periodontitis with the presence of a sinus tract. The canal was disinfected without mechanical instrumentation with the use of copious irrigation followed by a mixture of antibiotics. A blood clot was then produced to the level of the cementoenamel junction (CEJ), followed by a deep coronal restoration. With clinical and radiographic evidence of healing as early as 22 days, the large radiolucency had disappeared within 2 months, and at the 24-month recall, it was obvious that the root walls were thick and the development of the root apical to the restoration was similar to that of the adjacent and contralateral teeth.

CASE REPORTS

An 11-year-old boy of Japanese descent was referred to the endodontic clinic of the University of North Carolina by the pediatric dentistry department for evaluation on the lower right second premolar. The child had a lingual swelling of the right mandibular area 1 month previously with reported slight discomfort. On clinical examination, the patient was asymptomatic, and the tooth appeared intact, without caries (Fig. 1). The presence of occlusal tubercles on the other mandibular premolars suggested that one may have been present on this tooth, which was fractured during function, resulting in a microexposure and necrosis of the pulp. The tooth had an open apex associated with a large radiolucency (Fig. 2), and a lingual sinus tract was present that traced to the apex of the tooth (Fig. 3). Periodontal probings were within normal limits for all teeth in the lower right region. Diagnostic testing was inconclusive on cold and electric pulp testing, with sensitivity on percussion and palpation. Because of the presence of

Critical Reviews in Oral Biology & Medicine

Pulp Vascularization during Tooth Development, Regeneration, and Therapy

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Abstract

The pulp is a highly vascularized tissue situated in an inextensible environment surrounded by rigid dentin walls, with the apical foramina being the only access. The pulp vascular system is not only responsible for nutrient supply and waste removal but also contributes actively to the pulp inflammatory response and subsequent regeneration. This review discusses the underlying mechanisms of pulp vascularization during tooth development, regeneration, and therapeutic procedures, such as tissue engineering and tooth transplantation. Whereas the pulp vascular system is established by vasculogenesis during embryonic development, sprouting angiogenesis is the predominant process during regeneration and therapeutic processes. Hypoxia can be considered a common driving force. Dental pulp cells under hypoxic stress release proangiogenic factors, with vascular endothelial growth factor being one of the most potent. The benefit of exogenous vascular endothelial growth factor application in tissue engineering has been well demonstrated. Interestingly, dental pulp stem cells have an important role in pulp revascularization. Indeed, recent studies show that dental pulp stem cell secretome possesses angiogenic potential that actively contributes to the angiogenic process by guiding endothelial cells and even by differentiating themselves into the endothelial lineage. Although considerable insight has been obtained in the processes underlying pulp vascularization, many questions remain relating to the signaling pathways, timing, and influence of various stress conditions.

Keywords: angiogenesis, stem cell(s), pulp biology, tooth regeneration/transplantation, tissue engineering, vascular biology

Introduction

The vasculature is the earliest functional organ system of the human body (Udan et al. 2012) and is formed by vasculogenesis during embryonic development (Fig. 1A–D). Endothelial cells derived from the mesoderm aggregate and form vascular structures that are completed with a basement membrane and supporting cells, such as pericytes and vascular smooth muscle cells. At later developmental stages and in adult life, new blood vessels are mainly formed by angiogenesis, which is the sprouting of new vessels from existing ones (Fig. 1E–G). Both processes are controlled by angiogenic growth factors (Grant and Janigro 2006).

A well-functioning vascular system is vital, as it ensures gas exchange, nutrient supply, and waste removal for all organs, including the tooth. The tooth pulp tissue is highly vascularized and situated in an inextensible environment surrounded by rigid dentin walls with the apical foramina being the only access (Vongsavan and Matthews 1992). This renders unique properties to the pulp vasculature, which have been described previously (About 2014).

Pulp Vascularization: Parallels between Tooth Development and Engineering

During tooth development, vascularization of the tooth germ occurs by vasculogenesis, which has been demonstrated through the slice culture method. Indeed, it was observed that mesoderm-derived endothelial cell precursors invaded the developing papilla during the early bell stage, where they aggregate to form vascular structures. This invasion coincided with dental papilla size exceeding 200 µm (Rothova et al. 2011). It is known that oxygen diffusion through tissues is limited to 100 to 200 µm and that a vascular network is required to ensure all cells to be within this distance (Hoeben et al. 2004). Interestingly, the vascularization during entire tooth engineering procedures resembles what happens in vivo during tooth development (Fig. 2). Mesenchymal- and epithelial-derived stem cells cultured in vitro in a collagen gel allowed the formation of a tooth germ–like construct, similar to the bud and cap stage (Oshima et al. 2011). When this construct grows and exceeds 200 µm in size, vascularization becomes essential for its survival. To this end, the construct has to be implanted in vivo given that the in vitro culture lacks the necessary vascular supply. This step recalls the early bell stage—with the difference

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Treatment Options: Biological Basis of Regenerative Endodontic Procedures

Kenneth M. Hargreaves, DDS, PhD, Anibal Diogenes, DDS, MS, PhD, and Fabricio B. Teixeira, DDS, MS, PhD

Abstract

Dental trauma occurs frequently in children and often can lead to pulpal necrosis. The occurrence of pulpal necrosis in the permanent but immature tooth represents a challenging clinical situation because the thin and often short roots increase the risk of subsequent fracture. Current approaches for treating the traumatized immature tooth with pulpal necrosis do not reliably achieve the desired clinical outcomes, consisting of healing of apical periodontitis, promotion of continued root development, and restoration of the functional competence of pulpal tissue. An optimal approach for treating the immature permanent tooth with a necrotic pulp would be to regenerate functional pulpal tissue. This review summarizes the current literature supporting a biological rationale for considering regenerative endodontic treatment procedures in treating the immature permanent tooth with pulp necrosis. *(J Endod 2013;39:S30–S43)*

Key Words

Children, pulpal revascularization, regenerative endodontics, stem cells, trauma

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This Symposium includes a number of papers focused on diagnosis, prognosis, and treatment of the traumatized tooth. Here we provide a biological rationale for considering regenerative endodontic treatment procedures. A companion paper in this Symposium discusses considerations for the clinical protocol of a regenerative endodontic procedure (1).

Dental trauma occurs frequently in children and often can lead to pulpal necrosis. Population-based studies from around the world indicate that the prevalence of dental trauma injuries is about 4%–59%, with the majority of cases occurring in incisors (2). The broad range in estimated prevalence rates may be due in part to differences in sampling methods or study populations. In one study of 262 Swiss children aged 6–18 years, the prevalence of dental trauma was nearly 11%, and about 12% of enamel-dentin fractures led to pulpal necrosis (3). In another study of 889 permanent teeth with traumatic injuries, pulpal necrosis occurred in about 27% of the sampled population (4). The risk of developing pulpal necrosis is well recognized to be dependent on the type of dental trauma. In an analysis of 10,673 permanent teeth seen at a tertiary care center, pulpal necrosis was estimated to range from 0% (infraction), to 3% (concussion), to 26% (extrusion), to 58% (lateral luxation), to 92% (avulsion), to 94% (intrusion) (5).

The occurrence of pulpal necrosis in the permanent but immature tooth often represents a challenging clinical situation because the thin and often short roots increase the risk of subsequent fracture; indeed, overall survival of the replanted permanent teeth has been reported to range from 39%–89% (6). In treating the immature tooth with pulpal necrosis, the ideal clinical outcomes would be to prevent or heal the occurrence of apical periodontitis, promote continued root development, and restore the functional competence of pulpal tissue, particularly from both immunologic and sensory perspectives (7). These outcomes would very likely increase the long-term probability of retaining the natural dentition. Unfortunately, alternative procedures (eg, implants) are often contraindicated because of the still growing craniofacial skeleton in these young patients.

Treating the Immature Necrotic Tooth by Revascularization or Apexification

Current approaches (eg, replantation) for treating the traumatized immature tooth with pulpal necrosis do not reliably achieve healing of apical periodontitis, continued root development, and reestablishment of pulpal immunologic and sensorial competency. In one study, only 34% of replanted immature permanent teeth (32 of 94) exhibited pulpal healing (6). Another study reported an 8% revascularization rate (13 of 154) in replanted teeth, with this outcome defined as continued root development and an absence of radiographic signs of apical periodontitis or root resorption (8). These values are similar to a reported range of pulpal healing of about 4%–15% in a series of 470 replanted teeth reported by various authors (6). Importantly, the diameter of the apical opening ($\geq$1 mm), extraoral time (<45 minutes), and the arch (mandibular) were all significant predictors for improved revascularization of replanted avulsed teeth (8). Thus, the classic revascularization procedure of simply replanting an avulsed permanent tooth does not reliably achieve the goals of preventing apical periodontitis, triggering continued root development, and restoring functional competence of the pulp tissue.

An alternative approach for treating the immature permanent tooth is apexification procedures. The classic apexification method involves long-term application of

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Revascularization Outcomes: A Prospective Analysis of 16 Consecutive Cases

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Abstract

Introduction: Recent reviews lament the lack of evidence on the efficacy of regenerative procedures to induce further root maturation despite claims of a paradigm shift in the way infected, necrotic immature teeth with arrested root development can be endodontically treated. The majority of reports are either case series or successful case reports where nonstandardized images may make interpretation uncertain. **Methods:** This prospective clinical study reports on preliminary outcomes of regenerative endodontic procedures carried out on 16 teeth, 3 mandibular premolars and 13 traumatized central incisors, after 18-month reviews. Qualitative analysis of resolution of periapical radiolucencies and apical closure was undertaken. Quantitative analysis compared preoperative and recall radiographs by using a geometrical imaging program that calculated percentage changes in root length and dentin wall thickness. **Results:** Qualitative assessment showed 90.3% resolution of the periapical radiolucency. Apical closure was assessed as incomplete in 47.2% and complete apical closure in 19.4% of cases. Quantitative assessment showed change in root length varying from −2.7% to 25.3% and change for root dentin thickness of −1.9% to 72.6%. **Conclusions:** Patterns of continued root maturogenesis were variable at 18-month review. Reviews at 36 months showed continued root maturogenesis for 2 cases. Quantitative analysis can control for changes in angulation but may introduce other measurement errors. However, not all anterior teeth were suitable for TurboReg assessment because overlapping of the cementoenamel junctions and/or further eruption of teeth often precluded stable landmark positioning. Discoloration of the crown was a common consequence, with unaesthetic results in 10 of the 16 cases. *(J Endod 2014;40:333–338)*

Key Words

Regenerative endodontics, revascularization, traumatized teeth, tri-antibiotic paste, TurboReg

The promise and potential of regenerative endodontic therapies in necrotic teeth were first explored by Nygaard-Østby (1) in 1961 with limited success. During the last decade there has been renewed interest in regenerative endodontic procedures, which have been defined as "biologically based procedures designed to predictably replace damaged, diseased or missing structures, including dentine and root structures as well as cells of the pulp-dentine complex, with live viable tissues, preferably of the same origin, that restore the normal physiologic functions of the pulp dentine complex" (2, 3).

Numerous case reports have outlined successful outcomes when regenerative endodontic procedures have been used for the treatment of infected immature teeth (4–13). It has been reported that this treatment regime can result in apexogenesis, which is continued root maturation with closure of open apices, an increase in root length, and thickening of lateral dentin on root walls (14). Understandably, descriptions of these new techniques have created some excitement in the profession. A number of reviews have described this as a "paradigm shift for the treatment of immature permanent teeth" and as being the way of the future, going from traditional barrier formation approaches of calcium hydroxide and mineral trioxide aggregate (MTA) apexification to biologically based treatment protocols (15, 16). Therefore, it is surprising that 2 recent reviews are more circumspect in their recommendations for regenerative endodontic procedures (3, 17). A recent invited review in *Dental Traumatology* stated that "because of the lack of long-term evidence to support the use of endodontic regenerative procedures in traumatised teeth with open apices, revascularization regeneration procedures should only be attempted if the tooth is not suitable for root canal obturation, and after apexogenesis, apexification or partial pulpotomy treatments have already been attempted and have a poor prognosis" (3). Interestingly, the authors of that review have previously seemed to be avid proponents for regenerative techniques (2). The other recent review in the *Journal of Endodontics* states that "the outcome of revascularization procedures remains somewhat unpredictable and the clinical management of these teeth is challenging, when successful, they are an improvement to treatment protocols that leave the roots short and the walls of the root canal thin and prone to fracture" (17).

One identified drawback has been a lack of available evidence on outcomes (2). Although many case reports and some case series report favorable outcomes, these reports have to be considered a low level of evidence, particularly because one of the major problems with the interpretation of case reports is that often only successful individual outcomes are presented. Many of these reports also only describe outcomes limited to premolar teeth (4, 6, 8, 9, 11, 12). To date, there appears to be only one prospective pilot clinical study in the literature that examines outcomes for anterior

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Case Report

Dentistry Section

Revitalization of an Immature Permanent Mandibular Molar with a Necrotic Pulp Using Platelet-Rich Fibrin: A Case Report

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ABSTRACT

Any insult to the pulp during its development causes cessation of dentin formation and root growth. Pulpal status and degree of root development are the decisive factors in the treatment approach. Various treatment options have been tried like surgery with root-end sealing, calcium hydroxide–apexification, placement of apical plug and regenerative endodontic procedures to induce apexogenesis. An ideal scenario for a necrosed tooth with immature root would be continued root development coupled with regeneration of pulp tissue. We report a case, where revitalization was done using Platelet-Rich Fibrin (PRF) as a scaffold in immature mandibular molar tooth.

Keywords: Biodentine, Biomimetic, Open apex, Regenerative endodontics, Revascularisation

CASE REPORT

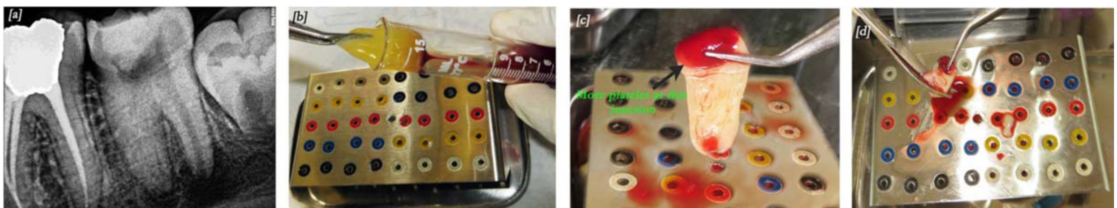
A 13-year-old male reported with a chief compliant of pain in the left posterior tooth region reported to the Department of Conservative Dentistry and Endodontics. The pain was intermittent, dull in nature for the past one month. History revealed that the pain started three months previously, which was continuous in nature for which he underwent treatment elsewhere, but did not complete it. There was no relevant medical history. Clinical examination revealed a temporary restoration in the second left mandibular molar (tooth# 37). The tooth was sensitive to both percussion and palpation tests. It did not respond to cold (Endo Ice, Coltene Whaledent, Ohio, USA) and Electric Pulp Tester (EPT). The contralateral tooth was negative to percussion and palpation tests and responded positively to cold and EPT. Periodontal probing depths were within normal limits. Radiographic examination revealed incomplete endodontic treatment with immature root and wide open apex in tooth #37 [Table/Fig-1a], periapical radiolucencies were also noted around the apices. On the basis of clinical and radiographic findings, a diagnosis of necrotic pulp and periapical diagnosis of symptomatic apical periodontitis were confirmed. So, a regenerative endodontic procedure was planned with the aid of Platelet-Rich Fibrin (PRF).

An informed consent was obtained from parents. Local anesthesia (2% lidocaine with 1:100,000 epinephrine) was administered. Rubber dam was applied and temporary restoration removed on tooth #37. Three canals were negotiated (mesio-buccal, mesio-lingual and distal). Working length was determined radiographically. Cleaning and shaping was performed in the mesial canals up to ProTaper F3 file (Dentsply, Pennsilvania, USA), to facilitate PRF placement. The canal was irrigated with approximately 10ml of 5.25% sodium hypochlorite, then neutralized with saline and dried with paper points. Triple antibiotic paste was prepared with mixing equal proportions of ciprofloxacin (Ranbaxy Lab, India), metronidazole (Unique Pharmaceuticals, Mumbai, India) and minocycline (Aurobindo, Andhra Pradesh, India) with propylene glycol into a thick paste. This antibiotic mixture was placed into the canals using a lentulo spiral up to the canal orifice. A cotton pellet was placed inside the pulp chamber and access cavity sealed with Cavit (ESPE, Cergy Pontoise, France).

The patient reported after 21 days without any symptoms. A 10ml sample of whole blood was drawn from the patient's right arm. The blood sample was transferred into a test tube without anti-coagulant and centrifuged immediately (REMI Model R-8c, India) at 3000rpm for 10 minutes to obtain the PRF. After centrifugation, PRF was removed from the test tube with the help of sterile tweezers [Table/Fig-1b]. Jelly-like PRF was converted into membrane in a modified endodontic file box [Table/Fig-1c]. This fibrin membrane was fragmented into small pieces with the help of surgical scissors [Table/Fig-1d]. So, that it can be easily placed inside the canals. In the mean time, local anaesthesia was given. After rubber dam isolation, the access cavity was reopened and saline irrigation done to remove the antibiotic mixture [Table/Fig-2a]. Each canal was dried with paper points and made ready for the placement of PRF.

The fragmented membrane was placed in the pulp chamber and pushed apically with the help of endodontic pluggers. This process was continued until the roof of the pulp chamber was filled with PRF [Table/Fig-2b]. Biodentine (Septodont, France) was placed directly over the fibrin clot and the reminder of the cavity was also restored fully with it [Table/Fig-2c]. During the 1st month follow-up the Biodentine was partially removed and a composite restoration placed over it.

The patient was reviewed at 3, 6, 9 and 12 months. The tooth# 37 was asymptomatic and was not sensitive to percussion. On the 9th month review, pulp sensibility tests with cold and EPT, elicited a positive response which was comparable to the contralateral molars. Radiographic examination revealed resolution of periapical lesion, thickening of the dentinal walls, root lengthening with apical closure showing a continuous lamina dura [Table/Fig-3a,b].



[Table/Fig-1]: a) Pre-operative intra-oral periapical radiograph showing wide root canal with open apex in mandibular molar tooth; b) PRF clot retrieved with the help of tweezers and placed on modified endo box for membrane preparation; c) Autologous fibrin membrane prepared; d) Fibrin membrane fragmented.

22.

Case Report

Pulp revascularization of a severely malformed immature maxillary canine

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Abstract: **Dens invaginatus (DI) is a dental anomaly exhibiting complex anatomical forms. Because of this anatomical complexity, immature DI teeth with necrotic pulp are difficult to treat via apexification. We used revascularization as an alternative treatment for a patient with DI. An 11-year-old boy visited our clinic with chief complaints of gingival swelling and pain in the left maxillary canine. Clinical and radiographic findings were consistent with a diagnosis of type III DI. Revascularization therapy was performed, and a 24-month follow-up examination confirmed healing of the periapical radiolucency and physiological root formation. (J Oral Sci 58, 295-298, 2016)**

Keywords: revascularization; dens invaginatus; antibiotics; regenerative endodontic treatment.

Introduction

Dens invaginatus (DI) is an anomaly that results in invagination of the enamel organ into the dental papilla before calcification. Most DI teeth exhibit deepening and development of a lingual pit. However, the size and morphology of the invaginated crown varies considerably (1).

A thin or incomplete enamel lining cannot prevent bacterial penetration of the pulp, and such penetration can lead to pulp necrosis and apical periodontitis. In addition, infected invaginations can result in necrosis of pulp tissue before the completion of root development, leaving the tooth with an open apex. Apexification has been used to close the open apex of an immature tooth with apical periodontitis. However, apexification of an immature invaginated tooth may be complicated by the irregular shape of the root canal system. The complex anatomy of DI can compromise instrumentation, disinfection, and application of calcium hydroxide and mineral trioxide aggregate (MTA) into the root canal. In some cases, it is not possible to access the invaginated area. In addition, teeth treated with apexification have thin, weak roots, which are susceptible to fracture. Therefore, treatment of an immature invaginated tooth with periapical abscess continues to be a challenge in pediatric dentistry.

Regenerative pulp therapy has recently been recommended as an alternative to apexification, and studies have reported the clinical success of regenerative pulp therapy for immature permanent teeth with pulp necrosis (2). Regenerative pulp therapy is a biologically based procedure designed to replace damaged structures, such as dentin and root structures-including the cells of the pulp-dentin complex. In the revascularization protocol, the infected canal is first disinfected by irrigation and application of combined antibiotics. Then, bleeding is induced from the mechanically irritated apex and sealed in the root canal to function as a scaffold for continuous regenerative tissue development (3). This report describes a case of an immature invaginated maxillary canine in an 11-year-old boy, which was treated successfully with regenerative pulp therapy.

Case Report

An 11-year-old boy with unremarkable systemic findings and family history visited our clinic with chief complaints of gingival swelling and pain near the left

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23.

Dental Traumatology

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Is revascularization of immature permanent teeth an effective and reproducible technique?

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Key words: treatment; revascularization; immature teeth; revitalization

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Abstract – *Background*: Revascularization has been proposed as an improved alternative treatment for irreversibly damaged pulp of immature teeth as it has been shown to preserve the potential for continued root growth in treated teeth. *Aim*: To review clinical cases of revascularization in humans to evaluate their utility and reproducibility. *Material and methods*: A structured electronic search of scientific articles published between 2001 and 2014 was carried out using the following keywords: 'pulp revascularization', 'pulp revitalization' and/or 'immature tooth'. Clinical revascularization cases conducted on human subjects were selected, reviewed and organized into two charts including patient information, diagnostic information, treatment and results in follow-up visits. *Results*: Ninety-seven of 101 teeth (96.0%) were successfully treated with the revascularization technique. The range of technique variations available for irrigation, disinfection and blood clot induction have a negligible impact on the clinical outcome variables tested in our analysis. During the follow-up visits, apical closure was detected in fewer cases (55.4%) as compared to the other apexogenesis phenomena (increased root length, 76.2%; increased root width, 79.2%). *Conclusions*: The review shows that the revascularization technique showed marked increase in the root length, width and apical closure in the cases that were reported independently of clinical variables such as operator and material selection and individual differences in protocols.

Root development of permanent teeth is not normally complete until 1–4 years following their eruption in the mouth (1). If during this period, in which teeth are considered to be immature, the pulp is irreversibly damaged due to caries or trauma, their root development will be arrested, resulting in teeth with open apices, thin dentinal walls and inappropriate crown–root ratio. This will result in fragile teeth, sensitive to external forces and prone to cervical fracture (2–19).

Treatment of immature permanent teeth with irreversibly damaged pulp is often technically complex. Conventional endodontic treatment has always been indicated as the recommended therapeutic strategy for mature permanent teeth with irreversibly damaged pulp with high success rate (78–98%) (19–25). However, it has traditionally been difficult to achieve in immature teeth due to the absence of apical constriction. This may lead to the extrusion of gutta-percha, and the dentinal walls may become even more fragile during the shaping of the root canal (2, 4–18, 26).

Apexification is the recommended endodontic procedure for these cases. It requires the intracanal placement of either calcium hydroxide ([$Ca(OH)_2$], over a long period of time in order to induce apical barrier formation), or mineral trioxide aggregate [(MTA), directly as the apical barrier]. Studies have shown that the barrier formed by $Ca(OH)_2$ is 'porous and inconsistent' and requires many clinical sessions to create it and that MTA is difficult to manipulate and is costly (52). It should also be noted that apexification with $Ca(OH)_2$ or MTA prevents the problem of extrusion of gutta-percha, whilst thin dentinal walls remain an issue. Thus, treated teeth continue to be prone to fractures (2, 4–19, 25, 27–33).

Recently, revascularization has been proposed as an improved alternative treatment for irreversibly damaged pulp of immature teeth as it has been shown to preserve the potential for continued root growth in treated teeth (5, 6, 24, 33–35). The root canal is cleaned and disinfected by irrigation with sodium hypochlorite and/or chlorhexidine. The second step included the placement of disinfectant paste [$Ca(OH)_2$ or triple antibiotic paste (TAP)], followed by sealing the tooth crown. In the follow-up appointment, the tooth is reopened and canal over-instrumentation with a k-file is carried out to provoke bleeding from the periapical region. This causes blood cells to be trapped in clot, followed by the induction of angiogenesis and new pulp-like tissue formation (Fig. 1) (4, 7–19, 26–32). MTA is placed over the clot and the tooth is permanently restored.

24.

Original Article

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Dentistry Setion

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

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ABSTRACT

Introduction: Treatment of a tooth with necrotic pulp and open apex is a special challenge to the clinicians. Apexification with calcium hydroxide and MTA barrier technique fails to induce continued root maturation which makes the tooth susceptible to root fracture. Hence, an ideal outcome for such a tooth should be regeneration of pulp like tissue into the root canal capable of continuing normal root maturation.

Aim: This study aims to compare the effect of Platelet Rich Fibrin (PRF), induced bleeding technique and Platelet Rich Plasma (PRP) in the revascularization of tooth with necrotic pulp and open apex. The main objectives of the study were to: (a) Radiographically evaluate the continuation of root development, increase in the dentin wall thickness and narrowing of canal space, apical closure and resolution of the periapical lesion; and to (b) To clinically evaluate the response to pulp sensibility testing and response to percussion and palpation tests.

Materials and Methods: Sixty patients (6 to 28 years) with necrotic immature permanent tooth were randomly categorised into three groups after the root canal disinfection procedure. PRF as scaffolding material (Group A: n=20), revascularization with conventional induced bleeding technique (Group B: n=20), and PRP as the biomaterial (Group C: n=20). The primary outcome variable was measured using Periapical Index (PAI) (for periapical healing), Chen and Chen index (for apical responses), Schei's ruler (for root lengthening and root thickening) and other clinical parameters. The Chi-square test was used to interpret the data among the three groups at the end of 12 months for the variables root lengthening and lateral wall thickness. ANOVA test was performed to compare the mean of the PAI scores of the three groups at preoperative stage and 12 months. If statistically significant, Bonferroni test was done to compare the outcome among the three groups. The significant level was set at $p<0.05$. Kappa agreement was used to see whether the clinical criteria of success (asymptomatic) were in agreement with radiographic criteria.

Results: At the end of 12 months, patients presented with no pain and no signs of reinfection or no radiographic enlargement of the pre-existing apical pathosis in all the three groups. PRP was better than PRF and induced bleeding technique with respect to periapical wound healing when used in the regenerative endodontic procedures. The groups were comparable on grounds of root lengthening and lateral wall thickening. Type 3 apical response was the most common apical response in all the three groups.

Conclusion: On analysing the pros and cons of the techniques performed, it is wise to establish induced bleeding technique as the standard endodontic procedure for revascularization of a non vital immature permanent tooth.

Keywords: Apical response, Pulp dentin complex, Root maturation, Schei's ruler, Triple antibiotic paste

INTRODUCTION

An archetypal outcome for a tooth with an immature root and necrotic pulp would be the regeneration of pulp tissue into the canal capable of bolstering the continuation of normal root development [1]. Three components contributing to the success of this procedure include, stem cells that are capable of hard tissue formation, signalling molecules for cellular stimulation, proliferation, and differentiation, and finally, a 3-dimensional physical scaffold that can fortify cell growth and differentiation [2]. The induced bleeding technique of revascularization is well documented in the literature [3-5].

PRP has been mentioned as a potentially ideal scaffold for regenerative endodontic treatment regimens because of its increased growth factor concentrations [1]. PRF is a second generation platelet concentrate which requires no biochemical handling of blood and is easy to procure [6]. The potential applications of PRF as a biologic scaffold to promote the regeneration of lost or injured dental pulp tissues and stimulate dentinogenesis was mentioned in a research study [7]. It has been mentioned that PRF is an ideal biomaterial for pulp dentin complex regeneration [8].

No study has compared the effect of PRP, PRF and induced bleeding in the revascularization of a tooth with necrotic pulp and open apex. The purpose of this study was to add knowledge to the existing literature in the field of regenerative endodontics by comparison of the effect of PRP, PRF and induced bleeding in the revascularization of a tooth with necrotic pulp and open apex. The main objectives of the study were to: (a) radiographically evaluate the continuation of root development, increase in the dentin wall thickness and narrowing of canal space, apical closure and resolution of the periapical lesion; and to (b) to clinically evaluate the response to pulp sensibility testing, percussion and palpation tests.

MATERIALS AND METHODS

A triple blind randomized controlled trial was conducted at Government Dental College, Calicut, Kerala over a period of one

25.

Influence of Age and Apical Diameter on the Success of Endodontic Regeneration Procedures

Bishoy Safwat Estefan, BDS,[*] Kariem Mostafa El Batouty, PhD,[*] Mohamed Mokhtar Nagy, PhD,[*] and Anibal Diogenes, DDS, MS, PhD[†]

Abstract

Introduction: Treatment of immature permanent teeth with necrotic pulp and apical pathosis constitutes a challenge for endodontists. The present study was done to evaluate the effect of age and apical diameter on the regenerative potential of young permanent immature teeth with necrotic pulps. **Methods:** Immature necrotic permanent maxillary incisors (*n* = 40) of patients 9–18 years old were divided into 2 groups according to the treatment protocol: group Y (younger age group), 9–13 years and group O (older age group), 14–18 years. Each group was further subdivided into 2 subgroups according to apical diameter, subgroup (n) (narrower diameter) between 0.5 and 1 mm and subgroup (w) (wider diameter) equal to or greater than 1 mm. Revascularization procedures were performed for all patients. Follow-up was done for up to 12 months. Standardized radiographs were digitally evaluated for increase in root length and thickness and decrease in apical diameter. **Results:** After the follow-up period, most of the cases demonstrated radiographic evidence of periapical healing. Group Y showed significant progressive increase in root length and width and decrease in apical diameter. Subgroup (w) representing wider apical diameter showed significant progress as well. **Conclusions:** It was found that revascularization procedures can be implemented in any age ranging from 9 to 18 years; however, younger age groups were better candidates for revascularization procedure than older ones. Regarding the apical diameter, regeneration procedures were successful with apical diameters as small as 0.5 mm. However, teeth with preoperative wider diameters (≥1 mm) demonstrated greater increase in root thickness, length, and apical narrowing. *(J Endod 2016;■:1–6)*

Key Words

Age, apical diameter, regeneration

Significance

Pulp regeneration is a biologic treatment modality for immature teeth with open apices. Regenerative ability depends on both age and apical diameter.

Treatment of immature permanent teeth with necrotic pulp and apical pathosis constitutes a challenge for endodontists. Such cases are commonly encountered in children because of trauma, dental anatomic variations (eg, dens evaginatus), and untreated carious lesions. Many of these teeth develop pulpal necrosis before completing root development. Such conditions are challenging not only in root canal debridement and filling but also for the thin dentinal walls increasing the risk of subsequent fracture (1).

Historically, immature apices have been managed with root-end closure through apexification (2, 3). Although these procedures have excellent clinical success for the resolution of infection and its associated symptoms, they do very little for continued root development. Thus, teeth remain with thin dentinal walls after apexification procedures (4). In addition, in highly immature teeth, clinician may elect the long-term use of calcium hydroxide as the method to develop an apical calcific barrier. This technique has several disadvantages because it requires multiple visits during long period of time, requiring compliance, and the long-term use of calcium hydroxide might alter the mechanical properties of dentin, making the tooth more susceptible to fractures (5). Therefore, even if apexification closure is successful, the long-term prognosis is questionable because of eventual root fracture (6).

Recently, the concept of immediate apical plug has been advocated. This technique is performed by placement of artificial apical plug obliterating the apical portion of the canal. Mineral trioxide aggregate (MTA) proved to be excellent candidate for this protocol. This protocol has the advantage of reduced number of visits, higher patient compliance, and high success rate. However, the problem of thin brittle roots was not solved (7–9).

An alternative approach to treating the immature necrotic permanent tooth is to regenerate pulpal tissue, allowing for continued root formation. This treatment modality was introduced as a biological alternative for continued maturation of the entire root rather than the formation of an apical calcific barrier through apexification (10–12).

In regenerative endodontics, one of the goals is to promote continued root development in addition to the crucial resolution of the disease process. Therefore, the advantage of this treatment is continued root lengthening and reinforcement of lateral dentinal walls with deposition of new hard tissue. Revascularization, regeneration, and revitalization are commonly used terms to describe the regrowth of *de novo* tissue within the root canal space (13–21).

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AAE Clinical Considerations for a Regenerative Procedure
Revised 4/1/2018

These considerations should be seen as one possible source of information and, given the rapid evolving nature of this field, clinicians should also actively review new findings elsewhere as they become available.

Case Selection:

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

Informed Consent

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

First Appointment

- Local anesthesia, dental dam isolation and access.
- Copious, gentle irrigation with 20ml NaOCl using an irrigation system that minimizes the possibility of extrusion of irrigants into the periapical space (e.g., needle with closed end and side-vents, or EndoVac™). Lower concentrations of NaOCl are advised [1.5% NaOCl (20mL/canal, 5 min) and then irrigated with saline or EDTA (20 mL/canal, 5 min), with irrigating needle positioned about 1 mm from root end, to minimize cytotoxicity to stem cells in the apical tissues.
- Dry canals with paper points.
    - Place calcium hydroxide or low concentration of triple antibiotic paste. If the triple antibiotic paste is used: 1) consider sealing pulp chamber with a dentin bonding agent [to minimize risk of staining] and 2) mix 1:1:1 ciprofloxacin: metronidazole: minocycline to a final concentration of 1-5 mg/ml. Triple antibiotic paste has been associated with tooth discoloration. Double antibiotic paste without minocycline paste or substitution of minocycline for other antibiotic (e.g., clindamycin; amoxicillin; cefaclor) is another possible alternative as root canal disinfectant. Clinicians should be aware that studies have been done using higher concentrations of TAP/DAP, but a recommendation to a higher concentration can't be made at this time due to limited studies.
- Deliver into canal system via syringe
- If triple antibiotic is used, ensure that it remains below CEJ (minimize crown staining).
- Seal with 3-4mm of a temporary restorative material such as Cavit™, IRM™, glass-ionomer or another temporary material. Dismiss patient for 1-4 week

27.

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2018 ASE UNDERGRADUATE ESSAY COMPETITION

A review of the prognostic value of irrigation on root canal treatment success

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Keywords
agitation, delivery, irrigant, irrigation, outcome, root canal treatment.

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Abstract

The aim of this review is to discuss the requirements for effective irrigation of the root canal system and its role in treatment outcome. A review of the literature regarding irrigants, methods of irrigation and regimens was undertaken. It indicates that irrigation is comprised of a multitude of factors, and that effective irrigant delivery and agitation achieves mechanical, chemical and microbiological functions, which are prerequisites for obtaining a clean canal system. However, most studies are ex vivo and assess intermediate outcome values rather than treatment success. Additionally, there is an absence of high-level evidence evaluating the effect of irrigation on the outcome of root canal treatment. Therefore, citing irrigation as a significant factor that affects root canal treatment success cannot be done. This highlights the need to further investigate the prognostic value of irrigation on root canal treatment success.

Introduction

Endodontic treatment is comprised of a set of guiding principles that generally include four consecutive steps: instrumentation, irrigation, medication and obturation. Treatment success is defined as the prevention or elimination of apical periodontitis and resolution of patient symptoms (1). The first three treatment processes are essential for microbial control in the root canal system. This is critical because the main causes of endodontic failure are remaining microorganisms or recolonisation (2,3). Sjogren (2) demonstrated that bacteria in the root canal, at the time of obturation, resulted in a statistically significant reduction in success. Canals yielding negative bacterial cultures had a 98% success rate, compared to 68% in those that yielded positive cultures (2). Thus, it can be surmised that ineffective application of any of these processes could potentially affect treatment outcome.

However, in regard to irrigation, a limitation to this assumption was presented in a meta-analysis conducted by Ng *et al*. It indicated that using irrigation as a prognostic factor for predicting root canal treatment success could not be done due to insufficient data (4). In contrast to insufficient high-level evidence related to the effect of irrigation on success, there is extensive literature – of lower level evidence – which has investigated the effect of irrigation on variables that are pertinent to endodontic treatment outcome. Additionally, these studies demonstrate that irrigation is a complex topic which can be deconstructed into constitutive parts. In doing this, it is appropriate to start with the question 'What elements determine effective irrigation?' The first step is to consider the goals of irrigation and the properties of an ideal irrigant, in relation to chemical and mechanical effects, as it is these effects which clean and disinfect canals.

Goals of irrigation and the ideal properties of an irrigant

Infections in root canal systems are polymicrobial and constantly undergoing selection pressure for the low-oxygen environment (5). Planktonic members aggregate in an extracellular polysaccharide matrix associated with the root canal wall to form biofilm (6). Additionally, the gram-negative bacteria have endotoxin in their cell walls

28.

Root Canal Irrigants

Matthias Zehnder, Dr. med. dent., PhD

Abstract

Local wound debridement in the diseased pulp space is the main step in root canal treatment to prevent the tooth from being a source of infection. In this review article, the specifics of the pulpal microenvironment and the resulting requirements for irrigating solutions are spelled out. Sodium hypochlorite solutions are recommended as the main irrigants. This is because of their broad antimicrobial spectrum as well as their unique capacity to dissolve necrotic tissue remnants. Chemical and toxicological concerns related to their use are discussed, including different approaches to enhance local efficacy without increasing the caustic potential. In addition, chelating solutions are recommended as adjunct irrigants to prevent the formation of a smear layer and/or remove it before filling the root canal system. Based on the actions and interactions of currently available solutions, a clinical irrigating regimen is proposed. Furthermore, some technical aspects of irrigating the root canal system are discussed, and recent trends are critically inspected. *(J Endod 2006;32:389–398)*

Key Words

Chelators, chlorhexidine, interactions, irrigants, review, sodium hypochlorite

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We are living in the age of evidence-based medicine. Any new concepts and techniques to be used in the clinic should ideally be assessed in randomized controlled clinical trials against their respective gold standards. This, however, poses a major problem in endodontic research. A favorable outcome of root canal treatment is defined as the reduction of a radiographic lesion and absence of clinical symptoms of the affected tooth after a minimal observation period of 1 yr (1). Alternatively, so-called surrogate outcome (dependent) variables yielding quicker results, such as the microbial load remaining in the root canal system after different treatment protocols, can be defined. However, these do not necessarily correlate with the "true" treatment outcome (2). Endodontic success is dependent on multiple factors (3), and a faulty treatment step can thus be compensated. For instance if cultivable microbiota remain after improper canal disinfection, they can theoretically be entombed in the canal system by a perfect root canal filling (4), and clinical success may still be achieved (5). On the other hand, in a methodologically sound clinical trial, single treatment steps have to be randomized and related to outcome. Otherwise, the results do not allow any conclusions and no causative relationships may be revealed (6).

The above issues may be viewed as the reason (or as an excuse) for the fact that no randomized controlled clinical trials exist on the effect of irrigating solutions on treatment outcome in the endodontic literature. As of yet, we largely depend on data from in vitro studies and clinical trials with microbial recovery after treatment as the surrogate outcome. Clinical recommendations based on such findings are merely deductive and need to be interpreted with care. Nevertheless, individual problems can be singled out in these investigations and basic information can be gained.

It was the purpose of this article to present an overview on irrigating solutions in endodontics, their actions and interactions. Based on data derived from basic science studies, results obtained in clinical investigations are discussed and some general recommendations are given.

Facing the Challenge

There can be no doubt today that microorganisms, either remaining in the root canal space after treatment or re-colonizing the filled canal system, are the main cause of endodontic failure (7, 8). The primary endodontic treatment goal must thus be to optimize root canal disinfection and to prevent re-infection.

Infection of the root canal space occurs most frequently as a sequela to a profound carious lesion (9). Cracks in the crown structure extending into the pulp chamber can also be identified as a cause of endodontic infection (10). Regardless of the microbial entryways, it should be differentiated between vital and nonvital cases (11). Pulpitis is the host reaction to opportunistic pathogens from the oral environment entering the endodontium (12). Vital pulp tissue can defend against microorganisms and is thus largely noninfected until it gradually becomes necrotic (9). In contrast, the pulp space of nonvital teeth with radiographic signs of periapical rarefaction always harbors cultivable microorganisms (13). Consequently, the treatment of vital cases should focus on *asepsis*, i.e. the prevention of infection entering a primarily sterile environment, which is the apical portion of the root canal. *Antisepsis*, which is the attempt to remove all microorganisms, is the key issue in nonvital cases. Vitality cannot always be predictably assessed with current sensitivity tests and radiologic methods before treatment (14). Once the pulp space is entered during access cavity preparation, however, the clinician can clearly discern between vital and nonvital pulp tissue (15), and further treatment decisions can be made accordingly.

Aseptic principles such as correct rubber dam placement and coronal disinfection of the tooth to be treated have long been accepted (16). Although asepsis is not the topic

29.

Translational Science in Disinfection for Regenerative Endodontics

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Abstract

The endodontic management of permanent immature teeth is fraught with challenges. Although treatment modalities for vital pulp therapy in these teeth provide long-term favorable outcome, the outcomes from the treatment of pulp necrosis and apical periodontitis are significantly less predictable. Immature teeth diagnosed with pulp necrosis have been traditionally treated with apexification or apexogenesis approaches. Unfortunately, these treatments provide little to no benefit in promoting continued root development. Regenerative endodontic procedures have emerged as an important alternative in treating teeth with otherwise questionable long-term prognosis because of thin, fragile dentinal walls and a lack of immunocompetency. These procedures rely heavily on root canal chemical disinfection of the root canal system. Traditionally, irrigants and medicaments have been chosen for their maximum antimicrobial effect without consideration for their effects on stem cells and the dentinal microenvironment. Translational research has been crucial to provide evidence for treatment modifications that aim to increase favorable outcome while steering away from common pitfalls in the currently used protocols. In this review, recent advances learned from translational research related to disinfection in regenerative endodontics are presented and discussed. *(J Endod 2014;40:S52–S57)*

Key Words

Disinfection, immature teeth, mesenchymal adult stem cells, pulp biology, regenerative endodontics, revascularization, root canal disinfection

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The developing dentition is at risk for pulpal necrosis because of trauma and developmental dental anomalies such as dens evaginatus (1–7). The loss of an immature permanent tooth in young patients with mixed dentition can be devastating, leading to loss of function, malocclusion, and inadequate maxillofacial development. These teeth traditionally have been treated with apexification procedures using either long-term calcium hydroxide treatment (8, 9) or immediate placement of a mineral trioxide aggregate (MTA) apical plug (10). Although these treatments often result in the resolution of signs and symptoms of pathosis, they provide little to no benefit for continued root development (11). Thus, immature teeth treated with these procedures are considered to be in a state of "arrested development," and no further root growth, normal pulpal nociception, and immune defense should be expected.

Regenerative endodontic procedures (REPs) have emerged as an alternative treatment alternative for these teeth that, in addition to healing of apical periodontitis, aims to promote normal pulpal physiologic functions. These include continued root development, immunocompetency, and normal nociception, as seen in some published cases (12). Thus, the ultimate goal of these procedures is to regenerate the components of the pulp-dentin complex. A significant number of case reports and case series have been published since the first reported case in 2001 (12). These published cases document the following (12):

1. Commonly observed clinical outcomes such as continued root development and sometimes normal nociceptive responses to vitality testing
2. Commonly found challenges such as technical pitfalls and unwanted adverse reactions such as coronal staining
3. Great variability in treatment protocols

Despite the lack of randomized clinical trials, these published clinical observations support the hypothesis that patients with otherwise limited treatment options could benefit from these procedures.

In 2011, a study showed that a substantial number of undifferentiated mesenchymal stem cells are delivered into root canal systems after REPs (13). This finding represented a turning point because treatment protocols previously used in REPs aimed to provide maximum disinfection without consideration for their impact on stem cells. Contemporary regenerative endodontics acknowledges and follows principles of bioengineering regarding the interplay between stem cells, scaffolds, and growth factors (14). Because stem cells represent 1 of the pillars of REPs, a series of translational studies evaluating the effect of disinfection on stem cell fate have been conducted. These studies have contributed to the foundational framework for the current American Association of Endodontists–recommended regenerative endodontic treatment protocol (15).

Translational Studies on Disinfection

Irrigants

Clinicians often face the challenge of adequately debriding large infected root canals in REPs. In these procedures, similar to conventional endodontic therapy, microbial control is crucial. These canals with compromised fragile underdeveloped dentinal walls represent a contraindication for mechanical instrumentation; thus, chemical debridement remains the main form of disinfection in REPs. Sodium hypochlorite (NaOCl) is the most widely used agent for chemical debridement in endodontic procedures, including REPs (12). It has several desirable characteristics including excellent bactericidal efficacy (16–18), tissue dissolution capacity (19–21), and effective

Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain

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Abstract

Sim TPC, Knowles JC, Ng Y-L, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *International Endodontic Journal*, **34**, 120–132, 2001.

Aim The aim of this study was to test the null hypothesis that sodium hypochlorite irrigation of root canals does not alter the properties of dentine and contribute to the weakening of root-treated teeth.

Methodology The effect of two concentrations (0.5%, 5.25%) of sodium hypochlorite (NaOCl) and saline on (i) the elastic modulus and flexural strength of machined dentine bars, and (ii) changes in strain of 'whole' extracted human teeth were evaluated. One hundred standardized plano-parallel dentine bars ($> 11.7 \times 0.8 \times 0.8$ mm) were randomly divided into the three groups, immersed for 2 h in the respective solutions and then subjected to a three-point bend test. Changes in strain of each of 10 teeth on cyclical nondestructive occlusal loading were measured using electrical resistance strain gauges bonded to the cervical aspects. Each tooth had its crown and enamel reduced and root canal prepared. These were irrigated sequentially in a series of four separate, 30-minute regimes; initial-saline, 0.5% NaOCl, 5.25% NaOCl and final-saline. The changes in strains after each irrigation regime were compared.

Results There was a significant decrease in elastic modulus of the dentine bars immersed in 5.25% NaOCl compared with the saline group ($P < 0.01$). There was also a significant decrease in flexural strength of the dentine bars in the 5.25% NaOCl group compared to both the saline and 0.5% NaOCl groups ($P < 0.01$). The strain data from the nondestructive tooth loading tests revealed significant increases in tensile strain between the initial-saline and the final-saline stages ($P < 0.01$). Significant increases in compressive strains were also found between initial-saline and 5.25% NaOCl; and between 0.5% NaOCl and 5.25% NaOCl stages ($P < 0.01$).

Conclusions The null hypothesis was rejected, 5.25% NaOCl reduced the elastic modulus and flexural strength of dentine. Irrigation of root canals of single, mature rooted premolars with 5.25% NaOCl affected their properties sufficiently to alter their strain characteristics when no enamel was present.

Keywords: dentine, irrigant, sodium hypochlorite, strain.

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Introduction

There is a widely held belief that root-treated teeth are weakened and more susceptible to fracture than vital teeth (Rosen 1961, Johnson *et al.* 1976, Gher *et al.* 1987), yet conclusive epidemiological evidence is still lacking. Nevertheless, there is convincing circumstantial evidence for the putative causes of nonvital and root-treated teeth to fracture (Burke 1992). The main causes may be divided broadly into three areas; loss of tooth tissue, altered physical properties of dentine and altered proprioception/nociception (Gutmann 1992, Gulabivala 1995). It is probable that these factors interact cumulatively to influence tooth loading and distribution of stresses, ultimately increasing the possibility of catastrophic failure.

Tooth tissue loss reduces the force required to strain and ultimately fracture teeth *in vitro* with the pattern of loss influencing the magnitudes of the induced strains (Vale 1956, Mondelli *et al.* 1980, Larson *et al.* 1981, Panitvisai & Messer 1995). Evidence from clinical

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31.

Concentration-dependent Effect of Sodium Hypochlorite on Stem Cells of Apical Papilla Survival and Differentiation

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Abstract

Introduction: Intracanal disinfection is a crucial step in regenerative endodontic procedures. Most published cases suggest the use of sodium hypochlorite (NaOCl) as the primary irrigant. However, the effect of clinically used concentrations of NaOCl on the survival and differentiation of stem cells is largely unknown. In this study, we tested the effect of various concentrations of NaOCl on the stem cells of the apical papilla (SCAPs) survival and dentin sialophosphoprotein (DSPP) expression. **Methods:** Standardized root canals were created in extracted human teeth and irrigated with NaOCl (0.5%, 1.5%, 3%, or 6%) followed by 17% EDTA or sterile saline. SCAPs in a hyaluronic acid–based scaffold were seeded into the canals and cultured for 7 days. Next, viable cells were quantified using a luminescence assay, and DSPP expression was evaluated using quantitative real-time polymerase chain reaction. **Results:** There was a significant reduction in survival and DSPP expression in the group treated with 6% NaOCl compared with the untreated control group. Comparable survival was observed in the groups treated with the lower concentrations of NaOCl, but greater DSPP expression was observed in the 1.5% NaOCl group. In addition, 17% EDTA resulted in increased survival and DSPP expression partially reversing the deleterious effects of NaOCl. **Conclusions:** Collectively, the results suggest that dentin conditioning with high concentrations of NaOCl has a profound negative effect on the survival and differentiation of SCAPs. However, this effect can be prevented with the use of 1.5% NaOCl followed by 17% EDTA. The inclusion of this irrigation regimen might be beneficial in regenerative endodontic procedures. *(J Endod 2014;40:51–55)*

Key Words

Dentin conditioning, dentin sialophosphoprotein, EDTA, gene expression, irrigant, regenerative endodontics, stem cells of apical papilla, sodium hypochlorite, survival, viability

The field of endodontics has seen a shift toward biologically based regeneration of pulpal tissues. Regenerative endodontic procedures rely on adequate disinfection while creating a microenvironment conducive for stem cell survival and differentiation (1). In current regenerative endodontic procedures, mesenchymal stem cells (MSCs) are delivered from the periradicular tissues into the root canal space via evoked bleeding (2). Postnatal MSCs include the dental pulp stem cells (DPSCs), stem cells from human exfoliating deciduous teeth (SHEDs), periodontal ligament stem cells, dental follicle progenitor cells, inflammatory periapical progenitor cells, and stem cells from the apical papilla (SCAPs) (3–6). Of special interest to endodontics are SCAPs. These cells can be found in the apical papilla immediately adjacent to the apex of the root canal, allowing for an easier incorporation of SCAPs in regenerative endodontic procedures (1, 2). SCAPs have also been reported to have high proliferation rates and odontogenic differentiation capacity (4).

Multiple case reports of regenerative endodontic procedures have been published, with significant differences in disinfection protocols. These have varied from the use of 5.25% sodium hypochlorite (NaOCl) followed by saline and 0.12% chlorhexidine (1, 7, 8) 5.25% NaOCl, or 2.5% NaOCl alone to 6% NaOCl followed by 2% chlorhexidine (1, 9). Although proven to show effectiveness as root canal irrigants for bactericidal, bacteriostatic, and tissue dissolution properties (10, 11), these irrigants have also been shown to be cytotoxic to fibroblasts, periodontal ligament stem cells, HeLA cells, SHEDs, and SCAPs (12–16). When NaOCl is used at 6%, it has a profound detrimental effect on SCAP survival using an organotype human root model (12) and on odontoblastic differentiation of DPSCs *in vivo* (17). Thus, dentin treatment with 6% NaOCl has a negative effect on the survival and differentiation of stem cells when in contact with the conditioned dentin.

Nonetheless, NaOCl is the most commonly used endodontic irrigant, and it has been used in the great majority of all the regenerative/revascularization reported cases (8, 18, 19). However, the effect of various clinically relevant concentrations of NaOCl on the survival and odontoblastic differentiation of stem cells has never been previously investigated. Thus, the aim of this study was to evaluate the effect of different concentrations of NaOCl on the survival and odontoblastic differentiation of SCAPs in an organotype root canal model.

Methods

Patient Recruitment

This study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, San Antonio, Texas. Third molar teeth diagnosed with vital pulp but with an indication for extraction were collected from the clinics

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32.

Effects of Morphogen and Scaffold Porogen on the Differentiation of Dental Pulp Stem Cells

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Abstract

Introduction: Dental pulp tissue engineering is an emerging field that can potentially have a major impact on oral health. However, the source of morphogens required for stem cell differentiation into odontoblasts and the scaffold characteristics that are more conducive to odontoblastic differentiation are still unclear. This study investigated the effect of dentin and scaffold porogen on the differentiation of human dental pulp stem cells (DPSCs) into odontoblasts. **Methods:** Poly-L-lactic acid (PLLA) scaffolds were prepared in pulp chambers of extracted human third molars using salt crystals or gelatin spheres as porogen. DPSCs seeded in tooth slice/scaffolds or control scaffolds (without tooth slice) were either cultured *in vitro* or implanted subcutaneously in immunodefficient mice. **Results:** DPSCs seeded in tooth slice/scaffolds but not in control scaffolds expressed putative odontoblastic markers (DMP-1, DSPP, and MEPE) *in vitro* and *in vivo*. DPSCs seeded in tooth/slice scaffolds presented lower proliferation rates than in control scaffolds between 7 and 21 days ($p < 0.05$). DPSCs seeded in tooth slice/scaffolds and transplanted into mice generated a tissue with morphological characteristics similar to those of human dental pulps. Scaffolds generated with gelatin or salt porogen resulted in similar DPSC proliferation. The porogen type had a relatively modest impact on the expression of the markers of odontoblastic differentiation. **Conclusions:** Collectively, this work shows that dentin-related morphogens are important for the differentiation of DPSC into odontoblasts and for the engineering of dental pulp-like tissues and suggest that environmental cues influence DPSC behavior and differentiation potential. *(J Endod 2010;36:1805–1811)*

Key Words

Dentin, endodontics, odontoblast, pulp regeneration, tissue engineering

Dental pulp stem cells (DPSCs) have the ability to differentiate into odontoblasts and secrete dentin matrix when odontoblasts are lost because of caries or injury (1). This fundamental observation serves as the biological rationale for the use of stem cells in dental pulp tissue engineering. However, one of the outstanding issues in dental pulp tissue engineering is the origin and nature of the signals required for the differentiation of stem cells into functional odontoblasts. It is not clear if these signals come from neighboring cells or the existing dentin matrix. In addition, the extent of the impact of the microenvironment of the scaffold in which the stem cells are cultured on the process of odontoblastic differentiation is not clear. It has become increasingly evident that further progress in dental pulp tissue engineering will require in-depth knowledge of the processes that result in the differentiation of stem cells into functional odontoblasts.

Stem cells can be found in the dental pulp tissue of permanent teeth (2), primary teeth (3), periodontal ligament (4), and apical papilla (5). It has been shown that stem cells from the pulp tissue are capable of generating an odontoblastic progeny, secrete dentin matrix, and differentiate into other cell lineages (6). In a seminal work, Young et al (7) have shown that cells originating from porcine third molar tooth buds seeded in a polymeric scaffold give rise to complex tooth structures after a period of 20 to 25 weeks. Notably, the recombination of bone marrow–derived stem cells and oral epithelium generated complex tooth-like structures 12 days after implantation in the renal capsule (8). We have shown that stem cells from exfoliated deciduous (SHEDs) teeth seeded in tooth slice/scaffolds and transplanted subcutaneously in immunodeficient mice generate a dental pulp-like tissue with morphological characteristics similar to those found in normal pulp (9).

The scaffold provides the three-dimensional substrate for developing cells and the template for tissue engineering (10). Polymers have shown good potential as scaffold material because of their design flexibility and degradability (11). It has been shown that the interconnectivity, format, and size of pores as well as the surface treatment of the scaffolds may have a significant influence on cell behavior (11–15). Synthetic polymers (eg, poly-L-lactic acid [PLLA]) are frequently used in tissue engineering, but they lack recognition signals for cells. Such signals can be found in natural polymers

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Review Article

Regenerative Endodontic Therapy: A Data Analysis of Clinical Protocols

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Abstract

Introduction: The aim of the present study was to systematically analyze the protocols that have been used in regenerative endodontic therapy and to detect any variations in clinical procedures. **Methods:** An electronic search was executed in PubMed using appropriate Medical Subject Heading terms covering the period from January 1993 to May 2014. Additional publications from hand searching and the reference section of each relevant article enriched the article list. The relevance of each article was initially evaluated by scanning all titles and corresponding abstracts. The definite inclusion of each article in the study was determined by using specific criteria applied independently by 3 reviewers. **Results:** Sixty relevant publications were finally included. The canal walls were not mechanically instrumented in 68% of the clinical articles. Sodium hypochlorite was included in 97% of the clinical studies either as the only irrigant or in combination with other irrigants. Antibiotic combination paste was used as the intracanal medicament in 80% of the clinical articles. Sodium hypochlorite, chlorhexidine, and EDTA were used in the final irrigation protocol in 75%, 4%, and 13% of the clinical studies, respectively. Neither the creation of a blood clot nor the use of platelet-rich plasma/platelet-rich fibrin was described in 13% of the clinical articles. Mineral trioxide aggregate was used as an intracanal coronal barrier in 85% of the relevant clinical studies. **Conclusions:** The variability of the clinical protocols applied during regenerative enododontic procedures is considerably high. A thorough analysis of regenerative protocols may constitute an additional source to provide useful clinical considerations for REPs. *(J Endod 2015;41:146–154)*

Key Words

Blood clot, mineral trioxide aggregate, regenerative endodontics, triple antibiotic paste

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The success of regenerative endodontic procedures (REPs) is undoubtedly related to the attainment of specific biological purposes (1). Healing of apical periodontitis, thickening and/or lengthening of root walls, and, finally, regaining a positive response to pulp sensibility tests are strongly correlated with a successful outcome of regenerative endodontic therapy (1). In cases of necrotic immature teeth with open apices, REPs promote root development and apical closure compared with calcium hydroxide apexification and the mineral trioxide aggregate (MTA) apical barrier technique (2–4).

REPs are based on 3 core principles of tissue engineering:

1. Appropriate sources of stem/progenitor cells
2. Growth factors that are capable of promoting stem cell differentiation
3. Appropriate scaffolds for the regulation of cell differentiation (5, 6)

Based on clinical studies related to REPs, the main sources of stem cells are the periapical tissues, and the main sources of growth factors are intracanal blood clot, platelet-rich plasma (PRP), and properly conditioned dentin surfaces with EDTA; in the majority of cases, scaffolds are either the intracanal blood clot or the PRP from the patient. The elimination of intracanal bacteria is required for the initiation of REPs, and prevention from reinfection of the root canal system is a prerequisite for the long-term success of the therapy (7, 8).

In the first clinical article related to REPs, the root canal walls were not instrumented mechanically in order to preserve the vitality of the stem cells of apical tissues (9). The control of root canal infection was achieved by using sodium hypochlorite (NaOCl), hydrogen peroxide (H_2O_2), and a mixture of antibiotics as intracanal medicament. Apical closure of the root and thickening of the root walls were observed in the 30-month postoperative radiography (9). Since then, a large amount of clinical studies, case series, and case reports related to regenerative endodontic therapy have been published, including a broad range of differences in treatment protocol.

Based on the best available evidence on regenerative endodontic therapy, it has not yet been totally evitable to determine a protocol that provides the most favorable outcome (10). This could be attributed to the fact that the vast majority of the clinical articles related to REPs are case series and clinical reports characterized by major differences among several pre-, intra-, and postoperative prognostic and clinical factors. (10). However, the lack of standardization of a clinical protocol for REPs may be a possible reason for withholding that treatment modality. It is imperative for clinicians and investigators to detect, via a systematic approach, the real magnitude of the variability observed in clinical protocols for regenerative endodontic therapy. Therefore, the present review was conducted with the aim of analyzing the protocols that have been used in regenerative endodontic therapy and detecting any variations in clinical procedures.

Materials and Methods

Search Methodology and Study Selection

An electronic search was conducted using the PubMed database (www.ncbi.nlm.nih.gov) covering the period from January 1993 to the second week of May 2014. The following appropriate Medical Subject Heading (MeSH) terms for the electronic search were formed by using 12 relevant guide articles: *((((("dental pulp"[MeSH Terms] OR ("dental"[All Fields] AND "pulp"[All Fields]) OR "dental pulp"[All Fields]*

34.

Drawbacks and Unfavorable Outcomes of Regenerative Endodontic Treatments of Necrotic Immature Teeth: A Literature Review and Report of a Case

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Abstract

Introduction: Endodontic treatment of immature necrotic teeth is challenging. Recently a biologically based treatment called regenerative endodontic treatment was introduced. Although regenerative endodontic treatment causes root development, there are several drawbacks and unfavorable outcomes that should be addressed. This article describes regenerative endodontic treatment of 2 maxillary central incisors with poor root development outcomes. **Methods:** A healthy 14-year-old female patient was referred. The patient had history of an impact trauma 6 years before the first visit. Clinical and radiographic examinations revealed that maxillary central incisors were immature and necrotic with symptomatic apical periodontitis. After local anesthesia, rubber dam isolation, and access cavity preparation each tooth was irrigated with 20 mL of NaOCl 5.25% and received triple antibiotic dressing (ciprofloxacin, metronidazole, minocycline) for 4 weeks. In the next visit, after eliminating antibiotic dressing, bleeding was induced inside the canals, and then the coronal thirds of the canals were sealed with mineral trioxide aggregate. A week later, both teeth were permanently restored. **Results:** In clinical and radiographic follow-ups, both teeth were functional, periapical lesions were healed, and the apices formed. However, the roots were not developed. After 6 years, because of moderate discoloration and caries, both teeth received root canal therapy and were permanently restored with casting dowel core and full crown restorations. **Conclusions:** Criteria for case selection in regenerative endodontic treatments should be determined. *(J Endod 2012;38:1428–1434)*

Key Words

Dental trauma, immature teeth, MTA, regenerative endodontics, root development, triple antibiotic paste, unfavorable outcome

Treatment of necrotic immature teeth is very challenging in endodontics. Immature roots are weak, short, and more susceptible to fracture. It is difficult to perform chemomechanical debridement and create an effective apical seal by using conventional endodontic treatment methods (1). Historically, multiple-visit apexification was the treatment of choice (2). Although this method was successful (3), it had several disadvantages including long-term treatment, increased root dentin brittleness, and increased risk of root fracture because of long-term presence of calcium hydroxide inside the root canal space (4). Apical barrier technique was introduced as a replacement for apexification with calcium hydroxide (5). In the apical barrier technique a barrier material is placed at the apex to facilitate obturation procedure. Considering its sealing ability (6), biocompatibility (7), hard-tissue induction potential (8), and the ability to set in the presence of moisture (9), mineral trioxide aggregate (MTA) is the material of choice for the apical barrier technique. Clinical studies have shown high success rates of this method (10). However, none of the aforementioned methods can promote root development.

Recently, a biologically based treatment called regenerative endodontic treatment was introduced (11). This approach is based on the presence of osteo/odonto progenitor stem cells in the apical papilla that are resistant to the infection and necrosis caused by proximity to periodontal blood supply (12). In this treatment, the ideal goal is to prepare an appropriate environment inside the root canal space (ie, absence of bacteria and necrotic pulp tissue, presence of a scaffold and a tight coronal seal) that promotes repopulation of these stem cells, regeneration of pulp tissue, and continuation of root development (13). The treatment procedure begins with chemical disinfection by copious irrigation of the root canal space with NaOCl (14), combination of NaOCl/chlorhexidine (15, 16) or NaOCl/hydrogen peroxide (17), followed by placement of an intracanal medicament at the first visit. Several medicaments like triple antibiotic mixture (metronidazole, ciprofloxacin, and minocycline) (18), calcium hydroxide (19), and formocresol (20) have been used successfully. At the next visit, which should be at least 1 week after the initial session (21) or more (14), in the absence of clinical signs of inflammation, the clinician removes the intracanal medicament and induces bleeding inside the root canal space by irritating the periradicular tissue. After clot formation, the clinician seals the root canal space by placing an MTA plug over the blood clot (18). There are several case studies that demonstrate successful clinical and radiographic outcome for this treatment approach in single-rooted (15, 22) and molar teeth (23, 24).

However, there are several drawbacks and unfavorable outcomes that are not addressed yet. The purpose of this study was to present these drawbacks and their

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35.

Direct Effect of Intracanal Medicaments on Survival of Stem Cells of the Apical Papilla

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Abstract

Introduction: Regenerative endodontic procedures are an alternative treatment for immature teeth with necrotic pulps. Typically, intracanal medicaments such as triple antibiotic paste (TAP) or double antibiotic paste (DAP) and calcium hydroxide ($Ca[OH]_2$) are used for disinfection. However, their effect on human stem cells of the apical papilla (SCAPs) is unknown. We hypothesized that intracanal medicaments at high concentrations are toxic to SCAPs. To test this hypothesis, a cell culture assay was used. **Methods:** Briefly, SCAPs were cultured and subjected to either no drug treatment or various concentrations including TAP, DAP, modified TAP (ciprofloxacin, metronidazole and cefaclor), Augmentin (Champs Pharmacy, San Antonio, TX), or $Ca(OH)_2$. Viable stem cells counts were obtained using an automated method of detecting trypan blue dye at 3 days after treatment. **Results:** All 4 antibiotics significantly reduced SCAP survival in a concentration-dependent fashion. Interestingly, $Ca(OH)_2$ was conducive with SCAP survival at all concentrations. **Conclusions:** Collectively, our data show that high concentrations of antibiotics have a detrimental effect on SCAP survival, whereas lower concentrations as well as $Ca(OH)_2$ at all tested concentrations are conducive with SCAP survival and proliferation. These studies highlight the clinically important point that intracanal medicaments must be used at concentrations that are bactericidal while having minimal effects on stem cell viability. *(J Endod 2012;38:1372–1375)*

Key Words

Apical papilla, biocompatible, calcium hydroxide, dental stem cells, double antibiotic, human stem cells of the apical papilla, regenerative, triple antibiotic

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Regenerative endodontic therapy has become a viable alternative in the treatment of immature teeth with pulpal necrosis. Since its inception, several successful case reports have been published (1–9) with substantial advancements in the understanding of the biological principles involved in the regeneration of the pulp-dentin complex, including the evaluation of stem cells, growth factors, and signaling molecules involved in the differentiation of stem cells into various cell types (10–12). Moreover, it has become evident from preclinical studies and clinical case reports that a successful regenerative procedure of a tooth with necrotic pulp requires adequate disinfection of the root canal system.

Clinical regenerative endodontic procedures depend primarily on chemical debridement of the canal space with minimal to no mechanical instrumentation (1–8). The most widely used intracanal medicament used in regenerativeendodontic/revascularization procedures is the mixture of ciprofloxacin, metronidazole, and minocycline, which is also called triple antibiotic paste (1, 13, 14). This combination has been shown to be highly efficacious against bacteria commonly found in infected root canal systems in both *in vitro* (13, 14) and *in vivo* studies (1, 3, 5, 9, 15). However, other intracanal medicaments have been used including $Ca(OH)_2$ (4); formocresol (7); the combination of metronidazole and ciprofloxacin (double antibiotic paste) (2); and the combination of metronidazole, ciprofloxacin, and cefaclor (modified triple antibiotic paste) (8). Although the combination of amoxicillin and clavulonic acid (Augmentin; Champs Pharmacy, San Antonio, TX) has not yet been used in a published regenerative endodontic case, it has been found to be effective against 100% of endodontic bacteria (16), and it may represent an important medicament alternative for patients without a history of a penicillin-like drug allergy. Collectively, the antibacterial effects of these drugs are well known; however, there is a large gap in knowledge regarding the potential toxic effect on human mesenchymal stem cells. Thus, the aim of this study was to evaluate the effect of commonly used intracanal medicaments on the survival of human stem cells of the apical papilla (SCAPs) *in vitro*.

Materials and Methods

Patient Recruitment

This study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. After verbal and written communication for informed consent, stem cells of the apical papilla were harvested from 2 extracted immature mandibular third molars (#17 and #32) from a 17-year-old female patient recruited from the clinic of the University of Texas Health Science Center at San Antonio School of Dentistry. The extracted teeth were immediately rinsed in sterile phosphate-buffered saline (PBS) and stored in a container with sterile PBS until SCAP harvesting.

Harvesting of SCAP and Cell Culture

SCAPs were obtained from the harvested apical papillae by enzymatic digestion as described previously (17). Cells in suspension were plated in 10-cm diameter poly-D lysine-treated culture plates (BD Biosciences, Bedford, MA). The cells formed single colonies and exhibited typical fibroblast-like morphology. Cells were allowed to expand in culture to 70% to 80% confluency followed by treatment with 0.05% trypsin (Gibco, Carlsbad, CA) and passing the culture to subsequent culture plates. SCAPs between the fifth and eighth passages were used in this study.

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The effect of calcium hydroxide on solubilisation of bio-active dentine matrix components

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Abstract

Calcium hydroxide ($Ca(OH)_2$) has been used extensively to induce dentine regeneration through formation of dentine bridges at sites of pulp exposure after dental tissue injury, however, the biological processes underpinning these events are unclear. We hypothesise that growth factors and other bio-active molecules, sequestered within dentine matrix, may be released by the action of $Ca(OH)_2$ and signal gene expression in pulp cells, which mediates the changes in cell behaviour observed during regeneration. Powdered sound, human dentine samples were extracted with either 0.02 M $Ca(OH)_2$, pH 11.7 or 10% EDTA, pH 7.2 (a control known extractant of bio-active and other ECM molecules from dentine) over a 14-day period. Extracts were compared for non-collagenous protein (NCP) and glycosaminoglycan (GAG) content using dye binding assays and protein compositions were analysed by 1D-polyacrylamide gel electrophoresis (1D-PAGE) and TGF-β1 ELISA. The effects of extracts on TGF-β1, Collagen-1α and Nestin gene expression were analysed using semi-quantitative RT-PCR in the dental MDPC-23, OD-21 and fibroblastic Swiss 3T3 cell lines following 24 h of exposure. $Ca(OH)_2$ solubilised NCPs and GAGs from the dentine ECM, although with a lower yield than the EDTA solution and with different kinetics. 1D-PAGE analysis demonstrated some differences in profiles for proteins solubilised from dentine by $Ca(OH)_2$ and EDTA. Both solutions released TGF-β1 from the dentine with higher concentrations present in the EDTA (1.395 ± 0.036 ng/mg) versus the $Ca(OH)_2$ (0.364 ± 0.012 ng/mg) extract. Notably, both extracts induced similar gene expression profiles in all cell lines. These data provide a rational explanation for the action of $Ca(OH)_2$ during pulp capping in which the cellular activities involved in dentine bridge formation may be mediated through release of growth factors and other bio-active molecules from the dentine by $Ca(OH)_2$.

Keywords: Calcium hydroxide; Dentine; Pulp; Regeneration; Growth factors; TGF

1. Introduction

Calcium hydroxide ($Ca(OH)_2$) is one of the most successful pulp capping materials for inducing repair of the dentine–pulp complex (dentine bridge formation) following injury [1], and is still the gold standard treatment against which new materials are tested [2]. Despite its wide use in clinical practice for over 60 years [3], the mechanism of action of $Ca(OH)_2$ in the induction of reparative dentinogenesis and dentine bridge formation is still unclear. Successful treatment with $Ca(OH)_2$ has been attributed to the release of hydroxyl ions [1] raising the pH of the exposed pulpal tissue. Supplementary to its bactericidal effects, this increase in pH induces local necrosis of the exposed pulpal tissue [4]. Thus, the effect of $Ca(OH)_2$ has been regarded as the result of chemical injury, which causes irritation of the vital pulpal tissue beneath the necrotic layer, stimulating the repair processes in the pulp in some unknown manner. In addition, it has also been postulated that the alkaline pH maintained at the injured/treated region creates favourable conditions for dentine formation [5] and that localised elevated calcium ion concentrations increase expression of mineralisation promoting genes (osteopontin and bone morphogenic protein-2) in pulp cells [6]. Studies by Davidson and Guo

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37.

International Endodontic Journal

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The effect of medicaments used in endodontic regeneration on root fracture and microhardness of radicular dentine

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Abstract

Yassen GH, Vail MM, Chu TG, Platt JA. The effect of medicaments used in endodontic regeneration on root fracture and microhardness of radicular dentine. *International Endodontic Journal*, **46**, 688–695, 2013.

Aim To investigate the effect of medicaments used in endodontic regeneration on root fracture resistance and microhardness of radicular dentine.

Methodology The root canals of mandibular premolars ($n = 180$) were instrumented and randomized into three treatment groups and an untreated control group. Each treatment group received either triple antibiotic paste (TAP), double antibiotic paste (DAP) or calcium hydroxide paste [$Ca(OH)_2$] intracanal medicament. Teeth were kept in saline for 1 week, 1 month or 3 months. After each time-point, 15 teeth were randomly selected from each group and two root cylinders were obtained from each tooth. One cylinder was subjected to a fracture resistance test, and the other cylinder was used for a microhardness test. Two-way ANOVA and Tukey's pairwise comparisons were used for statistical analysis.

Results For the microhardness test, the two-way interaction between group and time was significant ($P < 0.001$). The intracanal application of TAP and DAP caused significant and continuous decrease in root dentine microhardness after one ($P < 0.05$) and 3 months ($P < 0.001$), respectively. The three-month intracanal application of $Ca(OH)_2$ significantly increased the microhardness of root dentine ($P < 0.05$). The time factor had a significant effect on fracture resistance ($P < 0.001$). The three intracanal medicaments caused significant decreases in fracture resistance ranging between 19% and 30% after 3-month application compared to 1-week application.

Conclusion In this laboratory study, the 3-month application of triple antibiotic paste, double antibiotic paste or calcium hydroxide paste medicaments significantly reduced the root fracture resistance of extracted teeth compared to a 1-week application.

Keywords: calcium hydroxide, double antibiotic paste, fracture resistance, microhardness, regeneration, triple antibiotic paste..

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Introduction

Endodontic regeneration has been introduced as a treatment option for immature permanent teeth with necrotic pulps (Thibodeau 2009, Lenzi & Trope 2012). The technique has been suggested to reduce the risk of fracture associated with traditional apexification procedures by inducing a functional dentinopulpal complex that may lead to increased length and width of fragile immature roots (Jung *et al.* 2008). One of the essential elements for a successful endodontic regeneration protocol is the creation of a bacteria-free biological environment inside the root canal space through the use of intracanal antibacterial

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38.

Effects of Growth Factors on Dental Stem/Progenitor Cells

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KEYWORDS

• Growth factors • Dentin • Dental pulp • Dental pulp cells • Regeneration • Repair

KEY POINTS

- The goal of regenerative endodontics is to regain the vitality and functions of dental pulp–dentin complex. Dental pulp is the only vascularized tissue in mature, functional teeth in humans, and maintains homeostasis of the dentin.
- Current root canal therapy ends up with a devitalized tooth, therefore predisposing endodontically treated teeth to reinfections and fractures. Recent work showing regeneration of dental pulp-dentin–like tissues by cell homing that is orchestrated by growth factor delivery, without cell transplantation, provides one of the tangible pathways toward clinical translation.
- Growth factors regulate either transplanted cells or endogenously homed cells in dental pulp–dentin regeneration. Further understanding of the actions of growth factors is pivotal for dental pulp–dentin regeneration.

INTRODUCTION

Regenerative endodontics aims to restore the vitality and functions of the pulp–dentin complex that has been lost to trauma or infections (**Table 1**). Several recent reports have shown that dental pulp–like tissues can regenerate in vivo following the delivery of dental or nondental stem/progenitor cells.[1–3] An alternative approach is to orchestrate dental pulp–dentin regeneration by the homing of host endogenous cells relies on growth factor delivery, instead of cell delivery.[4] Regardless of cell transplantation or

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Conflict of interest: Columbia University is the owner of patents for several regenerative endodontic agents and methods on behalf of Dr Jeremy Mao's laboratory.
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39.

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Regeneration of Dental-Pulp-like Tissue by Chemotaxis-Induced Cell Homing

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Tooth infections or injuries involving dental pulp are treated routinely by root canal therapy. Endodontically treated teeth are devitalized, susceptible to re-infections, fractures, and subsequent tooth loss. Here, we report regeneration of dental-pulp-like tissue by cell homing and without cell transplantation. Upon *in vivo* implantation of endodontically treated real-size, native human teeth in mouse dorsum for the tested 3 weeks, delivery of basic fibroblast growth factor and/or vascular endothelial growth factor (bFGF and/or VEGF) yielded re-cellularized and revascularized connective tissue that integrated to native dentinal wall in root canals. Further, combined delivery of bFGF, VEGF, or platelet-derived growth factor (PDGF) with a basal set of nerve growth factor (NGF) and bone morphogenetic protein-7 (BMP7) generated cellularized and vascularized tissues positive of VEGF antibody staining and apparent neo-dentin formation over the surface of native dentinal wall in some, but not all, endodontically treated teeth. Newly formed dental pulp tissue appeared dense with disconnected cells surrounded by extracellular matrix. Erythrocyte-filled blood vessels were present with endothelial-like cell lining. Reconstructed, multiple microscopic images showed complete fill of dental-pulp-like tissue in the entire root canal from root apex to pulp chamber with tissue integration to dentinal wall upon delivery of bFGF, VEGF, or PDGF with a basal set of NGF and BMP7. Quantitative ELISA showed that combinatory delivery of bFGF, VEGF, or PDGF with basal NGF and BMP7 elaborated von Willerbrand factor, dentin sialoprotein, and NGF. These findings represent the first demonstration of regenerated dental-pulp-like tissue in endodontically treated root canals of real-size, native human teeth. The present chemotaxis-based approach has potent cell homing effects for re-cellularization and revascularization in endodontically treated root canals *in vivo*, although in an ectopic model. Regeneration of dental pulp by cell homing, rather than cell delivery, may accelerate clinical translation.

Introduction

Dental pulp is the only vascularized dental tissue encapsulated in highly mineralized structures, including dentin, enamel, and cementum, and maintains homeostasis of the tooth as a viable organ.[1] The overall health of the tooth is compromised upon dental pulp trauma or infections, frequently manifested as pulpitis.[2] A typical endodontic treatment or root canal therapy for irreversible pulpitis is pulpectomy, involving pulp extirpation followed by root canal enlargement and obturation with gutta percha, a bioinert thermoplastic material.[2] Despite reported clinical success, endodontically treated teeth become de-vitalized and brittle, susceptible to postoperative fracture and other complications, including re-infections due to coronal leakage or microleakage.[2,3] A substantial amount of tooth structures, including enamel and dentin, is removed during endodontic treatment, potentially leading to posttreatment tooth fracture and trauma.[2–4] Endodontically treated teeth have lost pulpal sensation, and are deprived of the ability to detect secondary infections.[2,3,5] The complications of current endodontic treatment are inevitable because of pulp devitalization or the loss of the tooth's innate homeostasis and defense mechanisms.

Existing effort in dental pulp regeneration has focused on cell transplantation (reviews[6–8]). Several reports have documented regeneration of dental-pulp-like tissue *in vitro* or ectopically by transplantation of dental pulp stem cells.[9–11] Deciduous and adult dental pulp stem cells seeded in a self-assembling peptide-amphiphile hydrogel showed distinctive behavior: greater proliferative rate for deciduous cells but greater osteogenic differentiation potential for adult cells.[7,8] Delivery of collagen scaffolds with dental pulp stem cells and dentin matrix protein-1 in dentin slices in mice led to

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40.

Revascularization Technique for the Treatment of External Inflammatory Root Resorption: A Report of 3 Cases

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Abstract

The current external inflammatory root resorption treatment protocol, which uses calcium hydroxide dressing, usually comprises multiple and long-term applications. In addition to the need for multiple appointments for calcium hydroxide replacement, the long-term maintenance of this compound in the root canal weakens dental structures. A modification of this therapy would be advisable. In this clinical investigation, 3 patients with external inflammatory root resorption were submitted to revascularization therapy protocol usually used in teeth with necrotic pulp and open apices. The teeth were treated with revascularization therapy protocol, which consisted of disinfecting the root canal system with triantibiotic paste, filling it with blood clot, and sealing of the root canal with mineral trioxide aggregate and bonded resin restoration. During the follow-up, the pathologic process was arrested with tissue repair in pre-existing radiolucent areas. Reduced mobility was observed in the treated teeth. The 3 cases were followed up for 30, 18, and 15 months, respectively. All teeth remained asymptomatic and retained function and physiological mobility. The therapy used in the revascularization procedure was efficient in the treatment of external inflammatory root resorption, reducing the number of appointments and increasing patient compliance. *(J Endod 2015;41:1560–1564)*

Key Words

Calcium hydroxide, revascularization, root resorption, trauma, triantibiotic paste

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External inflammatory root resorption (EIRR) represents a challenge in endodontic practice. EIRR commonly occurs after dental trauma that results in periodontal ligament injury, pulp necrosis, and subsequent infection. EIRR develops from an inflammatory process established along the root periodontium. It progresses rapidly and can lead to tooth loss within a few months (1).

Treatment of EIRR is based on disinfecting the root canal system (RCS) by chemomechanical procedures and then filling it with calcium hydroxide (2–4). Depending on the resorption pattern, calcium hydroxide may remain in the RCS for several months (2). In most cases, dental trauma occurs in young patients whose teeth are still not fully formed and have thin dentinal walls and open apices. Over time, the calcium hydroxide dressing in the RCS weakens the already fragile tooth structure, increasing the likelihood of fractures with consequent tooth loss (5, 6).

Revascularization therapy has proven to be suitable for the treatment of the RCS of teeth with pulp necrosis and open apices (7–10). The triantibiotic paste used in this therapy has proven to be effective in RCS disinfection (11–14). After disinfection according to the revascularization therapy protocol, blood clot formation is induced, and the canal is subsequently sealed, which provides an intracanal environment favorable to tissue repair (10).

Because of the aforementioned aspects, the modification of EIRR therapy is advisable, along with the replacement of calcium hydroxide as the antimicrobial agent used (5, 6). Thus, this study aims at evaluating the use of triantibiotic paste and the revascularization therapy protocol for EIRR treatment.

Case Reports

This study was approved by the CEP-UERJ research ethics committee under protocol no. 2699, September 2, 2010. After an explanation regarding the procedures to be performed, the patients' guardians were asked to sign the informed consent.

Three patients were referred to our endodontic clinic after suffering dental trauma in the maxillary central incisors and presenting with pulp necrosis, open apices, periapical lesions, and EIRR. After the positive result in the first case, 2 more cases were undertaken 1 year after the first case began. The cases are presented in sequence according to the adopted procedure and evolution.

Initial Clinical Description of Patients

Case 1

A 9-year-old boy suffered trauma to his maxillary central incisors, presenting dentinal and enamel fracture in both. These teeth did not receive any treatment at the time the trauma occurred. At the first appointment approximately 12 months after the trauma, the patient complained of a sinus tract. On clinical examination, the electric and thermal pulp sensibility tests were negative in both teeth. The teeth were not tender to percussion or palpation. Pulp necrosis was verified in both teeth. The right maxillary central incisor (RMCI) had class II mobility. On radiographic examination, RMCI presented EIRR in an advanced stage and had been diagnosed with chronic apical abscess. The left maxillary central incisor (LMCI) had asymptomatic apical periodontitis and EIRR.

41.

Dental Traumatology

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Review of current concepts of revascularization/revitalization

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Key words: Immature teeth; Revascularization; Revitalization; Root canal disinfection; Tissue scaffold

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Abstract – This review focuses on the current concepts on revascularization/revitalization therapy. Revascularization/revitalization procedures performed under current protocols have reportedly achieved successful clinical and radiographical outcomes for immature permanent teeth with non-vital pulps; however, randomized prospective studies are needed to develop evidence-based methodologies for regenerative endodontic treatment.

Why is revascularization/revitalization therapy needed?

Immature permanent teeth may become non-vital, usually as a result of trauma, caries, or congenital abnormalities. Apexification treatment has been a routine procedure to treat and preserve such teeth for many decades (1–3). Apexification is the process by which a suitable environment is created within the root canal and periapical tissue to allow for the formation of a calcific barrier across the open apex. Calcium hydroxide [$Ca(OH)_2$] has been the material of choice for apexification as Frank reported its capacity to induce physiological closure of immature pulpless teeth in 1966 (1–4). However, this technique has several disadvantages, including the unpredictability of apical barrier formation and the long duration of treatment, which often requires multiple visits (4–7). Complete removal of the paste represents another challenge, and residual $Ca(OH)_2$ in the canal has been shown to interact with zinc oxide-based sealers, resulting in poor cohesion (8, 9). While $Ca(OH)_2$-releasing gutta-percha points represent an innovate solution to this problem (10), no solution has been found to address another important issue related to $Ca(OH)_2$, namely that the hygroscopic and proteolytic properties of $Ca(OH)_2$ can cause tooth brittleness as a result of prolonged exposure (11). These concerns have led to a modification of the traditional $Ca(OH)_2$-based apexification procedure to achieve immediate obturation of the canal through the introduction of an artificial barrier of mineral trioxide aggregate (MTA) (12). MTA apexification reduces treatment time and results in favorable healing of periradicular tissue (13–15). In spite of these advantages, the outcomes of MTA apexification do not vary greatly from those of $Ca(OH)_2$ apexification. Thin dentinal walls still present a clinical problem, and the high costs associated with MTA and the difficulties in handling the material to the apical 3–4 mm may restrict it widespread use (16–18).

For immature teeth with non-vital pulps, the use of revascularization/revitalization techniques to induce apexogenesis and thus tissue regeneration, rather than tissue replacement using artificial substitutes, represents a relatively new treatment modality (17, 19). Revascularization/revitalization treatment is based on the theory that in the absence of bacteria and in the presence of an appropriate three-dimensional scaffold and stem/progenitor cells inside the root canal space, and with creation of a bacteria-tight seal, tissue repair can occur as in devitalized, uninfected, avulsed, immature permanent teeth (20, 21). Hypothetically, the close proximity of apical papilla stem cells (SCAP) to the periodontal blood supply may allow them to survive apical infection to form odontoblast-like, dentin-producing cells (22). Such biologically based treatment approaches could be of particular value in treating necrotic, immature permanent teeth in terms of restoring root development, and reinforcing dentinal walls to increase the likelihood of long-term tooth retention (17, 19).

According to Murray et al. (22), revascularization/revitalization is a technically simple, inexpensive procedure that is suited to currently available instruments and medicaments. A retrospective study by Jeruphuaan et al. (23) has shown a higher survival rate with regenerative endodontic treatment when compared to both MTA and $Ca(OH)_2$ apexification. In addition, revascularization/revitalization technique has

Evaluation of Blood Clot, Platelet-rich Plasma, Platelet-rich Fibrin, and Platelet Pellet as Scaffolds in Regenerative Endodontic Treatment: A Prospective Randomized Trial

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Abstract

Introduction: Regenerative endodontic procedures (REPs) using autologous platelet concentrates as scaffolds can improve the biologic outcome of treatment. This prospective, randomized trial compared the clinical and radiographic performance of REPs using platelet-rich plasma (PRP), platelet-rich fibrin (PRF), a platelet pellet (PP), and an induced blot clot (BC). **Methods:** Sixty-seven healthy children (aged 8–11 years) with 88 immature necrotic incisors were included. After the root canal disinfection step, the teeth were randomly assigned into 1 of the following groups (n = 22/group) according to the scaffold used: PRP, PRF, PP, and BC. In the PRP, PRF, and PP groups, the platelet concentrates were introduced into the root canal without prior induction of apical bleeding. Treatment outcomes were assessed using a combined clinical and radiographic scoring system, whereas the changes in root dimensions were compared using linear measurements of root length and width with ImageJ (National Institutes of Health, Bethesda, MD) and Turboreg (Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, Switzerland) and planar measurements using the radiographic root area (RRA) and radiographic canal area (RCA) techniques. One-way analysis of variance, the Duncan multiple range test, the Kruskal-Wallis test, the Mann-Whitney U test, and chi-square dependency tests were used for statistical analysis of data (all $P = .05$). **Results:** Except for 2 teeth in the PRF and BC groups, all teeth showed similar and high success scores (periapical healing, radiographic root development, and positive response to sensitivity tests) after an average follow-up time of 28.25 ± 1.2 months. Of all teeth, 73.9% showed complete apical closure with similar closure rates among groups ($P > .05$) and a greater tendency for conical-shaped apical closure than a blunt apex. Although linear measurements indicated a similar increase in root length and width among all groups ($P > .05$), the RRA of the BC group was significantly greater than those of the PRF and PP groups, and the RCA of the BC group was significantly greater than PRP, PRF, and PP (all $P < .05$) when the follow-up time was not used as a factor. Eighty-six percent of the teeth showed a positive response to sensitivity tests with similar initial response times ($P > .05$). **Conclusions:** PRP, PRF, and PP can yield similar clinical and radiographic outcomes to BC without the need for prior apical bleeding and with significantly less tendency for root canal obliteration. RRA and RCA may reveal minor differences that cannot be determined by linear measurements. *(J Endod 2019;■:1–7)*

Key Words

Clinical outcomes, image analysis, platelet-rich fibrin, platelet-rich plasma, regenerative endodontics, revascularization, revitalization

Significance

PRP, PRF, and PP can be useful alternatives to BC both in the presence and absence of apical bleeding.

In the last decade, the endodontic profession has witnessed a remarkable shift toward biologically based treatment strategies that aim to promote revitalization and root development in necrotic immature permanent teeth (1). Although current regenerative endodontic procedures (REPs) cannot restore the physiological structure and function, they can promote the formation of new vascularized tissue in the canal space, a guided endodontic repair process that results in continued root development, and complete resolution of apical periodontitis (2). With such clinical and radiographic outcomes, REPs may be considered first treatment options for immature teeth with pulpal necrosis (3).

Current REPs commonly use provoked apical bleeding into the pulp space as a possible source of stem cells and also for creating a blood clot (BC) that would act as a biologic scaffold (4). However, failure to induce apical bleeding or to achieve adequate blood volume within the canal space remains a common problem (5–7). Although transferring some blood volume from other root canals may offer a practical solution in molars (5, 6), this method cannot be used in single-rooted teeth.

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43.

Histologic Observation of a Human Immature Permanent Tooth with Irreversible Pulpitis after Revascularization/Regeneration Procedure

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Abstract

Introduction: Histological studies of immature human permanent necrotic teeth with or without apical periodontitis after revascularization have not been reported. This case report describes the histological findings of tissue formed in the canal space of an immature permanent tooth #9 with irreversible pulpitis without apical periodontitis after revascularization. **Methods:** An immature human permanent tooth #9 was fractured 3.5 weeks after revascularization and could not be retained. The tooth was extracted and prepared for routine histological and immunohistochemical evaluation in order to examine the nature of tissue formed in the root canal following the revascularization procedure. **Results:** At 3.5 weeks after revascularization, more than one half of the canal was filled with loose connective tissue similar to the pulp tissue. A layer of flattened odontoblast-like cells lined along the predentin. Layers of epithelial-like cells, similar to the Hertwig's epithelial root sheath, surrounded the root apex. No hard tissue was formed in the canal. **Conclusions:** Based on the histological findings in the present case, regeneration of pulp-like tissue is possible after revascularization. In this case, both the apical papilla and the Hertwig's epithelial root sheath survived in an immature permanent tooth despite irreversible pulpitis but without apical periodontitis. *(J Endod 2012;38:1293–1297)*

Key Words

Immature permanent tooth, irreversible pulpitis, pulp-like tissue regeneration, revascularization

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Iwaya et al (1) showed that a human immature permanent tooth with necrotic pulp and apical periodontitis/abscess after a revascularization procedure could induce increased thickening of the canal walls and continued root development. Since then, many similar cases have been reported (2–5). Radiographically, in some cases, revascularized human immature permanent teeth appear to show continued development as evidenced by the deposition of hard tissue on the canal walls and continued root development. Therefore, the revascularization of human immature permanent teeth with necrotic pulp and apical periodontitis/abscess has been considered to be a regenerative process (2–6). Regeneration is defined as the replacement of damaged tissue by the same parenchymal cells (7). However, regeneration is a histologic observation and cannot be determined radiographically. The nature of the tissue formed in the canal space in human revascularized immature permanent teeth with apical periodontitis is speculative because no histologic studies are available.

Currently, the available animal studies of immature teeth with pulp necrosis and apical periodontitis after revascularization procedures show that the tissues formed in the canal space are cementoid or osteoid tissue and periodontal ligament–like tissue (8–11). However, no studies have investigated the nature of the tissue present in immature teeth with irreversible pulpitis with normal periapical tissues after a revascularization/regeneration procedure in animals or humans. The purpose of this case report is to describe the histologic observation of a human immature permanent tooth clinically diagnosed as having irreversible pulpitis with normal periapical tissues after a revascularization/regeneration procedure. To our knowledge, this is the first histologic observation of a human revascularized immature permanent tooth with irreversible pulpitis.

Materials and Methods

A 10-year-old boy was referred from the Postgraduate Pediatric Clinic at New York University College of Dentistry to the Postgraduate Endodontic Clinic for the treatment of tooth #9. The child had a traumatic injury to his maxilla, which caused an uncomplicated crown fracture of tooth #8 and a complicated crown fracture with pulp exposure of tooth #9. The coronal one half of the crown of tooth #9 was horizontally fractured. According to the patient's mother, the general dentist removed part of the pulp, placed medication inside tooth #9, and advised the mother to bring the child to New York University College of Dentistry for further treatment. The patient and his mother visited the Postgraduate Pediatric Clinic approximately 1 month after treatment by the general dentist.

Pulp sensibility tests with Endo-Ice (Coltene/Whaledent Inc, Cuyahoga, OH), heated gutta-percha, and electric current with the Vitality Scanner (SybronEndo, Glendora, CA) of teeth #8, #9, and #10 were conducted in the Postgraduate Endodontic Clinic. Teeth #8 and #10 responded to heated gutta-percha, Endo-Ice, and electric current within normal limits and were not sensitive to palpation or percussion. The crown of tooth #9 had an Intermediate Restorative Material (Dentsply Internation, Milford, DE) restoration. It was asymptomatic and responded erratically to pulp sensibility tests because the patient was apprehensive and perhaps also because of the attempt at pulp therapy performed by the general dentist. The tooth had a large canal

Histologic Findings of a Human Immature Revascularized/Regenerated Tooth with Symptomatic Irreversible Pulpitis

Chufang Peng, DDS, Yuming Zhao, PhD, DDS, Wenjun Wang, PhD, Yuan Yang, DDDS, Man Qin, PhD, DDS, and Lihong Ge, PhD, DDS

Abstract

Introduction: Pulp revascularization/regeneration in immature permanent teeth with necrotic pulp and/or apical periodontitis is an effective approach for inducing root maturation. Previous histologic studies showed cementoid/osteoid tissue and/or periodontal ligament–like tissue formed within the root canals. This case report describes the histologic findings of a human symptomatic irreversible pulpitis immature permanent tooth with most of the pulp removed after a revascularization/regeneration procedure. **Methods:** A human immature permanent mandibular premolar (tooth #29) was diagnosed as symptomatic irreversible pulpitis with symptomatic apical periodontitis at the emergency department. Most of the pulp was removed. The tooth was treated with revascularization/regeneration. **Results:** At the 12-month recall, the radiographic examination revealed thickening of the root canal wall, narrowing of the root apex, and lengthening of the root. The tooth was extracted at 12 months for orthodontic treatment. The specimens were processed for histologic examination. Histologically, the apical third of the root canal was filled with newly formed dentinlike and pulplike tissue. There was a layer of flattened odontoblastlike cells lining the dentinal wall. In the midportion of the root canal, the newly formed dentinlike tissue gradually changed to cementumlike tissue. In the upper third of the root canal, there was a presence of cementocytelike cells housed in the lacunae of cementumlike tissue around the loose connective tissue. **Conclusions:** In the present case, regeneration of the pulplike tissue and the periodontium existed after a revascularization/regeneration procedure in an immature permanent tooth clinically diagnosed as symptomatic irreversible pulpitis. *(J Endod 2017;■:1–5)*

Key Words

Immature permanent teeth, irreversible pulpitis, regeneration, revascularization

Significance

This case report presents histologic findings of an immature permanent tooth with irreversible pulpitis after pulp revascularization, which is valuable to help clinicians understand the results of pulp revascularization and optimize the revascularization procedure accordingly.

The management of immature permanent teeth with necrotic pulp is a challenging task for dental professionals. The young pulpless tooth with an open apex frequently has thin, fragile walls, which make it difficult to debride adequately and obtain the necessary apical seal using conventional endodontic treatment methods. Calcium hydroxide–based apexification and mineral trioxide aggregate apexification are usually used for these cases. However, neither treatment can solve the problem of the cessation of root development and the subsequent thin and fragile root canal wall (1, 2).

A novel treatment of revascularization/regeneration of immature nonvital teeth was introduced by Iwaya et al (3) and Banchs and Trope (4). They showed that a human necrotic immature permanent tooth after a revascularization/regeneration procedure could achieve increased thickening of the canal walls and continued root development. Since then, many successful cases have been reported (5–8). Radiographically, revascularized human immature permanent teeth had apical closure, root length increasing, and canal wall thickening and gave the impression that a normal, functional pulp had regenerated. However, animal histologic studies of revascularized teeth showed that the new tissue growing in the pulp space was cementoid/osteoid tissue and/or periodontal ligament–like tissue (9–14) regardless of the presence or absence of apical lesion, with or without the introduction of stem cells and scaffold. Almost all human studies of immature teeth with pulp necrosis and apical periodontitis after a revascularization/regeneration procedure showed that the tissues formed in the canal space were similar to the animal studies (15–18), except Torabinejad and Faras (19) in which pulplike tissue was observed in a revitalized tooth.

In an irreversible pulpitis tooth with normal periapical tissues, which was treated with revascularization/regeneration after a deep pulpotomy or a partial pulpectomy, the canal was filled with loose connective tissue similar to the pulp tissue (20). Based on the histologic findings in the case, regeneration of pulplike tissue is possible after a revascularization/regeneration procedure.

This case report describes the histologic observation of a human immature permanent tooth clinically diagnosed as symptomatic irreversible pulpitis after a revascularization/regeneration procedure.

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Revascularization of an immature permanent tooth with apical periodontitis and sinus tract

Iwaya S, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol 2001; 17: 185–187. © Munksgaard, 2001.

Abstract – A necrotic immature mandibular second premolar with periapical involvement in a 13-year-old patient was treated. Instead of the standard root canal treatment protocol and apexification, antimicrobial agents were used in the canal, after which the canal was left empty. Radiographic examination showed the start of apical closure 5 months after the completion of the antimicrobial protocol. Thickening of the canal wall and complete apical closure was confirmed 30 months after the treatment, indicating the revascularization potential of a young permanent tooth pulp into a bacteria-free root canal space.

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Key words: revascularization; immature tooth; periapical involvement; root development

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In the avulsed immature tooth with an open apex, revascularization is the most desirable healing response because the tooth continues to develop to form strong root dentin. In the infected immature tooth with periapical involvement, pulp revascularization has been considered impossible due to the presence of bacteria in the root canal space and the lack of vital pulp progenitor cells necessary for the proliferation of pulpal tissue. Such a tooth receives an apexification procedure, because revascularization of the pulp chamber is not expected. Apexification induces further development of an apex to close the foramina, but does not promote the thickness of the entire canal wall dentin. A tooth with this kind of thin root dentin and large canal lumen is prone to fracture (1).

Theoretically, when an extremely large communication from the pulp space to the periapical tissues exists, as with a very young tooth, it may be possible for periapical disease to be present when the pulp is only partially necrotic and infected. Vital pulp may still be present in the most apical part of the canal. If this were the case, successful removal and disinfection of the necrotic infected coronal pulp would still leave vital pulpal cells with the potential to proliferate new pulp into the coronal pulp space. Thus obturation of the root canal space, temporarily with a medicament or with gutta-percha, would remove the possibility for revascularization to occur and thus be counterproductive.

In this case report, we treated an immature mandibular second premolar with an intraoral sinus tract. Instead of the standard root canal treatment and apexification, the root canal was not mechanically cleaned to the apex but copious irrigation and antimicrobial agents were used in the canal. Thus necrotic and infected pulp was removed coronally, leaving residual pulp tissue apically in the canal to promote revascularization. The tooth responded to the treatment as hoped and continued root development and apical closure were confirmed.

Case report

The patient was a 13-year-old female. She had a history of swelling of the right mandibular buccal vestibule for which she received an incision for drainage at a nearby dental office in the summer of 1995. Because the periapical symptoms and swelling returned after some time, she visited Tohoku Welfare Hospital for advanced treatment in January 1996. A sinus tract was found apically on the mesiobuccal aspect of the right mandibular first molar (Fig. 1). The premolars and molars were free of caries, but fracture of the central cusp of the second premolar was noted on

46.

Case Report

Pulp Revascularization of a Necrotic Infected Immature Permanent Tooth: Case Report and Review of the Literature

Blayne Thibodeau, DMD, MS[1] • Martin Trope DMD[2]

Abstract: *The purpose of this report was to present the case of a patient wherein revascularization of the necrotic infected pulp space of an immature permanent maxillary central incisor tooth was induced in vivo by stimulation of a blood clot from the periapical tissues into the canal space. This was achieved after disinfection of the canal space with a topical antibiotic paste followed by a blood clot scaffold induced from the periapical tissues. This treatment approach offers clinicians great potential to avoid the need for traditional apexification with calcium hydroxide or the need to achieve an artificial apical barrier with mineral trioxide aggregate. Furthermore, this treatment approach can help rescue infected immature teeth by physiologically strengthening the root walls. (Pediatr Dent 2007;29:47-50)*

KEYWORDS: PULP REVASCULARIZATION, PULP THERAPY, APEXIFICATION

Pulpal necrosis of an immature tooth secondary to caries or trauma poses many potential complications. The traditional techniques of chemomechanical instrumentation and disinfection of the root canal system used in mature teeth are limited by the anatomy of the immature tooth. It is difficult to instrument immature canal spaces with conventional endodontic techniques. The open apex is difficult or impossible to seal with conventional root filling methods because of the absence of an apical stop. Furthermore, the arrested development of the dentinal walls at the time of pulp necrosis leaves a weak tooth with thin dentinal walls that are susceptible to fracture. Root reinforcement techniques for immature, thin-walled teeth are available. They can make root canal re-treatment difficult or even impossible, however, if the need arises in the future.[1] Finally, the traditional treatment of immature teeth with necrotic pulps and apical periodontitis involves long-term application of $Ca(OH)_2$ (calcium hydroxide) to induce apexification. Andreasen and others have shown that long-term $Ca(OH)_2$ treatment may leave the tooth even weaker and more susceptible to fracture, emphasizing the need for an improved treatment technique.[2]

Studies on traumatic injuries to immature teeth show promise for revascularization of the root canal system.[3,4] Under such a scenario, the uninfected necrotic pulp may act as a scaffold for the ingrowth of new tissue. The key factor for the success of this process in necrotic, infected, immature teeth is disinfection of the root canal system, since it is theorized to be essential to create an environment conducive to revascularization of the root canal system.[5,6]

Various combinations of topical antibiotics have the ability to disinfect carious dentin and necrotic, infected root canals. One combination that is effective against the bacteria commonly found in infected root canals is the use of: (1) ciprofloxacin; (2) metronidazole; and (3) cefaclor.[7] By application of a treatment protocol using this combination, it is expected that necrotic infected immature teeth can be rendered to the same starting point as avulsed immature teeth with necrotic but uninfected pulps (ie, a severed vascular supply secondary to avulsion). If this approach is successful, revascularization of the pulp space may be a predictable outcome in disinfected, necrotic, immature teeth.

A bacteria-free canal is a prerequisite for tissue regeneration, but tissue will not grow into an empty space.[8,9] Rather, a scaffold is essential to aid the ingrowth of new tissue into the canal space. Induction of a blood clot, with its constituent growth and differentiation factors[10,11] from the periapical tissues, may act as a scaffold for the ingrowth of new tissue in the disinfected necrotic immature tooth—not unlike uninfected necrotic pulp does in an avulsion scenario. The blood clot consists of cross-linked fibrin. It serves as a pathway for the migration of cells,[12,13] including macrophages and fibro-

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47.

Regenerative Treatment of an Immature, Traumatized Tooth With Apical Periodontitis: Report of a Case

Elisabetta Cotti, DDS, MS, Manuela Mereu, DDS, and Daniela Lusso, DDS

Abstract

This case report describes the treatment of a necrotic immature permanent central incisor with complete crown fracture, suspected root fracture, and sinus tract, which was not treated with conventional apexification techniques. Instead, a regenerative approach based on the trauma literature's methods for revascularization was provided. The root canal was gently debrided of necrotic tissue with a sharp spoon excavator and irrigated for only one third of its length with NaOCl and then medicated with calcium hydroxide. After 15 days the sinus tract had healed, and the tooth was asymptomatic. The tooth was accessed, calcium hydroxide was removed, bleeding was stimulated to form an intracanal blood clot, and mineral trioxide aggregate was placed coronally to the blood clot. After 8 months, a coronal calcified barrier was radiographically evident and accompanied with progressive thickening of the root wall and apical closure. Two and a half years after treatment was initiated, the tooth remained asymptomatic, and the sinus tract had not reappeared. The progressive increase in the thickness of the dentinal walls and subsequent apical development suggest that appropriate biologic responses can occur with this type of treatment of the necrotic immature permanent tooth with sinus tract. *(J Endod 2008;34:611–616)*

Key Words

Apical periodontitis, open apex, regeneration, revascularization

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The traumatic injury of an immature permanent tooth can lead to the loss of pulp vitality and arrested root development. The consequences of interrupted development include a poor crown-root ratio, a root with very thin walls, an increased risk of fracture, and an apex that is open. The traditional endodontic management of such cases typically includes debriding the root canal, disinfecting the space, and final obturation of the system preceded either by an apexification procedure or by developing an apical barrier by using materials such as mineral trioxide aggregate (MTA) (1–4).

The apexification clinical procedure, which consists of applying calcium hydroxide as an intracanal medication to induce an apical closure over time, has a certain predictability of success (1–3). Its disadvantages are the necessity of multiple visits during a relatively long period of time (an average of 12 months) and the fact that there is no expectation that the root canal walls will be strengthened (3, 5).

An alternative to traditional apexification is to place an artificial barrier at the apex to prevent the extrusion of filling materials during obturation. The material of choice is MTA for its sealing ability and its biocompatibility (4). This latest technique is convenient because it is faster than the traditional apexification. The case can be finalized within 2 appointments, and a hard tissue barrier eventually forms against the MTA (4). However, even this alternative approach has the same disadvantage of a tooth with thin dentinal walls and no further root development.

The ideal treatment to obtain further root development and thickening of dentinal walls in an immature tooth with apical periodontitis would be to stimulate the regeneration of a functional pulp-dentin complex (6–8). This outcome has been observed after reimplantation in avulsed immature permanent teeth (9). It has been proposed that reimplantation of the tooth with an open apex permits coronal proliferation of tissue, leading to replacement of the necrotized pulp and subsequent continued development of the root (9). Although the histologic identity of this pulp-like tissue is generally unknown, radiographic presentation often includes progressive thickening of the dentinal walls and apical closure. Some reports, mostly published in recent years, have shown that even the immature permanent tooth with nonvital pulp and apical periodontitis can undergo pulp regeneration or revascularization (6–8). Because the term *regeneration* is based on clinical and radiographic outcomes and not histologic or biochemically based assessments, one can only make a clinically functional interpretation of the healing process. It is not known whether a complete pulp-dentin complex has been regenerated. However, once the regeneration protocol is completed, these teeth can continue to develop, with the radiographic presentation of full tooth development and the clinical presentation of an asymptomatic functional tooth.

The typical revascularization protocol advocates that the immature tooth, diagnosed with apical periodontitis (7), should be accessed and irrigated with either 5% NaOCl + 3% H_2O_2 (7) or 5.25% NaOCl (8) and PeridexTM (Procter & Gamble, Cincinnati, OH) (6). An antimicrobial agent (either an antibiotic such as metronidazole + ciprofloxacin (7) or ciprofloxacin+ metronidazole+ minocycline (6) or Ca (OH)2 (8)) should be then applied into the root canal system, and the access cavity is sealed. After an average of 3 weeks, in the absence of symptoms, the tooth is re-entered, the tissue is irritated until bleeding is started and a blood clot produced, and then MTA is placed over the blood clot (6), and the access is sealed. Within the next 2 years a gradual increase in root development can be observed (6–8).

In the present case report, we describe the use of this protocol to stimulate the continued root development in a case of trauma-induced necrosis and sinus tract of an immature permanent central incisor.

48.

Biologically Based Treatment of Immature Permanent Teeth with Pulpal Necrosis: A Case Series

Il-Young Jung, DDS, MS, Seung-Jong Lee, DDS, MS,* and Kenneth M. Hargreaves, DDS, PhD†*

Abstract

This case series reports the outcomes of 8 patients (ages 9–14 years) who presented with 9 immature permanent teeth with pulpal necrosis and apical periodontitis. During treatment, 5 of the teeth were found to have at least some residual vital tissue remaining in the root canal systems. After NaOCl irrigation and medication with ciprofloxacin, metronidazole, and minocycline, these teeth were sealed with mineral trioxide aggregate and restored. The other group of 4 teeth had no evidence of any residual vital pulp tissue. This second group of teeth was treated with NaOCl irrigation and medicated with ciprofloxacin, metronidazole, and minocycline followed by a revascularization procedure adopted from the trauma literature (bleeding evoked to form an intracanal blood clot). In both groups of patients, there was evidence of satisfactory postoperative clinical outcomes (1–5 years); the patients were asymptomatic, no sinus tracts were evident, apical periodontitis was resolved, and there was radiographic evidence of continuing thickness of dentinal walls, apical closure, or increased root length. *(J Endod 2008;34:876–887)*

Key Words

Endodontics, immature permanent tooth, open apex, regenerative, revascularization, stem cell

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Although contemporary nonsurgical endodontic procedures confer high degrees of clinical success (1, 2), the root canal system is obturated with synthetic materials, preventing any of the advantages that might ensue by regeneration of a functional pulp-dentin complex (3). This is a particular problem when treating the necrotic but immature permanent tooth, where conventional treatment often leads to resolution of apical periodontitis, but the tooth remains susceptible to fracture (4) as a result of interruption of apical and dentinal wall development. Thus, one alternative approach would be to develop and validate biologically based endodontic procedures designed to restore a functional pulp-dentin complex.

For more than 50 years, clinicians have evaluated biologically based methods to restore a functional pulp-dentin complex in teeth with necrotic root canal systems caused primarily by trauma or caries. Although case series from the 1960s–1970s in general were not successful in producing this outcome (5, 6), it should be appreciated that they were performed without contemporary instruments or materials and without insight generated from the trauma or tissue engineering fields (7). More recent case reports, published during the last 15 years, have demonstrated that it is possible in humans to restore a functional pulp-dentin complex in the necrotic immature permanent tooth (8–13). Human histologic studies have not yet been reported, so it is not known whether these treatments truly recapitulate the normal pulp-dentin complex. However, these case studies provide some measure of achieving satisfactory functional outcomes, because postoperative recalls indicate that the patient is asymptomatic, no sinus tracts are present, apical periodontitis is resolved, and there is radiographic evidence of continuing thickness of dentinal walls, apical closure, or development of root length.

Although case series do not provide definitive evidence to support a given treatment modality, they do have the advantage of being conducted in actual patients and thus provide greater insight than preclinical studies. Moreover, the results from case series can be used to identify potentially important parameters that can guide the design of future prospective clinical trials. For example, in nearly all published case series on pulpal regeneration, an effort was made to evoke an intracanal blood clot to trigger tissue ingrowth. In this case series, we report conditions in which it was not necessary to evoke intracanal bleeding to have continued root development.

Pulp Regeneration without Formation of a Blood Clot

Case 1

A 10-year-old girl was referred to the Department of Conservative Dentistry of the Dental Hospital of Yonsei University by an oral and maxillofacial surgeon for evaluation on the right second mandibular premolar (tooth #29). The girl had a history of swelling of the right mandibular buccal vestibule, for which she received an incision for drainage procedure at the Department of Oral and Maxillofacial Surgery 2 months earlier. On clinical examination, the patient was slightly symptomatic to percussion, and a sinus tract was present that traced to the apex of tooth #29. The first and second premolars were free of caries, but a fracture of an occlusal tubercle of tooth #29 was noted on visual inspection. Periodontal probings were within normal limits for all teeth in the lower right region. Diagnostic testing was inconclusive on cold and electric pulp testing, but the patient reported sensitivity to percussion or palpation. Periradicular radiographic examination revealed that tooth #29 had an incompletely developed apex and a periradicular radiolucency (Fig. 1*A*). The diagnosis of pulp necrosis and chronic apical abscess with a sinus tract was made for tooth #29.

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Conference Paper

Case Report: Pulp Revascularization of a Necrotic, Infected, Immature, Permanent Tooth

Blayne Thibodeau, DMD, MS

Abstract: *The purpose of this report is to present the case of a patient wherein revascularization of the necrotic infected pulp space of an immature permanent maxillary central incisor tooth was induced in vivo by stimulation of a blood clot from the periapical tissues into the canal space. This was achieved after disinfecting the canal space with a topical antibiotic paste followed by inducing a blood clot scaffold from the periapical tissues. This treatment approach offers great potential to avoid the need for traditional apexification with calcium hydroxide or the need to achieve an artificial apical barrier with mineral trioxide aggregate. Furthermore, this treatment approach can help rescue infected immature teeth by physiologically strengthening the root walls. (Pediatr Dent 2009;31:145-8)*

KEYWORDS: PULP REVASCULARIZATION, PULP THERAPY, APEXIFICATION

Pulpal necrosis of an immature permanent tooth secondary to caries or trauma poses many potential complications. The traditional techniques of chemomechanical instrumentation and disinfection of the root canal system used in mature teeth are limited by the immature tooth's anatomy. It is difficult to instrument immature canal spaces with conventional endodontic techniques. The open apex is difficult or impossible to seal with conventional root filling methods because of the absence of an apical stop. Furthermore, the arrested development of the dentinal walls at the time of pulp necrosis leaves a weak tooth with thin dentinal walls that are susceptible to fracture.

Root reinforcement techniques for immature, thin-walled teeth are available; however, they can make root canal retreatment difficult or even impossible if the need arises in the future.[1] Finally, the traditional treatment of immature teeth with necrotic pulps and apical periodontitis involves long-term application of calcium hydroxide ($Ca[OH]_2$) to induce apexification. Andreasen and others have shown that long-term $Ca(OH)_2$ treatment may leave the tooth even weaker and more susceptible to fracture, thus emphasizing the need for an improved treatment technique.[2]

Studies on traumatic injuries to immature teeth show promise for revascularization of the root canal system.[3,4] Under this scenario, the uninfected necrotic pulp may act as a scaffold for the in-growth of new tissue. The key factor for the success of this process in necrotic, infected, immature teeth is disinfection of the root canal system, since it is theorized to be essential to create an environment conducive to revascularization of the root canal system.[5,6]

Various combinations of topical antibiotics have the ability to disinfect carious dentin and necrotic, infected root canals. One combination that is effective against the bacteria commonly found in infected root canals is the use of ciprofloxacin, metronidazole, and cefaclor.[7] Using this combination, it is expected that necrotic, infected, immature teeth can be returned to the same starting point as avulsed immature teeth with necrotic but uninfected pulps (ie, a severed vascular supply secondary to avulsion). If this treatment approach were successful, revascularization of the pulp space may be a predictable outcome in disinfected, necrotic, immature teeth.

A bacteria-free canal is a prerequisite for tissue regeneration, but tissue will not grow into an empty space.[8,9] Rather, a scaffold is essential to aid the in-growth of new tissue into the canal space. Induction of a blood clot, with its constituent growth and differentiation factors[10,11] from the periapical tissues, may act as a scaffold for the in-growth of new tissue in the disinfected necrotic immature tooth. This is similar to uninfected necrotic pulp in an avulsion scenario. The blood clot consists of cross-linked fibrin. It serves as a pathway for the migration of cells,[12,13] including macrophages and fibroblasts from the periapical area. However, a blood clot consists of more than an inactive scaffold. Its cells contain many growth and differentiation factors important in the wound healing process.[10,11,14] Human periapical granulation tissue also contains osteogenic cells.[14]

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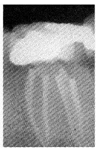

CASE REPORT

Pulp revascularization of necrotic bilateral bicuspids using a modified novel technique to eliminate potential coronal discolouration: a case report

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Abstract

Reynolds K, Johnson JD, Cohenca N. Pulp revascularization of necrotic bilateral bicuspids using a modified novel technique to eliminate potential coronal discolouration: a case report. *International Endodontic Journal*, **42**, 84–92, 2009.

Aim To present a case report in which the pulp of two bilateral mandibular premolars with dens evaginatus were revascularized using a modified novel technique to avoid undesired crown discolouration.

Summary Recently, regeneration of necrotic pulps has become an alternative conservative treatment option for young permanent teeth with immature roots and is a subject of great interest in the field of endodontics. This novel procedure exploits the full potential of the pulp for dentine deposition and produces a stronger mature root that is better able to withstand the forces than can result in fracture. However, the current protocol has potential clinical and biological complications. Amongst them, crown discolouration, development of resistant bacterial strains and allergic reaction to the intracanal medication. In the case presented, a modified technique to avoid undesired crown discolouration was applied sealing the dentinal tubules of the chamber, thus avoiding any contact between the tri-antibiotic paste and the dentinal walls.

Key learning points

• Sealing the dentinal tubules of the chamber prevents the undesirable crown discolouration produced by tri-antibiotic medication whilst maintaining the revascularization potential of the pulp.

• Further research is warranted to seek an alternative infection control protocol capable of preventing possible allergic reactions and development of resistant strains of bacteria, as well as a biological material capable of inducing angiogenesis and allow a more predictable scaffold and tissue regeneration.

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51.

Pulp Revascularization of Immature Teeth With Apical Periodontitis: A Clinical Study

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Abstract

Introduction: The purpose of this study was to examine the effect of a pulpal revascularization procedure for immature necrotic teeth with apical periodontitis. **Methods:** Twelve patients, each with an immature permanent tooth with chronic or acute apical periodontitis, were recruited. A triantibiotic mix (ciprofloxacin, metronidazole, and minocycline) was used to disinfect the pulp for 1 week. Then a blood clot was created in the canal, over which grey mineral trioxide aggregate was placed. Patients were recalled periodically. **Results:** Six patients dropped from the study (as a result of pain or failure to induce bleeding after canal disinfection) and instead received a standard apexification procedure. Another 3 patients did not attend any recall appointments. The remaining teeth (n = 3) were found to exhibit complete root development, with a positive response to pulp testing. **Conclusions:** Revascularization could be effective for managing immature permanent teeth with apical periodontitis with appropriate case selection. *(J Endod 2009;35:745–749)*

Key Words

Healing, open apex, periapical lesion, pulp revascularization, treatment outcome

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Pulp necrosis of an immature tooth as a result of caries or trauma could arrest further development of the root, leaving the tooth with thin root canal walls and blunderbuss apices (1). The absence of an apical constriction makes root canal treatment problematic because of the difficulty to obtain a seal with conventional obturation methods. The thin root canal walls render it susceptible to fracture. Traditionally for such cases, apexification is used as a method to induce a calcified barrier that serves as a matrix against which the root filling material is compacted (2). Calcium hydroxide has been the most often advocated material for this purpose (3). However, apexification with calcium hydroxide has several disadvantages, including variability of treatment time, patient compliance (for attending the recalls), and increased risk of tooth fracture after dressing with the material for extended periods (4, 5). Although the open apex might be "closed" by a calcific barrier, apexification does not promote the continued development of the root.

Human case series of avulsed teeth (6) and animal studies (7, 8) have provided radiographic and histologic evidence, respectively, of successful revascularization of immature permanent teeth after replantation. In these cases, the uninfected but necrotic pulp (after avulsion) probably has acted as a scaffold, permitting the ingrowth of new tissue from the periapical area. In addition, the wide open apex and short root length had allowed the tissues to grow into the root canal relatively easily. The crown of these teeth (in the clinical studies above) was intact and thus had ensured some protection against bacterial penetration into the pulpal space (9). All in all, revascularization was favored in the competition between ingrowth of new tissue and bacterial invasion for avulsed immature teeth that were replanted and remained uninfected.

Regeneration of the pulpal tissue of an infected immature tooth with apical periodontitis had once been thought to be impossible. However, if a suitable environment could be achieved, ie, absence of intracanal infection and presence of a scaffold conducive to tissue ingrowth, then regeneration of pulp might take place. The key factor for the success of this process is disinfection of the root canal system, because tissue growth will halt at the level where bacteria are found (8, 10). Various combinations of antibiotics have been shown to be able to disinfect the infected root canals; one example is a mixture of ciprofloxacin, metronidazole, and minocycline (11). Then the pulpal space might become repopulated with mesenchymal tissue resembling the dental pulp (12). The aim of this clinical study was to examine the effect of a revascularization procedure on immature teeth with apical periodontitis.

Materials and Methods

The study population comprised 12 patients (8–11 years of age), consisting of 5 incisors and 7 premolars with or without signs and/or symptoms of periapical pathology, who attended the Pediatric Dentistry clinic of the School of Stomatology of Peking University, Beijing, China. The patients presented with either a fractured dens invaginatus or trauma of the premolar(s), which was associated with symptoms of acute or chronic apical periodontitis (ie, pain, diffuse facial and/or mucosal swelling, tenderness to percussion, or intraoral sinuses). Radiographically, the tooth had an immature apex, either blunderbuss or in the form of a wide canal with parallel walls and an open apex. After clinical and radiographic examination, an electric pulp test was performed on the affected tooth and a control (contralateral but unaffected) tooth. The patients and their parents were informed of the treatment plan (for revascularization) and the potential risks before giving their consent to the treatment.

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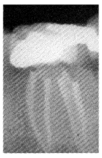

CASE REPORT

One step pulp revascularization treatment of an immature permanent tooth with chronic apical abscess: a case report

S. Y. Shin, J. S. Albert & R. E. Mortman

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Abstract

Shin SY, Albert JS, Mortman RE. One step pulp revascularization treatment of an immature permanent tooth with chronic apical abscess: a case report. *International Endodontic Journal*, **42**, 1118–1126, 2009.

Aim To describe a case in which a mandibular right second premolar with a necrotic pulp, sinus tract, periradicular radiolucency and an immature apex underwent revascularization via a single treatment approach.

Summary Revascularization procedures have the potential to heal a partially necrotic pulp, which can be beneficial for the continued root development of immature teeth. However, it is not clear which revascularization protocols are the most effective. This case report details the outcome of a successful revascularization procedure on tooth 45 (FDI) in a 12-year-old patient, eliminating the associated periapical pathosis within 19 months. The tooth was treated using coronal root irrigation with 6% NaOCl and 2% chlorhexidine without instrumentation in a single visit. The successful outcome of this case report suggests that this conservative revascularization treatment approach can preserve the vitality of the dental pulp stem cells and create a suitable environment for pulp regeneration, resulting in the completion of root maturation.

Key learning points

- The noninstrumentation procedure using 6% NaOCl and 2% chlorhexidine coronal irrigation may help preserve the remaining vital dental pulp stem cells believed to be critical for pulp revascularization.
- A single visit pulp revascularization protocol can be a favourable treatment option for an immature permanent tooth with a partially necrotic pulp.

Keywords: dental pulp stem cells, immature apex, pulp regeneration, revascularization, stem cells of the apical papilla.

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Revascularization of an immature permanent tooth with periradicular abscess after luxation

CASE REPORT

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Accepted 25 October, 2010

Abstract – An immature permanent mandibular central incisor with periapical involvement in a 7-year-old boy was treated to promote revascularization. The tooth suffered from acute apical periodontitis after periodontal treatment by a general practitioner. An access cavity was prepared in the tooth and the cavity was left open until the next visit to achieve drainage through the canal. The root canal was not mechanically cleaned during the treatment period, but was irrigated with hydrogen peroxide and sodium hypochlorite. Calcium hydroxide compound was used for disinfection. At the fifth visit vital tissue appeared in the canal near the apical region, and calcium hydroxide compound was placed in contact with the soft tissue in the root canal. The access cavity was sealed with glass–ionomer cement followed by an adhesive composite resin filling. Radiographic examination 30 months after the initial treatment confirmed closure of the apex and thickening of the root wall. The case was observed for up to 13 years and root development was confirmed.

Successful cases of revascularization of immature permanent teeth with infected root canals have been reported (1–11). As it has been observed in these reports, revascularization probably occurs predictably in teeth with open apices under appropriate treatment.

In the case report here, an immature mandibular central incisor with periapical involvement in a 7-year-old boy was treated to promote revascularization using copious irrigation with dressing of calcium hydroxide compound for disinfection. The case was observed for up to 13 years and root development was confirmed. The follow-up period in the reports in the past are mostly shorter than 3 years. Most of the reported cases have been mandibular premolar teeth, and the rest have been mainly maxillary incisors.

Case report

The patient was a 7-year-old boy. He received a scaling of lower incisors with a hand instrument by a general dentist in May of 1996. Soon after the treatment, he complained of pain and movement of both mandibular central incisors. He visited Tohoku Welfare Pension Hospital for special treatment in June of 1996.

The clinical findings of the patient were as follows; gingival swelling around the mandibular anterior teeth with mobilities of these teeth $+_1$ for the right central incisor and lateral incisors and $+_2$ for the left central incisor. Radiographic examination revealed that both central and lateral incisors had incomplete root formation and a periodontal ligament space in the left central incisor was widened (Fig. 1). Positive response to thermal (Pulper Dental Coolant, GC, Tokyo, Japan) and electric (Pulp Tester; AT Analytic Technology, Redmond, WA, USA) sensitivity testing were confirmed from the right central and lateral incisors but not from the left central incisor. A diagnosis of subluxation was made for the right mandibular central incisors and lateral incisors and a diagnosis of lateral luxation and suspected apical periodontitis was made for the left central incisor.

Based on the diagnosis, the anterior mandibular teeth were stabilized using adhesive resin, and antibiotics (Amoxicillin 600 mg per day for 3 days) were prescribed for the patient. 19 days after the initial visit, gingival swelling and abscess formation occurred on the left central incisor (Figs 2 and 3). Therefore, the diagnosis of the left central incisor was changed to acute apical periodontitis, and root canal treatment was initiated. The fixation was prolonged for 2 weeks for a total of 4 weeks.

When the access cavity was prepared on tooth 31, bloody and purulent exudate discharged from the pulp chamber. The patient had no pain sensation when the access cavity was prepared and also when a smooth broach was inserted into the canal. The tooth was left open until the next visit to achieve drainage through the canal.

The patient visited the hospital weekly and the discharge of exudates stopped by the second visit. Until the fifth visit the access cavity was opened to the canal orifice and the upper part of the root canal was irrigated using 5% sodium hypochlorite and 3% hydrogen

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Continued Development of the Root Separated from the Main Root

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Abstract

Introduction: Endodontic infection or physical trauma of a developing tooth might lead to unusual situation for root development. **Methods:** Two cases are presented. Both cases involved immature mandibular second premolars with infected pulps. In the first case, the tooth was treated by apexification, whereas revascularization procedures were performed in the second case. Interestingly, a separated root tip was observed with the main root at the initial presentation of case 2. **Results:** At recall after apexification, a separate root tip was observed apically to the main root end, and root development continued in case 1. In the second case, the separate root displayed continuing root formation, but the main root did not gain root thickness or length. **Conclusions:** These cases revealed that Hertwig's epithelial root sheath and stem cells from the apical papilla can be separated from the main tooth structure by an external force or iatrogenic factors and thereafter produce a separate root tip. *(J Endod 2011;37:711–714)*

Key Words

Immature teeth, open apex, revascularization, separated root

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Since Iwaya et al (1) first reported a case of revascularization in an immature permanent tooth with apical periodontitis, a significant number of case reports with similar results have been published (2–6). Although there were some differences in treatment protocol, most researchers reported that the teeth gained root thickness and length. Accordingly, continued root formation after disinfection of the root canal system of immature teeth with an infected pulp is not an exceptional incident, and it is believed that stem cells from the apical papilla (SCAP) are responsible for the development (7).

After trauma to an immature tooth, a new root tip might form separately from the main root and can continue development (8–12). It is believed by the authors that this continued development is also associated with SCAP, and the separation is due to detachment of SCAP and Hertwig's epithelial root sheath (HERS) by a traumatic episode. Yang et al (13) reported a separate growing root with an odontoblastic layer, predentin, dentin, cementum, and an apical foramen. The authors believed that the separation was attributable to damage to HERS caused by the apexification procedures. However, because very few cases of a separate root growing in immature teeth with an infected pulp have been reported, the underlying mechanism is still unclear. The following report describes 2 cases of separately growing roots that were not directly associated with a traumatic episode.

Case 1

A 14-year-old girl presented to the Department of Conservative Dentistry at Yonsei University Dental Hospital for emergency care of the left mandibular second premolar (tooth #20). The girl had experienced intermittent spontaneous pain in that area for previous months. A clinical examination revealed tooth #20 to be quite sensitive to percussion and palpation. On all aspects of the crown, the probing depths measured 6–10 mm; the mobility of the tooth was +3. The tooth was free of caries, but a pinpoint defect was seen on the occlusal surface, which seemed to be caused by the ground down dens evaginatus. The tooth did not respond to the cold and electric pulp vitality tests. A radiographic examination revealed that tooth #20 had an incompletely developed apex and periradicular radiolucency (Fig. 1*A*). A diagnosis of pulp necrosis and symptomatic apical periodontitis was made for tooth #20.

When the access cavity was made under rubber dam isolation, the pulp was found to be completely necrotic. The canal length was determined by a parallel preoperative radiograph. The root canal was cleaned by using a #30 file in an ultrasonic unit and 2.5% NaOCl. After drying the pulp canal, it was filled with Vitapex (Neo Dental Chemical Products, Tokyo, Japan), and IRM (Dentsply Caulk, Milford, DE) was used as a temporary filling material (Fig. 1*B*). The patient returned 3 months later and was asymptomatic and reported no pain. Periodontal probings of tooth #20 were 2–3 mm deep, and the mobility of the tooth was normal. A radiographic examination revealed some decrease in radiolucency around tooth #20 (Fig. 1*C*). At the next appointment 3 months later, the patient was asymptomatic. After opening the tooth, the canal was evaluated for the presence of apical closure by using a #30 file, and a hard tissue barrier was confirmed. The canal was cleaned and obturated with gutta-percha and a sealer by using vertical compaction. The access cavity was filled with amalgam. At the 14-month recall, the patient was still asymptomatic, and healing of the periradicular bone was apparent radiograpically

55.

Regenerative Endodontic Treatment (Revascularization) for Necrotic Immature Permanent Molars: A Review and Report of Two Cases with a New Biomaterial

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Abstract

Introduction: Revascularization is a valuable treatment in immature necrotic teeth that allows the continuation of root development. In this article we describe successful revascularization treatment of 2 necrotic immature first mandibular molars. **Methods:** The clinical and radiographic examinations showed extensive coronal caries, immature roots, and periapical radiolucencies in mandibular first molars of a 9-year-old boy and an 8-year-old girl. The exam findings suggested revascularization treatment in both cases, which was started with irrigation of the canals by using NaOCl 5.25% for 20 minutes, followed by 3 weeks of triple antibiotic (metronidazole, ciprofloxacin, and minocycline) paste dressing. Next, the antibiotic paste was removed, bleeding was induced in the canals, and calcium enriched mixture (CEM) cement was placed over blood clots. **Results:** In radiographic and clinical follow-ups both cases were asymptomatic and functional, periapical radiolucencies were healed, and roots continued to develop. **Conclusions:** Revascularization is a realistic treatment in immature necrotic molars. In addition, placing CEM cement as a new endodontic biomaterial over the blood clot formed inside the canals provided good seal and favorable outcomes. *(J Endod 2011;37:562–567)*

Key Words

Apexification, calcium enriched mixture, CEM cement, open apex, regenerative treatment, revascularization, triple antibiotic paste

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Treatment of necrotic immature teeth has always been a challenge in endodontics. It is difficult to get an appropriate apical seal in teeth with open apices by using the conventional endodontic treatment methods. The discontinued development of dentinal walls after the pulp necrosis can also lead to a weak root structure with thin dentinal walls, which makes the tooth susceptible to future fractures (1). Traditionally, multiple-visit apexification with calcium hydroxide was the treatment of choice in necrotic immature teeth, which would induce formation of an apical hard tissue barrier (2). Although this approach was predictable and successful (3), long-term use of calcium hydroxide has several disadvantages such as multiple treatment appointments, probable recontamination of the root canal system during treatment period, and increased brittleness of root dentin, which increases the risk of future cervical root fractures (3, 4).

An alternative technique for apexification with calcium hydroxide is artificial apical barrier technique, which is done by placing barrier material in apical portion of the canal. The material of choice for this technique is mineral trioxide aggregate (MTA) (5), which has been shown to have high success rates (6–8) and reduce the number of required clinical sessions. Both of the mentioned methods (ie, apexification and artificial apical barrier techniques) share the same disadvantage of not allowing the continuation of root development, which leads to a fragile root structure.

Revascularization is a regenerative treatment and a biologically based alternative approach to treat necrotic immature teeth that, unlike apexification and artificial apical barrier techniques, allows continuation of root development (9, 10). Several types of stem cells including dental pulp stem cells (DPSCs), which are more populated in central cell rich zone of the pulp, bone marrow stem cells (BMSCs), stem cells from human exfoliated deciduous teeth (SHED), and stem cells from apical papilla (SCAPs) have exhibited different levels of ability to generate osteoid and odontoid structures (11). SCAPs are the source of primary odontoblasts that are responsible for continuation of root development (12, 13) and, as a result of proximity to the periodontal blood supply, can survive pulp necrosis even in the presence of periradicular infection (12, 14). In the optimal situation (ie, elimination of microorganisms and their by-products and necrotic tissues and in the presence of a protein scaffold and a tight coronal seal) these stem cells can populate in the root canal space of necrotic immature tooth (15, 16).

The proposed regenerative treatment generally starts with chemical disinfection of the canals by using passive NaOCl (17, 18), NaOCl-chlorhexidine (15, 19, 20), or NaOCl–hydrogen peroxide 3% (21, 22) irrigation without any instrumentation. Different concentrations of NaOCl including 6% (20, 23), 5.25% (15, 17, 19), 2.5% (21, 24, 25), and 1.25% (18) and different concentrations of chlorhexidine including 2% (20, 23) and 0.12% (19) have successfully been used for this purpose. The procedure continues by a triple antibiotic dressing including ciprofloxacin + metronidazole+ minocycline, as suggested by Hoshino et al (26). The required time of dressing varies between few days to few months in different studies (15, 17–19). In the absence of clinical signs and symptoms of periradicular diseases (ie, sensitivity to percussion or palpation, presence of swelling, redness, or sinus tract, and suppuration or exudation from canals), the treatment continues with removing the paste and inducing bleeding inside the canals by irritating the apical tissues with a sterile file

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Case Report

Emerging Therapies for the Management of Traumatized Immature Permanent Incisors

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Abstract: *Early loss of immature permanent teeth due to pulpal necrosis secondary to trauma can have dire consequences for a child's growth and development. The treatment alternatives include surgical endodontics, traditional calcium hydroxide apexification, and mineral trioxide aggregate (MTA) apexification. These options pose potential complications, including: arrest of root development; weakened dentinal walls; and increased potential for fracture. Revascularization of the dentin-pulp complex is a new approach that involves disinfecting the root canal system followed by tissue repair and regeneration while allowing for continued root development and thickening of the lateral dentinal walls through deposition of new hard tissue. The purpose of this report was to present the revascularization of an immature permanent maxillary central incisor that had evidence of external root resorption. Six months later, internal bleaching was performed to remove cervical discoloration from the triple antibiotic paste. At 18 months, the tooth remained vital and had evidence of continued root development.* *(Pediatr Dent 2012;34:66-9) Received May 17, 2010 / Last Revision August 24, 2010 / Accepted August 25, 2010*

KEYWORDS: TRAUMA, AVULSION, ROOT RESORPTION, REVASCULARIZATION, TRIPLE ANTIBIOTIC PASTE

Dental injuries in the permanent dentition occur most commonly in 8- to 12-year-olds at a time when there is loosely structured periodontal ligament surrounding erupting teeth that often exhibit short, incompletely formed roots.[1] Because root completion takes place 2 to 3 years after a tooth fully erupts, management of avulsed immature teeth can be a challenge.

Pulpal necrosis and/or inflammatory root resorption following avulsion can pose potential problems and complications that are exacerbated in a growing child. Treatment options for avulsed immature teeth include pulpotomy and apexogenesis treatment for vital teeth and either calcium hydroxide or mineral trioxide aggregate (MTA; Dentsply, Tulsa Dental, York, Pa) apexifications for nonvital teeth. When the pulp of avulsed immature teeth becomes necrotic or nonvital, treatment options are limited and prognosis is guarded due to instrumentation difficulty, thin dentinal walls, and arrested tooth development, leaving the tooth weak and fracture-prone.[2] Recently, pulp revascularization has become a viable treatment option for traumatized immature teeth with devitalized pulp.[3-6]

Revascularization in immature permanent teeth. An immature or open apex tooth has the potential to establish revascularization when there is a minimum of a 1.0 mm apical opening.[7] Complete pulpal revascularization has been shown to occur at a rate of 18% among immature teeth.[8]

The revascularization procedure involves disinfecting the root canal system using a mixed paste of antibiotics and then allowing the remaining tissues to serve as a scaffold for the ingrowth of connective tissue. To induce pulp revascularization, a blood clot is created; its growth factors and cross-linked fibrin will then act as a scaffold. It is speculated that the new tissue resembles that of periodontal ligament (PDL) cells. The traditional triple antibiotic mix used consists of ciprofloxacin, metronidazole, and minocycline.[9] Recently, the use of cefaclor (cephalosporin) instead of minocycline has been recommended to avoid discoloration.

Published case reports[3-6] have shown in vivo that it is possible to disinfect immature teeth with necrotic pulps and/or healing of inflammatory root resorption using combinations of antibiotic pastes.

The purpose of this report was to add to the literature of successful revascularization using the triple antibiotic paste as a method to control external inflammatory root resorption and to illustrate the use of vital internal bleaching treatment for the discoloration expected from this treatment protocol.

Case report

Initial appointment. A healthy 9 year-old male presented to the emergency department's (ED) Pediatric Dental Service of the University of North Carolina at Chapel Hill, Chapel Hill, NC, with dental trauma. The patient had sustained orofacial trauma approximately 2 hours earlier while riding his skateboard. He was seen by an ED physician, but not cleared medically until head and spine computed tomography scans were obtained. The

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Case Report

Conventional apexification and revascularization induced maturogenesis of two non-vital, immature teeth in same patient: 24 months follow up of a case

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Abstract

Various authors have demonstrated the regenerative process in immature, non vital teeth by revascularization induced maturogenesis. The aim of this case report is to compare calcium hydroxide apexification and pulp revascularization induced maturation procedures in the same patient, in two different teeth. The right maxillary central incisor in this individual was treated with conventional calcium hydroxide induced apexification procedure followed by guttaperchaobturation, and the left maxillary central incisor was treated by pulp revascularization induced maturation procedures. 24 months follow-up shows root elongation and apical closure in the tooth treated with revascularization induced maturation procedures. Revascularization induced maturogenesis, where indicated, can provide several advantages over conventional apexification procedures.

Keywords: Apexification; apex formation; maturogenesis; revascularization

INTRODUCTION

Management of immature non vital teeth poses a great challenge to the clinician. Most of the clinicians rely on traditional calcium hydroxide apexification. Calcium hydroxide induced apexification has its own limitations such as:.[1] long term therapy for barrier formation; and, strengthening or reinforcing of the thin fragile blunderbuss canals is not achieved. Instead, its long term therapy has shown to make the tooth brittle due to its hygroscopic and proteolytic properties.[2,3] Even after apexification procedure, the clinician has to go for conventional root canal obturation as the barrier formed is often porous and not continuous.[4,5] Mineral trioxide aggregate (MTA) has also been used to provide an artificial barrier; however, it also has the limitations of non-reinforcement of root canal dentin and a high cost.[6]

Recently some case reports have shown that non vital, infected immature teeth can be alternatively treated by pulp regenerative process.[7-16] The authors have termed this regenerative process as revascularization,[7-11] revitalization or maturogenesis.[14,15] The common aspect of all regenerative modalities is intra canal disinfection using copious irrigation, placement of antibiotic pastes and formation of a sterile blood clot inside the pulp cavity. The concept of pulp regeneration was first noted in traumatic avulsed and replanted immature teeth.[16-18] Rule[19] documented root development and apical barrier formation in cases of pulpal necrosis. The authors emphasized on the importance of sterile blood clot and granulomatous tissue within the pulpal cavity. Various possible explanations have been given to explain why apexogenesis/maturogenesis can occur in these infected immature permanent teeth. These include the presence of mesenchymal stem cells residing in the apical papilla, also known as stem cells of apical papilla (SCAP),[20] which are the multi-potent dental pulp stem cells,[21] and resistant to necrosis/infection.[22,23] The exact etiology, pathogenesis or histo-pathological events that occur in this regenerative process are still not known.

Till date very few cases have been reported using pulp regeneration as a therapeutic therapy for the treatment of immature non vital teeth. It is necessary to compare the outcomes of conventional calcium hydroxide therapy and pulp revascularization procedure in same individuals, so that the general practitioners can compare both therapies. It is difficult to clinically find bilateral non-vital, immature, infected teeth with approximately similar root development stage. A case report is presented with bilateral immature

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Case Report

Platelet Rich Fibrin in the revitalization of tooth with necrotic pulp and open apex

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Abstract

Regeneration of pulp-dentin complex in an infected necrotic tooth with an open apex is possible if the canal is effectively disinfected. The purpose of this case report is to add a regenerative endodontic case to the existing literature about using Platelet Rich Fibrin (PRF). A nine year old boy who accidently broke his immature maxillary central incisor tooth, developed pulpal necrosis with apical periodontitis. After the access cavity preparation, the canal was effectively irrigated with 20 ml of 5.25% sodium hypochlorite solution and 10ml of 0.2% chlorhexidine solution and dried with paper points. Triple antibiotic paste was placed inside the canal and left for 21 days. 12 ml of whole blood was drawn from the patient's right antecubital vein and centrifuged for 10 minutes to obtain the Choukroun's PRF. After the removal of the triple antibiotic paste, the PRF was placed into the canal till the level of cementoenamel junction and 3mm of grey MTA was placed directly over the PRF clot. The setting of MTA was confirmed 3 days later and the tooth was double sealed with GIC and Composite restoration.After 1 year the clinical examination revealed negative responses to percussion and palpation tests. The tooth responded positively to cold and electric pulp tests. Radiographic examination revealed continued thickening of the dentinal walls, root lengthening, regression of the periapical lesion and apical closure. On the basis of the results obtained in our case report we conclude that revitalization of necrotic infected immature tooth is possible under conditions of total canal disinfection and PRF is an ideal biomaterial for pulp-dentin complex regeneration

Keywords: Open apex, revitalization, Platelet Rich Fibrin

INTRODUCTION

Regenerative endodontic procedures are biologically based procedures which deals with the regeneration of pulp like tissue, more idealistically the pulp-dentin complex, damaged coronal dentin such as that following a carious exposure or trauma; and regenerate resorbed root, cervical or apical dentin.[1] The mechanics behind the revitalization endodontic procedure is that, despite the tooth being necrotic, some pulp tissue can survive apically which under favorable conditions proliferate to aid in the process of regeneration.[2-4] Although there are no established standardized treatment protocols for endodontic regeneration, many of these cases have shown favorable results, with continued radiographic evidence of development of the dentin-pulp complex and an absence of clinical symptoms.[3,5-11]

The conventional method of revitalization procedure was done by inducing bleeding into the pulp canal by mechanically irritating the periapical tissues.[3] In necrotic teeth with open apices, some amount of pulp tissue along with Hertwigs Epithelial Root Sheath may survive apically and these tissues can proliferate once the inflammatory condition are reversed and the canal becomes totally disinfected.[3] The created blood clot acts as a matrix for the in growth of new tissues into the pulp canal. However, this procedure will cause discomfort for the patient while mechanically irritating the periapical tissues.

In the past two decades, an increased understanding of the physiological roles of platelets in wound healing and after tissue injury has led to the idea of using platelets as therapeutic tools. Platelet-Rich Plasma (PRP) consists of a limited volume of plasma enriched with platelets, which is obtained from the patient. The use of PRP as a potentially ideal scaffold for regenerative endodontic therapy has been documented in the literature.[12] However, the use of bovine thrombin for the activation of Platelet Rich Plasma (PRP) has

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Clinical Article

CASE SERIES

Clinical Complications in the Revascularization of Immature Necrotic Permanent Teeth

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Abstract: *The purpose of this case series was to report on the use of a technique of revascularization for necrotic immature permanent teeth, several problems encountered, and solutions to those problems. Eighteen pulp revascularizations were performed in 2009 using the original protocol of revascularization (adapted from the AAE/AAPD joint meeting in 2007 in Chicago). The protocol consisted of opening the canal and disinfecting it with sodium hypochlorite, sealing in a triple antibiotic paste for 2-6 weeks, re-opening, re-irrigating, creating a blood clot in the canal, and sealing with an MTA barrier over the clot. Three problems were encountered during the treatment: (1) bluish discoloration of the crown; (2) failure to produce bleeding; and (3) collapse of the mineral trioxide aggregate (MTA) material into the canal. Modifications to solve these problems included: changing one of the antibiotics, using a local anesthesia without epinephrine, and adding collagen matrix to the blood clot.* *(Pediatr Dent 2012;34:414-7) Received December 23, 2010 | Last Revision May 4, 2011 | Accepted May 5, 2011*

KEYWORDS: REVASCULARIZATION, TOOTH, ENDODONTICS, ABSCESS

Permanent teeth are still immature when they erupt into the oral cavity of children; their roots are not complete and have thin, divergent, and fragile walls and wide open apices. Thus, the treatment of necrotic immature permanent teeth has always presented many challenges. Current endodontic treatments for nonvital immature teeth have included calcium hydroxide apexification or an immediate apexification procedure with a mineral trioxide aggregate (**MTA**) apical barrier. One major problem of performing an apexification with calcium hydroxide is the multiple appointments required and the long treatment period. It has also been reported that long-term calcium hydroxide treatment can alter the properties of dentin.[1] The use of ProRoot® MTA (Dentsply Tulsa Dental, Tulsa, Okla., USA) to create an artificial apical barrier at the root apex has proven to be effective but is technique sensitive. Both calcium hydroxide and MTA apexification procedures serve to achieve an apical stop to facilitate 3-dimensional root canal obturation with gutta-percha and ultimately obtain the resolution of the periapical lesion. The immature tooth remains fragile, however, because the root remains short with thin, radicular walls. It is more susceptible to fracture.

The ideal treatment for an immature necrosed tooth is to regenerate a healthy pulp-dentin complex that would allow the continued maturation of the root. This concept was first called revascularization and was unsuccessfully attempted in the 1960s.[2] In 1978, it was demonstrated that revascularization could occur immediately after the reimplantation of extracted dog teeth, a process which would be completed in 45 days.[3]

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Recent advances in the development of new materials have allowed clinical researchers to achieve closure with continued root maturation in immature necrotic permanent teeth. Since 2004, sporadic case reports have shown the possibility of revascularization, allowing the formation of the root to continue.[4-10] Most of the cases reported had favourable outcomes; they presented resolution of the periapical lesion, significant root lengthening, thickening of the dentinal walls, and closure of the apex. Eighteen cases of revascularization of necrotic immature teeth were started in 2009 at the Montreal Children's Hospital in the hope of achieving similar success. The purpose of this study was to report the technique used to threat these teeth, the problems encountered during treatment and follow-up, and some modifications made to the procedure to solve the problems.

Report of Case Series

Fourteen children needing apexification treatment, with a total of 18 immature teeth, were selected from the Department of Dentistry of Montreal Children's Hospital, McGill University Health Center, Montreal, Quebec, Canada. All of these patients presented with chronic or acute odontogenic infections, of which 14 were the result of a trauma, 2 had been caused by caries, and 2 originated from dental anomalies (dens evaginatus and dens invaginatus).

The initial protocol was based on a relevant literature review and was developed by an endodontist and a pediatric dentist. All patients were treated by the same clinician. The project was approved by the Montreal Children's Hospital Research Ethic Board, and an informed consent was obtained from the children's parents. Only necrotic teeth were selected for this study, and the teeth were evaluated clinically and radiographically for pulpal and periapical pathology .

The first step was to standardize the radiographic technique by securing the same angulation and the same position of the radiograph with respect to the teeth at each appointment.

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Revascularization of Immature Permanent Incisors after Severe Extrusive Luxation Injury

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Posted on January 19, 2012

Tags: children endodontics injury treatment

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ABSTRACT

Pulp necrosis is an uncommon sequel to extrusive luxation in immature teeth with incomplete apical closure. In this report, we describe the management of severely extruded immature maxillary incisors and the outcome of revascularization to treat subsequent pulp necrosis. An 8.5-year-old boy with severe dentoalveolar trauma to the anterior maxillary region as a result of a fall was provided emergency treatment consisting of reduction of the dislodged labial cortical bone and repositioning of the central incisors, which had suffered extrusive luxation. When he presented with spontaneous pain involving the traumatized incisors a week later, the teeth were treated via a revascularization protocol using sodium hypochlorite irrigation followed by 3 weeks of intracanal calcium hydroxide, then a coronal seal of mineral trioxide aggregate and resin composite. Complete periradicular healing was observed after 3 months, followed by progressive thickening of the root walls and apical closure. Follow-up observations confirmed the efficacy of the regenerative treatment as a viable alternative to conventional apexification in endodontically involved, traumatized immature teeth.

Introduction

Extrusion is an injury characterized by partial axial displacement of a tooth.[1] Clinically, the affected tooth appears elongated, is usually displaced in the palatal direction and demonstrates excessive mobility.[2,3] Radiographically, extruded teeth appear to have an increased periodontal ligament space. Based on severance of the periodontal ligament that has not yet been exposed to desiccation or disarticulation of the tooth from the blood supply, Andreasen[4] described extrusive luxation as "partial avulsion." According to Lee and colleagues,[5] this term is useful in terms of treatment approach, as the pulpal outcome of severe extrusion may be comparable to that of a replanted tooth.

The stage of apical development is a key factor in pulp healing after extrusive luxation.[3,5,6] In teeth with open apices, the pulp has greater potential for healing, commonly followed by pulp canal obliteration; in patients with closed apices, the likelihood of pulp revascularization is low, usually leading to pulp necrosis.[1,3,5,6] Once pulp necrosis is diagnosed, endodontic therapy should be initiated to eliminate infection and facilitate healing and retention of the tooth.[3] If root development is incomplete, apexification is indicated to induce formation of a calcific barrier at the apex. However, this technique has several disadvantages, including up to 24 months of treatment, which often requires multiple visits and renewal of the intracanal dressing.[7,8] Apical closure is unpredictable,[9] and the tooth is susceptible to root fracture after prolonged exposure to calcium hydroxide ($Ca(OH)_2$).[10,11] Because of these concerns, the traditional $Ca(OH)_2$-based apexification procedure has been modified by the introduction of an artificial apical barrier using mineral trioxide aggregate (MTA).[12–15] Obturation of open apices with MTA plugs significantly reduces treatment time and results in favourable healing of periradicular tissues.[12,14,16,17] However, MTA plugs cannot stimulate physiologic apical closure and thickening of radicular dentin, leaving the tooth's structural integrity compromised.[18,19]

Revascularization is an emerging regenerative endodontic treatment approach that aims to allow continuation of root development and tissue regeneration in immature necrotic teeth.[20,21] The root canal is disinfected with sodium hypochlorite, followed by placement of an intracanal medicament, such as calcium hydroxide or a combination of ciprofloxacin, metronidazole and minocycline.[22] After disinfection, the antibiotic paste is removed and apical bleeding is induced to form a blood clot below the coronal level. The root canal orifice is then sealed with MTA, and the tooth crown is restored permanently.

Revitalization Procedures in Two Traumatized Incisors with Different Biological Outcomes

Renato Lenzi, DDS, and Martin Trope, DMD†*

Abstract

Introduction: The revitalization of a previously necrotic pulp space has been shown to be possible and even considered predictable. However, exact criteria for success are still lacking, and, in fact, some cases do not respond as predicted. **Methods:** In this case, the same operator treated 2 teeth similarly according to the principles laid out by Banch and Trope. The tooth that according to our expectations was more likely to be revitalized successfully failed to do so, whereas the second tooth that, in our estimation, was less likely to succeed was successful. In the tooth that failed to revitalize, auto-apexification occurred. **Conclusions:** Complete understanding for the criteria for predictable revitalization and apexification is still lacking. *(J Endod 2012;38:411–414)*

Key Words

Apexification, pulp, revascularization, revitalization

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The revitalization of immature teeth with necrotic infected pulps and apical periodontitis has been shown to be possible and even predictable (1–5). The procedure requires disinfection of the pulp space with an effective antibacterial regimen commonly with the use of a bi- or triantibiotic paste (6, 7), the production of a scaffold into the pulp space (usually by means of a blood clot), and the placement of a bacterial tight restoration above the root canal (1).

This case report describes the treatment of 2 nonvital immature teeth with infected pulp spaces as a result of trauma. The same operator treated both teeth similarly. In one tooth, the procedure was successful in revitalization and continued root thickening and closure of the apex. In the adjacent tooth that in fact appeared preoperatively more likely to succeed, revitalization of the pulp space was unsuccessful. However, the disinfection protocol was successful in that the apical inflammation resolved and auto-apexification resulted.

Case Report

An 8-year-old boy was referred to Dr Lenzi for root canal treatment on the upper left and right maxillary central incisors. At presentation, both teeth had crowns with fractures from a fall at school (Fig. 1*G*). The reported accident had occurred 2.5 months previously, but he had not received any treatment. Medically, the child was under cardiac observation after a surgical repair for an interventricular communication at age 5. The surgery was successful, and the child was considered in very good health.

The physical examination revealed complicated crown fractures on the upper left maxillary incisor (ULI) and the upper right maxillary incisor (URI) (Fig. 1*G*). The URI appeared slightly infraoccluded with a suspected intrusive injury in addition to the crown fracture. The teeth were asymptomatic with no signs of acute infection, swelling, or sinus tract. The patient had no symptoms. Both teeth tested nonvital to cold and electric pulp tests. Palpation, percussion, and periodontal probing were all within normal limits.

In the periapical radiograph (Fig. 1*A*), the URI showed a complicated crown fracture. The tooth appeared to be of normal width and length with a slightly open apex. The presence of an apical lesion was inconclusive from this radiograph. The ULI had thin dentinal walls with a wide-open apex, indicating that the tooth had become nonvital because of a previous accident although the child and parents denied any previous traumatic injury. There was a distinct radiolucency apically on this tooth.

A cone-beam computed tomography (CBCT) radiograph taken at the initial visit (Fig. 1*B–F*) showed that the URI incisor had normal development for the child's age, with the apex being almost closed. The tooth appeared slightly intruded. The ULI had arrested development, thin dentinal walls, and an extremely wide apical foramen. A large radiolucency was present, suggesting a long-standing root canal infection previous to the latest traumatic injury.

Treatment

Because of the patient's age and short attention span, the teeth were treated on different days but similarly as described later. The teeth were anesthetized and isolated with a rubber dam. Access was made, and the access cavities and surrounding rubber dam were disinfected with a 2% chlorhexidine rinse on a cotton swab.

For the URI, there was some minor bleeding around the apex of the tooth, and care was made not to disturb any vital tissue in the apical part of the root. For the ULI, serous fluid was present in the canal with no apparent bleeding in the canal. A working length

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CASE REPORT

Long-term follow-ups of revascularized immature necrotic teeth: three case reports

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Revascularization of immature necrotic teeth is a reliable treatment alternative to conventional apexogenesis or apexification. In case 1, a 12-year-old boy had his necrotic, immature mandibular left second premolar treated with a revascularization technique. At a 24-month follow-up, periapical radiolucency had disappeared and thickening of the root wall was observed. In cases 2 and 3, a 10-year-old boy had his necrotic, immature, bilateral mandibular second premolars treated with the same modality. At 48-month (in case 2) and 42-month (in case 3) follow-ups, loss of periapical radiolucencies and increases in the root wall thickness were also observed.

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Keywords: revascularization; pulp necrosis; mineral trioxide aggregate; antibiotic mixture; immature tooth

INTRODUCTION

Pulp necrosis of immature teeth may give rise to complications.[1] It prevents further development of the root, leaving a tooth with thin root walls. Conventional root canal treatment techniques used in mature teeth are difficult to use in immature teeth which have various anatomical complexities.[2] The instrumentation and obturation of immature root canals are difficult or impossible with conventional techniques.[3] Furthermore, weak teeth with thin root walls are susceptible to fracture.

Conventional apexification uses calcium hydroxide as intracanal medicament to induce apical closure over time. This is a successful treatment technique for immature teeth, but it has several disadvantages, including the need for multiple visits over a relatively long period of time (an average of 12 months). In addition, the root canal may not be reinforced.[4] An alternative to conventional apexification with calcium hydroxide is to make an artificial apical barrier to prevent the extrusion of root canal filling materials. The material of choice is mineral trioxide aggregate (MTA) which has good sealing ability and biocompatibility.[5] However, it does not also promote further root development.

Recently, an alternative biologically based treatment has been introduced for immature teeth with necrotic pulp.[6] Procedures that preserve the remaining dental pulp stem cells and mesenchymal stem cells of the apical papilla can result in intracanal revascularization and continued root development.[7] The revascularization of these teeth is based on the concept that vital stem cells in the apical papilla can survive pulpal necrosis, even in the presence of periapical infection, because the open apex provides good communication to the periapical tissues.[8–9] The present case reports evaluated the long-term prognosis of revascularization in necrotic immature teeth, with the aim of providing reliable evidences for the revascularization technique.

CASE REPORTS

Case 1

A 12-year-old boy was referred to our department for treatment of his mandibular left second premolar. His medical history was unremarkable. When taking the dental history, the patient stated that prior to the first visit, he experienced severe pain, which had decreased over the past 5 days, but increased with chewing. Clinical examination revealed that the tooth was not responsive to electric pulp test and was sensitive to percussion. Periodontal probing depths were within normal limits ($<$3 mm). Radiographic examination revealed a immature tooth with periapical radiolucency (Figure 1a).

Based on the results of the clinical and radiographic examinations, a diagnosis was made of pulp necrosis with symptomatic apical periodontitis. Under local anesthesia, the tooth was isolated with a rubber dam and the access cavity was prepared. The root canal was irrigated with 10 mL of 3% sodium hypochlorite for 2 min, and then dried with sterile paper points. A creamy paste mixture of metronidazole (Samil Pharm, Seoul, Korea), ciprofloxacin (Sinil Pharm, Seoul, Korea) and cefaclor (Myungin Pharm, Seoul, Korea) in sterile saline was applied using a lentulo-spiral and tapped down into the canal with the blunt end of sterile paper points. The tooth was temporarily restored using Caviton (GC, Aichi, Japan).

The patient was asymptomatic 2 weeks later. The Caviton was removed under rubber dam isolation. The mixture of antibiotics was completely removed with 3% sodium hypochlorite and sterile saline. The apical tissue was stimulated with a No. 10 K-file to induce

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Endodontic Clinical Management of a Dens Invaginatus Case by Using a Unique Treatment Approach: A Case Report

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Abstract

Introduction: Dens invaginatus is a developmental anomaly that poses treatment challenges when nonsurgical root canal therapy is deemed necessary. Teeth with complex root canal morphology often require a comprehensive treatment approach to effectively clean, shape, and disinfect the pulp canal space before obturation. Endodontic clinical management of a tooth with dens invaginatus might include using cone-beam computed tomography (CBCT) to aid in the diagnosis and treatment-planning phase and use of the surgical operating microscope in performing the clinical phase of treatment. A novel approach could include using the revascularization technique as the final treatment step in the management of a dens invaginatus case in which the root apex has not completed formation. **Methods:** This case report will provide both an overview of the feasibility of using CBCT scans in diagnosis and treatment planning and a step-by-step clinical technique, by using surgical operating microscope and the revascularization technique, in the successful endodontic management of a complex dens invaginatus case. **Conclusions:** Five-month and 12-month follow-up clinical and radiographic findings will provide a candid view of inherent advantages and challenges of this technique. *(J Endod 2012;38:1145–1148)*

Key Words

Cone beam computerized tomography, dens invaginatus

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Endodontists are constantly challenged in the treatment of teeth presenting with complex root canal morphologic variations. It is important for the clinician to understand and be aware of variations in root canal morphology and various treatment options (1). Dens invaginatus has complex root canal morphology and has been described as a tooth within a tooth or as an invagination of an enamel-lined tract extending into the root, with or without exposure of the dental pulp. For endodontic treatment to be successful, it is necessary to identify and successfully debride and disinfect the entire root canal system (1). A number of case reports have indicated the difficulty in treating teeth with the dens invaginatus morphologic variation (2–4).

The etiology of this developmental anomaly is still unclear. Possible etiologic factors include trauma, infection, growth retardation of specific cells, disruption in factors regulating the formation of enamel organ, and links to genetic factors (5–7). Variations in presentation are classified in a number of ways, with Oehlers classification being most popular because of its simplicity (8). Treatment of this entity is dictated by the anatomic complexity of each individual case (5, 9).

Until recently, imaging in endodontics was limited to 2-dimensional, standard intraoral radiographs. The advent and feasibility of cone-beam computed tomography (CBCT), a 3-dimensional imaging modality, and its application in clinical cases have helped not only in diagnosis but also in assisting the clinician in better treatment planning of complex cases requiring endodontic therapy (3, 10). The benefits of CBCT to study root canal morphology and various other applications have been reported (11, 12).

Pulp revascularization techniques are studied (13) and practiced by using current basic principles. Thus far, the outcomes have been solely documented in the form of animal studies (14), case reports (15–17), and case series (18, 19), and although this technique is in the early stages of research and development, these case reports and case series demonstrate much promise (13).

This case report will provide both an overview of the feasibility of using CBCT scans in diagnosis and treatment planning as well as a step-by-step clinical technique by using the surgical operating microscope and the revascularization technique in the successful endodontic management of a complex dens invaginatus case. Five-month and 12-month follow-up clinical and radiographic findings will provide a candid view of inherent advantages and challenges of this technique.

Case Report

An 11-year-old male patient was referred for evaluation and treatment of tooth #7. The patient gave a history of frequent episodes of pain (sensitivity to cold beverages) in the upper right front tooth for the previous 2 weeks. The patient was seen by his general dentist a week before, who prescribed antibiotics (amoxicillin 250 mg 3 times a day for 7 days), analgesic medication (acetaminophen 160 mg 4 times a day for 7 days), and referred the patient to our office for evaluation and treatment.

On presenting to our office, the patient reported the pain was temporarily relieved with the medication, and for the past week he had not experienced much discomfort when drinking cold beverages. He was presently unable to chew or bite food with tooth #7, and the tooth was tender to touch. The patient was in good general health. On clinical examination, tooth #7 was tender to direct vertical percussion and had normal mobility and a normal response to labial and palatal palpation. The tooth did not respond to a cold thermal test (Endo Ice; Coltene Whaldent Inc, Cuyahoga Falls,

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INTERNATIONAL JOURNAL OF PAEDIATRIC DENTISTRY

CASE REPORT

Revascularization for a necrotic immature permanent lateral incisor: a case report and literature review

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Background. Revascularization is a valuable treatment in immature necrotic teeth that allows the continuation of root development.
Case Report. This article describes the successful revascularization treatment of an immature maxillary lateral incisor that was initially diagnosed with apical periodontitis. The tooth was asymptomatic and functional clinically and radiographically during the follow-up period of 5 years.
Conclusion. The follow-up showed evidence of progressive thickening of the dentinal walls, development of root length and apical closure. The article also discusses the currently available literature regarding revascularization of immature permanent teeth.

Introduction

The treatment of pulpal necrosis in an immature tooth with an open apex presents a unique challenge to the dentist. Traditionally, multiple-visit apexification with calcium hydroxide was the treatment of choice in necrotic immature teeth, which aimed at formation of an apical hard tissue barrier[1]. An alternative technique for apexification is by placing an artificial barrier in apical portion of the root canal. The material of choice for this technique is mineral trioxide aggregate (MTA), which has been shown to have high success rates and reduce the number of required clinical sessions[2,3]. Both of these apexification methods share the same disadvantage of not allowing the continuation of root development resulting in fragile root structure[3].

Revascularization is a regenerative treatment and a biologically based alternative approach to treat necrotic immature teeth. Unlike apexification and artificial apical barrier techniques, it allows for continuation of root development[4]. This article describes a case of successful revascularization in necrotic immature lateral incisor using MTA with a follow-up period of 5 years.

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Case report

An 11-year-old boy reported for evaluation and treatment of a maxillary right lateral incisor (tooth 12).The patient was accompanied by his mother who reported that her son had suffered a traumatic injury to the upper right front tooth about a month ago with loss of the coronal fragment. The medical history of the patient was non-contributory, and the patient had no prior dental visit to the dental school. Clinical examination revealed that the tooth had an Ellis class III fracture (Fig. 1), and mobility and periodontal probing were within physiological limits. Pulp vitality was negative on cold and electric pulp testing (EPT), but the patient reported sensitivity to percussion and palpation. Periradicular radiographic examination revealed that the tooth had an incompletely developed apex and a periradicular radiolucency (Fig. 1). A diagnosis of pulpal necrosis with symptomatic apical periodontitis was made for tooth 12. The mother was informed about the limitations and advantages of revascularization as a

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Case Report/Clinical Techniques

Histological Findings of Revascularized/Revitalized Immature Permanent Molar with Apical Periodontitis Using Platelet-rich Plasma

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Abstract

Introduction: An immature mandibular right first molar (#30) with apical periodontitis of a 9-year-old boy was treated with a revascularization/revitalization procedure using either a mixture of platelet-rich plasma (PRP) and a blood clot or a blood clot alone on the same tooth. **Methods:** Tooth #30 fractured 2 years and 1 month after the revascularization/revitalization procedure and could not be saved. The tooth was extracted and processed for histologic examination to determine the nature of the tissues that formed in the canals. **Results:** Clinically, the endodontic treatment of the case was successful based on the resolution of apical periodontitis and the absence of clinical signs and symptoms. Histologically, the tissues formed in the distal and mesial canals were mineralized tissue similar to cementoid/osteoid tissue and uninflamed fibrous connective tissue regardless of PRP or no PRP treatment. No pulp-like tissue characterized by the presence of odontoblast-like cells polarized along the dentin-like mineralized tissue was observed. **Conclusions:** The tissues formed in the canals were mineralized tissue and some fibrous connective tissue. No pulp-like tissue characterized by the presence of odontoblast-like cells was observed lining the dentin-like mineralized tissue. *(J Endod 2013;39:138–144)*

Key Words

Apical periodontitis, cementoid/osteoid tissue, immature permanent tooth, platelet-rich plasma, revascularization/revitalization

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Traditionally, the treatment of immature permanent teeth with apical periodontitis is accomplished by an apexification procedure to achieve apical closure followed by nonsurgical root canal therapy (1). Since Iwaya et al (2) reported that a revascularization procedure using an antibiotic paste (metronidazole and ciprofloxacin) in immature permanent tooth with apical periodontitis could result in increased thickening of the canal walls, continued root development, and restoration of pulp vitality, revascularization has become a preferable treatment choice over apexification as shown in many recent case series reports (3).

In addition to elimination of the root canal infection, which is the most important aspect of the revascularization/revitalization procedure, an additional step regarded necessary is the induction of blood clot formation in the canal by provoking bleeding from the periapical tissue (4, 5). It is hypothesized that the blood clot serves as a matrix for migration of progenitor cells into the canal, possibly from the apical papilla (6). This procedure is similar to inducing bleeding and clot formation in the bony crypt after apical surgery to initiate bone wound healing. Bleeding and clot formation is an initial step of tissue wound healing, which will lead to granulation tissue formation, which is an essential component of wound healing. The purpose of the induction of bleeding into the canal space in the revascularization/revitalization procedure is to recreate the events of tissue wound healing (regeneration or repair). It has also been shown histologically that the outcome of revascularization of immature dog teeth with apical periodontitis either in the presence or absence of a blood clot in the canal space is not statistically significant (7). However, cell migration or movement requires extracellular matrix in the canal space.

The nature of the tissues formed in the canals of the revascularized/revitalized immature permanent teeth with apical periodontitis in humans is not currently known because no histologic studies have been reported. However, in animal studies, the tissues formed in the canal of the revascularized immature teeth with apical periodontitis were described as cementoid/osteoid tissue and periodontal ligament–like tissue (7–10). Surprisingly, no pulp-like tissue as characterized by the presence of odontoblast-like cells lining the dentin-like mineralized tissue was observed in animal studies. This suggests the possibility that the apical papilla did not survive in apical periodontitis; this is thought to provide a source of the progenitor cells driving root pulp development.

Recently, platelet-rich plasma (PRP) was used as a matrix instead of a blood clot in revascularization of the tooth with necrotic pulp and an open apex (11). PRP contains concentrated growth factors such as platelet-derived growth factor, transforming growth factor, vascular endothelial growth factor, epidermal growth factor, and insulin-like growth factor (12, 13). The target cells of the growth factors are not very clear at this point because there are multiple potential targets for each factor (14). PRP has been shown to enhance wound healing if the parenchymal tissue of the organs was not completely destroyed (13, 15). However, it is not known whether PRP could induce tissue regeneration if the parenchymal tissue was completely destroyed. Torabinejad and Faras (16) showed that pulp-like tissue could be generated in a human tooth with necrotic pulp and an open apex by using PRP as a scaffold in regenerative endodontic procedures. The generated tissue was removed from the canal of the tooth 14 months after the revascularization procedure, and histologic examination revealed vital pulp-like connective tissue although with no evidence of

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Pulp Revascularization after Root Canal Decontamination with Calcium Hydroxide and 2% Chlorhexidine Gel

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Abstract

Introduction: Pulp revascularization may be considered a promising alternative for necrotic immature teeth. Many studies have accomplished passive decontamination associated with an antibiotic paste. To date, there is no report evaluating calcium hydroxide associated with 2% chlorhexidine gel for revascularization therapy. The aim of this case report was to describe a new proposal for pulp revascularization with mechanical decontamination and intracanal medication composed of calcium hydroxide and 2% chlorhexidine gel. **Methods:** The patient, a 9-year-old girl, suffered an intrusion associated with pulp exposure caused by an enamel-dentin fracture in her maxillary left central incisor. After diagnosis, treatment consisted of revascularization therapy with gentle manual instrumentation of the cervical and medium thirds of the root in addition to intracanal medication with calcium hydroxide and 2% chlorhexidine gel for 21 days. In the second session, a blood clot was stimulated up to the cervical third of the root canal. Mineral trioxide aggregate (MTA; Angelus, Londrina, Paraná, Brazil) was used for cervical sealing of the canal. Coronal sealing was performed with temporary filling material and composite resin. **Results:** During the follow-up period, the root canal space showed a progressive decrease in width, mineralized tissue deposition on root canal walls, and apical closure. A cone-beam computed tomography scan taken at the 2-year follow-up confirmed these findings and did not show complete root canal calcification. **Conclusions:** This new proposal for revascularization therapy with 2% chlorhexidine gel may be used for the treatment of necrotic immature root canals. *(J Endod 2013;39:417–420)*

Key Words

Chlorhexidine, immature teeth, pulp revascularization

Pulp revascularization may be a promising alternative for the treatment of immature teeth according to animal model studies (1–3) and case reports (4–8) that showed the continuation of root development with thickening of root walls and apical closure. This therapy promotes invagination of stem cells from the apical region in young patients with open apexes (9, 10). Compared with apexification, pulp revascularization requires a shorter treatment time and offers a shorter time to apical closure (11).

A variety of root canal decontamination protocols have been discussed in several case reports (6, 8, 12, 13). They mainly involve passive decontamination with irrigants (7, 14) in an attempt to maintain cell viability. These cells could differentiate and promote root end development in association with an intracanal dressing. In case reports (11, 13, 14), the most investigated intracanal dressing up to now has been a paste containing 3 antibiotics (ie, metronidazole, minocycline, and ciprofloxacin) that showed antimicrobial ability against the main endodontic pathogens (2) and root development. However, this paste may have some disadvantages such as the development of bacterial resistance and allergic reactions and one of its components (ie, minocycline) may produce crown discoloration (7). Considering these disadvantages, calcium hydroxide may be a promising alternative for intracanal medication because of its antimicrobial properties (15, 16), the unlikelihood of crown discoloration (17), the possible release of growth factors and biomolecules from dentin (18), and the availability of this medication in routine clinical practice. Recently, some case reports have shown satisfactory results with the use of calcium hydroxide medications in aqueous vehicles (8, 19, 20). Besides the use of aqueous vehicles, some studies have shown antimicrobial properties of different associations of intracanal medicaments such as calcium hydroxide and 2% chlorhexidine gel (21, 22). Chlorhexidine has antimicrobial properties and low cytotoxicity; this may be a safe alternative if this medication came into contact with periapical tissues in immature teeth (23, 24). Nevertheless, to date, no study has shown remission of symptomatology and root development with the use of endodontic instruments in the root canal in association with intracanal dressing of calcium hydroxide and 2% chlorhexidine gel. Thus, the aim of this case report was to discuss a clinical case treated with a new proposal for root decontamination with pulp revascularization therapy.

Case Report

The patient, a 9-year-old girl, was referred to the Dental Trauma Service of the Piracicaba Dental School, State University of Campinas, Piracicaba, São Paulo, Brazil,

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67.

Pulp Revascularization of Immature Dens Invaginatus with Periapical Periodontitis

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Abstract

Introduction: Dens invaginatus is a rare developmental malformation of a tooth caused by the invagination of the tooth crown before biological mineralization occurs. The complex anatomy of these teeth makes nonsurgical endodontic treatment difficult and more so when there is presence of periapical periodontitis with open apex. The endodontic treatment of dens invaginatus is a challenge, especially in the case of periapical periodontitis with open apex. Pulp revascularization is a conservative endodontic treatment that has been introduced in recent years. Presented here is a variant approach for the treatment of immature dens invaginatus type II with periapical periodontitis, which combines filling of the invagination and pulp revascularization. **Methods:** After accessing the pulp chamber, the main canal and the invagination were explored. The root was thoroughly disinfected by irrigating and medication, invagination was filled, and the main canal was revascularized. Then the coronal sealing was made by glass ionomer cement and composite resin. Radiograph taken regularly and computed tomography scan were used to investigate the healing of the periapical lesion and development of the root. **Results:** In the subsequent follow-up, the periapical lesion was completely eliminated, the open apex was closed, and the wall of the root was thickened. **Conclusions:** For type II immature dens invaginatus with large periapical lesion, conservative endodontic treatment should be considered before periapical surgery. With sufficient infection control, pulp revascularization can be an effective alternative method. *(J Endod 2013;39:288–292)*

Key Words

Conservative endodontic treatment, dens invaginatus, immature permanent tooth, periapical periodontitis, pulp revascularization

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Dens invaginatus is a rare malformation of a tooth caused by the invagination of the tooth crown before biological mineralization occurs. Other names for this type of malformation are dens in dente, invaginated odontoma, dilated composite odontoma, tooth inclusion, and dentoid in dente (1). In the published literature, controversy exists regarding the terminology of this anatomic defect because of lack of consensus on the etiology of the anomaly.

Currently, there are several theories giving different hypotheses on the etiology of dens invaginatus; however, no agreement has been reached, and it remains unclear (1, 2). Hülsmann (1) presented a number of theories that have been proposed to explain the mechanisms of dental coronal invagination, including growth pressure of the dental arch, focal failure of growth of the internal enamel epithelium, rapid and aggressive proliferation of a part of the internal enamel epithelium, distortion of the enamel organ, fusion of 2 tooth germs, infection, and trauma.

The incidence of dens invaginatus has been reported to be in the range of 0.04%–10% (3, 4), with the upper lateral incisors most commonly involved. Isolated cases have been reported in the mandibular region and in the deciduous dentition (5–7).

The most popular and commonly used classification of invaginated teeth has been proposed by Oehlers (2): type I, invagination confined within the crown; type II, invagination as a blind sac extending beyond the cementoenamel junction but not reaching the periodontal ligament; and type III, invagination extending beyond the cementoenamel junction, with the second foramen extending into the periapical tissues or into the periapical area.

In most cases, the thin or incomplete enamel lining of the invagination cannot prevent the entry of bacteria into the pulp, which leads to pulp necrosis and eventually develops into periapical inflammation. The complex anatomy of dens invaginatus may compromise instrumentation, disinfection, and obturation of the root canals. Moreover, the infected invaginations may also lead to the early necrosis of pulp tissue before the completion of root development, leaving the tooth with an open apical foramen.

The treatment options for an invaginated tooth include preventive sealing or filling of the invagination, root canal treatment, endodontic periapical surgery, intentional replantation, and extraction. Treatment of an invaginated tooth can be combined with periapical surgery after insufficient root canal treatment or intentional replantation after failed periapical surgery (8, 9). In certain cases, endodontic treatment techniques may involve removal of the dens invaginatus from the root canal, apexification, obturation of the invagination alone while maintaining pulp vitality, and surgical treatment of the invagination alone (10–14).

The treatment of immature permanent tooth with periapical periodontitis is a challenge in pediatric dentistry, especially in the anatomic abnormal tooth such as dens invaginatus. The traditional treatment of immature permanent tooth with periapical periodontitis is apexification because there is little to no expectation of continued root development, and because of the likelihood of root fracture and reinfection, the prognosis of this method is not ideal.

In recent years, several clinical case reports proved that for immature permanent tooth with pulp necrosis or periapical periodontitis, continued root development can be accomplished by conservative treatment after disinfection of the root canal system, evoked bleeding into the root canal, and adequate coronal seal. Those procedures are called pulp revascularization of immature permanent tooth (15–18). Increasing

Revascularızatıon/Regeneratıon Performed in Immature Molars: Case Reports

Sönmez IS*/ Akbay Oba A**/ Erkmen Almaz M***

These 3 case reports the outcome of revascularization treatment in necrotic immature molars. During treatment, a tri antibiotic mix was used to disinfect the pulp for 2 weeks. Then a blood clot was created in the canal, over which mineral trioxide aggregate was placed. After 24 months, the immature molars showed continuation of root development. The patients were asymptomatic, no sinus tracts were evident and apical periodontitis was resolved. Results from these cases show that revascularization/regeneration using 3Mix-MP method could be effective for managing immature permanent molar teeth with pulpal necrosis.

Keywords: *Immature permanent teeth, MTA, pulpal necrosis, revascularization*

INTRODUCTION

Endodontic treatment of an immature permanent tooth with pulp necrosis is a very difficult and time consuming procedure for both patients and dentists . The traditional treatment of such a tooth is apexification, that involves long-term application of calcium hydroxide.[1,2] Apexification induces further development of an apex to close the foramina with a calcific barrier,[3,4] but has a disadvantage of a requirement for multiple visits and a long time period before completion of a root filling. More recently, placement of mineral trioxide aggregate (MTA) acting as an artificial barrier for the alternative apexification protocol has been used. With this technique, the duration of treatment is shortened and is generally completed in one or two appointments.[5] However, both calcium hydroxide or MTA placement do not promote the continued development of the root6, so with a thin root dentin and large canal lumen, the tooth is prone to fracture.[3]

Pulp tissue of an immature open-apex tooth has a great potential to regenerate in response to damage, owing to its rich blood supply and stem cells. Therefore to allow successful apexogenesis (normal development of the root apex of a tooth) to occur, diseased open-apex teeth should be treated as conservatively as possible.[2] Contrary to apexification, apexogenesis encourages a longer and thicker dentinal composed root to develop.[5] In recent years, the concept of 'Pulp Revascularization/regeneration' therapy has been developed, that attempts to obtain a longer and thicker root.[7] The key factor for the success of revascularization/regeneration procedure is disinfection of the root canal system.[1] For the disinfection of the infected root canals; various combinations of antibiotics have been shown to be effective, one example is a mixture of ciprofloxacin, metronidazole, and minocycline. Also in an *in vitro* study the tri-antibiotic paste (mixture of ciprofloxacin, metronidazole, and minocycline) has been shown to sufficiently potent to eradicate bacteria from the infected dentin root canals.[8]

The purpose of these case reports was to examine the effects of pulp revascularization/regeneration with 3Mix-MP paste (including ciprofloxacin, metronidazole, minocycline with propylene glycol and macrogol) for immature necrotic permanent molars.

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Case Reports

Three patients, each with an immature permanent molar tooth with pulp necrosis, who referred to the Pediatric Dentistry Clinic of Kırıkkale University Faculty of Dentistry, Kırıkkale, Turkey, were attended to the study. Clinically, all teeth presented deep carious lesions and radiographically, the teeth had an immature apex with periapical radiolucency. Vitality, percussion and palpation exams were performed on the teeth and adjacent teeth. The diagnosis of pulp necrosis and chronic apical abscess was made for the teeth. As the parents did not want extraction of the teeth, other treatment options were offered to the parents. The treatments were carried out with institutional review board approval. The procedures, possible discomforts or risks, as well as possible benefits were explained fully to the parents' of children involved, and their informed consent was obtained prior to the investigation.

The teeth were anesthetized with a local injection of Ultracaine DS (articaine hydrochloride 4% with epinephrine 1:100,000) and isolated with rubber dam. The access cavity was prepared and the root canal was gently flushed with 10 ml of 5.25% NaOCl solution positioned 1-2 mm below the root canal orifice. Then the canal was dried with sterile paper points (DiaDent, Burnaby, BC, Canada) and 3Mix-MP paste was prepared as described by Hoshino *et al*,[8] placed in the root canal and left for 2 weeks. The access cavity was sealed with Cavit G (3M ESPE, St. Paul, MN, USA). In the next visit, if the teeth were symptom free, the paste was removed by rinsing with

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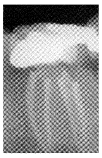

CASE REPORT

Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin: a case report

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Abstract

Keswani D, Pandey RK. Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin: a case report. *International Endodontic Journal*.

Aim To discuss the clinical and radiological outcome of a revascularization procedure in an immature tooth with a necrotic pulp using platelet-rich fibrin (PRF), a second-generation platelet concentrate.

Summary A 7-year-old male reported with pain in the maxillary anterior region as a result of the injury to his immature right maxillary central incisor tooth. After preparation of the access cavity, the necrotic pulp was removed and the canal irrigated using 5.25% sodium hypochlorite solution. The canal was then dried and packed with triantibiotic paste for 3 weeks. After removal of the antibiotic paste, a 5 mL sample of whole venous blood was drawn from the patient's forearm to prepare PRF. The freshly prepared PRF was condensed in the canal until the cementoenamel junction. Three millimetres of white MTA was placed directly over the PRF clot. Three days later, the tooth was restored using a permanent restoration. At 7-, 12- and 15-month recall, the tooth was asymptomatic with no sensitivity to percussion or palpation. At 12- and 15-month follow-up, the tooth responded positively to cold and an electric pulp test, and similarly to adjacent lateral incisor and canine teeth. Radiographic examination demonstrated continued thickening of root canal walls, root lengthening and apical closure.

Key learning point

- Platelet-rich fibrin might serve as a potentially ideal scaffold in revascularization of immature permanent teeth with necrotic pulps as it is rich in growth factors, enhances cellular proliferation and differentiation, and acts as a matrix for tissue ingrowth.

Keywords: growth factors, immature tooth, open apex, platelet-rich fibrin, revascularization.

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Platelet-rich fibrin-mediated revitalization of immature necrotic tooth

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Abstract

Contemporary studies have shown that the regeneration of tissues and root elongation is possible in necrotic immature permanent teeth. The purpose of this case report is to add a new vista in regenerative endodontic therapy by using platelet rich fibrin for revitalization of immature non vital tooth. An 11year old boy with the history of trauma was diagnosed with the pulpal necrosis and symptomatic apical periodontitis in tooth #21. Intra oral periapical radiograph showed open apex and associated immature supernumerary tooth with respect to tooth #21. Access preparation and minimal instrumentation was done to remove necrotic debris under copious irrigation with 2.5% sodium hypochlorite. Triple antibiotic paste was packed in the canal for four weeks. During second visit, 5 mL of whole blood was drawn from the medial cubital vein of the patient and blood was then subjected to centrifugation at 2400 rpm for 12 minutes for the preparation of Platelet rich fibrin (PRF) utilizing Choukroun's method. Triple antibiotic paste was removed and canal was dried. PRF clot was pushed to the apical region of tooth #21 using hand pluggers. Three millimetres of Mineral trioxide (MTA) was placed in cervical part of the root canal and permanent restoration was done three days later. Clinical examination at 6 and 12 months revealed no sensitivity to percussion and palpation in tooth #21and it responded positively to both electric pulp and cold tests. Radiographic examination showed resolution of periapical rarefaction, further root development and apical closure of the tooth #21 and its associated supernumerary tooth. On the basis of successful outcome of the present case it can be stated that PRF clot may serve as a scaffold for regeneration of necrotic immature teeth.

Keywords: Platelet-rich fibrin, revitalization, triple antibiotic paste

Introduction

The contemporary non-surgical endodontic management of mature teeth has shown favorable outcome rates of 95% in teeth diagnosed with irreversible pulpitis[1] and 85% in necrotic cases.[2] But this management in necrotic immature permanent teeth offers limited predictability and has a questionable prognosis due to thin dentinal walls.[3,4] Moreover open apex is difficult to seal either by thermo-plasticized or lateral condensation methods.[5] Traditionally such teeth were managed with apexification (Frank AL 1966 and Steiner 1968) which entailed the long-term treatment with calcium hydroxide resulting in formation of porous hard tissue apical barrier[6,7] but it has been shown that either the short-term (Rosenberg *et al.*, 2007)[8] or long-term (Andreason *et al.*, 2002 and 2006)[9,10] use of calcium hydroxide can reduce the root strength due to denaturation of collagen. This finding is consistent with the large case series by Cvek M in 1992 which showed that major reason for tooth loss following such apexification was tooth fracture.[3] To overcome the drawbacks of patient compliance and incomplete barrier, Torabinejad[11] in 2000 and Witherspoon *et al.*,[12] in 2000 pursued the single-step apexification using MTA. Ballesio *et al.*,[13] and Felippe *et al.*,[14] in 2006 showed that one-step apexification also does not generally result in further root development and thin dentinal walls still pose a challenge to the clinicians.

The teeth with poor crown root ratio and thin dentinal walls are more susceptible to fracture by secondary injuries and are also poor candidates for the restorative procedures.[4,15]

Therefore, there was a paradigm shift in the treatment protocol of such teeth from apexification to regenerative procedures.[16,17] This approach was extrapolated from successful revascularization of avulsed immature permanent teeth after replantation in animals[18] and humans.[19] Hargreaves *et al.*, in 2008 reported that regenerative endodontic procedures are possible by application of the principles of the tissue engineering that requires the spatial orientation of stem cells, signaling molecules, and the scaffold.[20,21] It has been reported that the remnants of Hertwig's epithelial root sheath or cell rests of Mallasez are resistant to peri-apical infections.[22] Thus, the signaling networks from these remnant cells may stimulate various stem cells like stem cells from apical papilla (SCAP),[23] bone marrow,[24] and multipotent pulp stem cells[25] to form odontoblasts-like cells in non-vital, immature,

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Case Report

Comparative outcome of revascularization in bilateral, non-vital, immature maxillary anterior teeth supplemented with or without platelet rich plasma: A case series

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Abstract

Loss of pulp vitality in an immature permanent tooth arrests root development. This leads to tooth with open apex and weak lateral dentinal walls. Management of such necrotic teeth with immature roots poses several treatment challenges. The documented study was performed to evaluate and compare apexogenesis induced by revascularization, with and without platelet rich plasma (PRP) in non-vital, immature anterior teeth. Three patients having bilateral, non-vital, immature maxillary central incisors with apical periodontitis were recruited after institutional ethical clearance. Subsequent to chemo-mechanical preparation, revascularization with and without PRP was randomly induced in either of the tooth. The cases were followed-up clinically and radiographically at 6 and 12 months. There was a marked difference in periapical healing, apical closure and dentinal wall thickening of teeth treated by revascularization with PRP.

Keywords: Open apex; platelet rich plasma and chemo-mechanical preparation; revascularization

INTRODUCTION

In young children, anterior teeth by virtue of their position in the dental arch are prone to trauma. In cases of complicated tooth fracture with the loss of pulp vitality, cessation of root development with an open apex is a serious sequel. Management of such immature, non-vital teeth is a challenge. Until date, treatment protocols mentioned were surgery and retrograde sealing, apical barrier formation with calcium hydroxide[1] or mineral trioxide aggregate (MTA)[2] and customized or thermoplasticized gutta-percha obturation technique.

Recently, there has been a paradigm shift in the management of these teeth based on the "regenerative concept." Case series/reports have documented the efficacy of revascularization. This involves intentional induction of bleeding from the peri-apex and formation of an intra-canal blood clot, which acts as a scaffold. Platelet rich plasma (PRP), an autologous first generation platelet concentrate with a rich source of growth factors has been proposed as a potential addendum/substitute scaffold to improve the outcome of this procedure.[3,4] Until date only few case reports on the use of PRP in the revascularization are cited in the literature.[5] This case series comparatively evaluates the outcome of revascularization with and without PRP in three cases of bilateral, non-vital, immature, anterior teeth in terms of periapical healing (PAH), apical closure (AC), lateral dentinal wall thickening and root lengthening (RL).

CASE REPORTS

Case I

A healthy 10-year-old boy was referred for evaluation and management of his broken upper anterior teeth, subsequent to a fall 3-year prior. Root canal therapy was initiated, but not completed by a general dental practitioner. Intraoral examination revealed Ellis class III fracture and a well-defined, localized swelling in relation to both the upper central incisors. Radiographic examination showed immature open apices with thin dentinal walls in relation to both the teeth [Figure 1a]. Based on clinical and radiographic examination, a diagnosis of acute periapical abscess in relation to both the central incisor

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Case report

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RDE
Restorative Dentistry & Endodontics

Apexogenesis and revascularization treatment procedures for two traumatized immature permanent maxillary incisors: a case report

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Traumatic injuries to an immature permanent tooth may result in cessation of dentin deposition and root maturation. Endodontic treatment is often complicated in premature tooth with an uncertain prognosis. This article describes successful treatment of two traumatized maxillary central incisors with complicated crown fracture three months after trauma. The radiographic examination showed immature roots in maxillary central incisors of a 9-year-old boy with a radiolucent lesion adjacent to the right central incisor. Apexogenesis was performed for the left central incisor and revascularization treatment was considered for the right one. In 18-month clinical and radiographic follow-up both teeth were asymptomatic, roots continued to develop, and periapical radiolucency of the right central incisor healed. Considering the root development of these contralateral teeth it can be concluded that revascularization is an appropriate treatment method in immature necrotic teeth. (*Restor Dent Endod* 2013;38(3):178-181)

Key words: Apexogenesis; Open apex; Pulpotomy; Pulp revascularization; Trauma

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Introduction

Traumatic injuries to the teeth may result in pulpal and periapical disease. Most dental traumas occur in the 7 - 10 year-old age group with incomplete apical root development.[1,2] Complicated crown fractures which involve the enamel, dentin and pulp occur in 0.9 - 13% of all dental injuries.[3,4] Vital pulp therapy (VPT) is the treatment of choice for traumatized immature teeth with pulp exposure.[5,6] VPT allows continuation of root development, which leads to apical closure and strengthening of the root structure.[7] If the pulp vitality of a traumatized immature tooth is lost, the treatment will be a challenge in endodontics. It is difficult to obtain an appropriate apical seal in these teeth by using the conventional obturation methods. Furthermore, thin root canal walls make the teeth susceptible to future fractures.[8] Several different clinical methods have been used to treat these teeth, such as long-term calcium hydroxide apexification and one-visit apexification (use of a material to create an artificial barrier). Recently, regenerative endodontic procedures have drawn much attention. The advantage of this treatment modality over apexification is that it allows root maturation to continue by generating vital tissue.[9]

This case report presented regenerative therapy of a traumatized immature maxillary central incisor with apical abscess. The outcome of root development in this tooth was compared with contralateral tooth that treated using vital pulp therapy.

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Clinical, Radiographic, and Histological Observation of a Human Immature Permanent Tooth with Chronic Apical Abscess after Revitalization Treatment

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Abstract

Introduction: Revitalization procedures have been widely used for the treatment of immature permanent teeth with apical periodontitis. The treatment procedures appear to be capable of encouraging continued root development and thickening of the canal walls. The nature of tissues formed in the canal space and at the root apex after revitalization has been shown histologically in several animal studies; similar studies in humans were recently reported. **Methods:** A 9-year-old boy had a traumatic injury to his upper anterior teeth. Tooth #9 suffered a complicated crown fracture with a pulp exposure, which was restored with a composite resin. The tooth developed a chronic apical abscess. Revitalization procedures were performed on tooth #9 because it was an immature permanent tooth with an open apex and thin canal walls. Twenty-six months after revitalization, the tooth had a horizontal crown fracture at the cervical level and could not be restored. The tooth was extracted and processed for routine histological and immunohistochemical examination to identify the nature of tissues formed in the canal space. **Results:** Clinically and radiographically, the revitalization of the present case was successful because of the absence of signs and symptoms and the resolution of periapical lesion as well as thickening of the canal walls and continued root development. The tissue formed in the canal was well-mineralized cementum- or bone-like tissue identified by routine histology and immunohistochemistry. No pulp-like tissue characterized by the presence of polarized odontoblast-like cells aligning dentin-like hard tissue was observed. **Conclusions:** The tissues formed in the canal of revitalized human tooth are similar to cementum- or bone-like tissue and fibrous connective tissue. *(J Endod 2013;39:1078–1083)*

Key Words

Apical periodontitis, bone-like tissue, cementum-like tissue, immature permanent tooth, revitalization

Since the report of Iwaya et al (1) that a type of treatment termed "revascularization" was applied to treat human immature permanent tooth with apical periodontitis and sinus tract and resulted in not only resolution of apical periodontitis but also thickening of the root canal walls and continued root development, many human immature permanent teeth with apical periodontitis have been treated with revascularization procedures instead of traditional apexification procedures (2). It has been discussed that the term "revitalization" is more appropriate to describe such clinical treatment; therefore, revitalization will be used herein (3, 4). The nature of tissues formed in the root canals of immature teeth with apical periodontitis after revitalization was described histologically as mineralized tissue resembling cementum or bone and periodontal ligament-like tissue in several animal studies (5–8). The nature of the tissue formed in the root canals of human revitalized teeth was recently reported (9–11). We report clinical, radiographic, and histological observation of a human immature permanent tooth with chronic apical abscess after revascularization treatment.

Case Report

Diagnosis and Treatment Planning Visit

A 9-year old boy was referred from the Postgraduate Pediatric Clinic to the Postgraduate Endodontic Clinic at New York University College of Dentistry for the evaluation of tooth #9. The patient had a history of trauma to the upper anterior teeth that occurred from a fall approximately 3 months ago. The patient's chief complaint was "I hit my front teeth 3 months ago, and now I have an infection." The patient did not report any symptoms. Clinical examination showed that tooth #9 had a complicated crown fracture with a pulp exposure, which was restored with a composite resin. A sinus tract was present in the apical area of tooth #9. The tooth was not sensitive to percussion and palpation. It also did not respond to Endo Ice (Coltene/Whaledent Inc, Cuyahoga, OH) or an electric pulp tester (Vitality Scanner; SybronEndo, Glemdora, CA). Radiographic examination revealed a well-circumscribed large periapical radiolucent lesion measuring approximately 8×8 mm around the apex of tooth #9 (Fig. 1*A*). The immature tooth had thin canal walls and an open apex (Fig. 1*A*). The clinical diagnosis for tooth #9 was pulpal necrosis and chronic apical abscess. Treatment options including revitalization,

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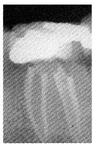

CASE REPORT

Concentrated platelet-rich plasma used in root canal revascularization: 2 case reports

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Abstract

Bezgin T, Yılmaz AD, Çelik BN, Sönmez H. Concentrated platelet-rich plasma used in root canal revascularization: 2 case reports. *International Endodontic Journal.*

Aim To present two case reports describing the treatment of immature teeth with necrotic pulps using concentrated platelet-rich plasma (cPRP) with 12-month clinical and radiographic follow-up.
Summary Root canal revascularization was performed on immature permanent teeth clinically and radiographically diagnosed as requiring root canal treatment. Following disinfection of the canal space with triple antibiotic paste (1 : 1 : 1: ciprofloxacin, metronidazole and cefaclor), a tissue scaffold was created with cPRP prepared using 2-step centrifugation. The final restoration was completed with white mineral trioxide aggregate and composite resin. The patients were recalled for clinical and radiographic evaluations every 3 months. At the 12-month follow-up apical closure by narrowing of the apical foramen and convergence of the apical walls in the treated teeth was observed.
Key learning points
- Concentrated platelet-rich plasma (cPRP) was successful as a scaffold in revascularization treatment.
- 2-step centrifugation was a simple and useful technique to prepare PRP.

Keywords: concentrated platelet-rich plasma, immature teeth, revascularization.

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Introduction

Regenerative endodontics refers to the biologically based treatment that is designed to replace damaged structures and cells in the pulp–dentine complex with live viable tissue, which restore normal physiologic functions. These procedures may be of particular value in the treatment of immature permanent teeth with necrotic pulps in terms of

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75.

Histologic Study of a Human Immature Permanent Premolar with Chronic Apical Abscess after Revascularization/Revitalization

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Abstract

Introduction: Histologic studies of teeth from animal models of revascularization/revitalization are available; however, specimens from human studies are lacking. The nature of tissues formed in the canal of human revascularized/revitalized teeth was not well established. **Methods:** An immature mandibular premolar with infected necrotic pulp and a chronic apical abscess was treated with revascularization/revitalization procedures. At both the 18-month and 2-year follow-up visits, radiographic examination showed complete resolution of the periapical lesion, narrowing of the root apex without root lengthening, and minimal thickening of the canal walls. The revascularized/revitalized tooth was removed because of orthodontic treatment and processed for histologic examination. **Results:** The large canal space of revascularized/revitalized tooth was not empty and filled with fibrous connective tissue. The apical closure was caused by cementum deposition without dentin. Some cementum-like tissue was formed on the canal dentin walls. Inflammatory cells were observed in the coronal and middle third of revascularized/revitalized tissue. **Conclusions:** In the present case, the tissue formed in the canal of a human revascularized/revitalized tooth was soft connective tissue similar to that in the periodontal ligament and cementum-like or bone-like hard tissue, which is comparable with the histology observed in the canals of teeth from animal models of revascularization/revitalization. *(J Endod 2014;40:133–139)*

Key Words

Bone-like tissue, cementum-like tissue, chronic apical abscess, human immature permanent tooth, revascularization/revitalization

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Many case reports and case series of revascularization/revitalization of human immature permanent teeth with apical periodontitis have been reported in the literature. However, a high level of evidence concerning the outcome of revascularization/revitalization is lacking. The resolution of apical periodontitis, thickening of the canal walls, and continued root development are often reported in published cases after revascularization/revitalization of immature permanent teeth with apical periodontitis. Five types of hard-tissue responses of human immature permanent teeth with apical periodontitis after revascularization/revitalization have been described (1). In some cases, no to minimal thickening of the canal walls and closure of the root apex without root lengthening were observed radiographically (1–4). Some authors speculated that this was because of the blood clot in the canal breaking down, leaving no scaffold for new tissue to grow into the empty canal space (4). Histologic studies of revascularized teeth in animals showed that the tissue growing in the pulp space was fibrous soft connective tissue and cementum-like or bone-like hard tissue (5–8). Most recently, 2 case reports described the histology after successful revascularization procedures in humans (9, 10). It was confirmed that the tissue formed in the canal space was cementum-like or bone-like tissue. No dentin-like tissue or odontoblast-like cells were observed.

The purpose of this case report was to describe histologically a human immature mandibular premolar after a revascularization/revitalization procedure that initially had infected necrotic pulp and a chronic apical abscess. Radiographically, the revascularized/revitalized tooth showed resolution of the periapical lesion, minimal thickening of the canal walls, and narrowing of the root apex without root lengthening.

Case Report

An 11-year-old girl was brought to the dental office because she noted a localized swelling on the mucosa in the area of the mandibular left premolars. The patient did not have any spontaneous or evoked pain or other symptoms. Clinical examination showed that the crown of tooth #20 was caries free but had a dens evaginatus with a fractured cusp. A sinus tract was present buccally in association with the mandibular second premolar. The tooth was not tender to percussion (vertical and lateral) or palpation. No mobility was noted. The tooth showed no response to sensibility tests (ie, heat, cold, and electric pulp test). A periapical radiograph of tooth #20, taken after tracing the sinus tract with a gutta-percha point, showed that a large radiolucent lesion was present in the periapical area (Fig. 1*A*). The root apex was open, and the canal walls appeared to be thin (Fig. 1*A*).

The clinical diagnosis of tooth #20 was pulpal necrosis and a chronic apical abscess with a sinus tract. Treatment options and procedures, including apexification and revascularization/revitalization, were explained to the patient and patient's parents. They chose the revascularization/revitalization procedure of the tooth for the child, and informed consent was obtained.

First Session

No anesthesia was administered to see if vital tissue was present in the apical portion of the canal in the first session. The tooth was isolated with a rubber dam, and an adequate access cavity was prepared. No vital tissue was observed in the apical

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Histologic and Histobacteriologic Observations of Failed Revascularization/Revitalization Therapy: A Case Report

Louis M. Lin, BDS, DMD, PhD, Emi Shimizu, DDS, PhD,* Jennifer L. Gibbs, MAS, DDS, PhD,* Simona Loghin, DDS,† and Domenico Ricucci, MD, DDS†*

Abstract

Introduction: Mechanical debridement plays an important role in eliminating intracanal bacteria, such as biofilm on the canal walls and bacteria in the dentinal tubules. Mechanical debridement is not recommended for root canal disinfection in revascularization/revitalization therapy. Here we report a failed revascularization/revitalization case, which could be due to inadequate root canal disinfection without mechanical removal of biofilm and bacteria in dentinal tubules. **Methods:** A 6-year-old boy had a traumatic injury to tooth #9, which was avulsed and replanted within 40 minutes. The tooth subsequently developed a local swelling in the periapical area. The patient was referred to the Postgraduate Endodontic Clinic for revascularization/revitalization therapy on tooth #9. The treated tooth remained asymptomatic for 16 months and then developed pain and local periapical swelling. The oral surgeon extracted the revascularized/revitalized tooth. On request, the extracted tooth was processed for histologic and histobacteriologic examination. **Results:** The tissue in the canal was completely destroyed. Most bacteria were observed in the apical portion and not in the coronal portion of the canal and formed biofilm on the canal walls and penetrated into the dentinal tubules. **Conclusions:** On the basis of histobacteriologic observations, the failure of revascularized/revitalized tooth could be due to inadequate root canal disinfection without mechanical debridement. It may be important to perform mechanical debridement as part of the revascularization/revitalization therapy to disrupt the biofilm on the canal walls and remove bacteria in the dentinal tubules because revascularization/revitalization therapy is able to increase thickening of the canal walls. *(J Endod 2014;40:291–295)*

Key Words

Immature permanent tooth, reinfection, revascularization/revitalization, root canal disinfection

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Bacterial infection is the primary cause of pulpal and periapical disease (1). When the root canal is infected, bacteria colonize the canal walls as biofilm (2, 3) and penetrate into the dentinal tubules (4, 5) and lateral/accessory canals (6) as well as isthmuses (7). The number and depth of dentinal tubules invaded by bacteria are higher and deeper in the teeth of young than of old people (8, 9). Bacteria in biofilm firmly attached to the canal walls and in the canal dentinal tubules are very hard to eliminate by canal irrigants and intracanal medicaments during root canal therapy (10–14). In addition, biofilm can evade the host's innate and adaptive defense mechanisms and resist antimicrobial chemotherapy (15).

Similar to open flap debridement for marginal periodontitis, root canal therapy requires mechanical instrumentation or debridement to remove biofilms, canal walls contaminated by bacteria toxins, and bacteria in the dentinal tubules (16, 17). The current protocol used to control root canal infection of immature permanent teeth with infected necrotic pulp in revascularization/revitalization procedures is by irrigation with sodium hypochlorite and intracanal medication with calcium hydroxide or triple antibiotics without mechanical debridement (18). Regardless of apexification or revascularization/revitalization procedures, effective control of root canal infection is the key to the success of both treatments. Infection/inflammation prevents tissue regeneration and stem cell differentiation (19) and therefore has to be under control for wound healing to take place. It has been suggested that control of root canal infection in revascularization/revitalization procedures might have to be more thorough than that of regular root canal therapy for pulp tissue regeneration to occur (20). No failures of revascularization/revitalization cases have been reported by using sodium hypochlorite irrigation and triple antibiotics intracanal medication without mechanical debridement in human (21) and in animal studies (22–24), which are of short-term observations. These studies give the impression that the clinical outcome of revascularization/revitalization therapy is a 100% success.

We have experienced failed revascularization/revitalization cases by using current root canal disinfection protocol and had to re-treat the failed cases with apexification procedures. The purpose of the present case report is to describe a failed immature permanent tooth with infected necrotic pulp and acute apical abscess after revascularization/revitalization procedures by histologic and histobacteriologic examination. The failure of the present case could be caused by an inadequate control of intracanal infection by using sodium hypochlorite irrigation and calcium hydroxide as well as triple antibiotics paste intracanal dressing without mechanical debridement of the infected canal. Although this is a single case report, we hope that it will call attention to reevaluate the effectiveness of root canal disinfection protocol of revascularization/revitalization procedures *in vivo* to improve the success of revascularization/revitalization therapy.

Case Report

A 6-year-old boy was referred to the Postgraduate Endodontic Clinic at New York University College of Dentistry from a university-affiliated City Hospital for treatment of tooth #9. The patient's medical history was not contributory. Tooth #9 was avulsed and replanted with rigid splint by an oral surgeon at the City Hospital within 40 minutes of avulsion approximately 4 months prior. The general dentist at the City Hospital performed instrumentation of the root canal space because the patient developed a localized swelling in the periapical area of tooth #9 about 2 months after tooth replantation.

77.

Regenerative Potential of Immature Permanent Teeth with Necrotic Pulps after Different Regenerative Protocols

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Ahmed Abdel Rahman Hashem, BDS, MSc, PhD,[*†] *and Ashraf M. Abu-Seida, BVSc, MVSc, PhD*[‡]

Abstract

Introduction: Regenerative endodontics is a promising alternative treatment for immature teeth with necrotic pulps. The present study was performed to assess the regenerative potential of young permanent immature teeth with necrotic pulp after the following treatment protocols: (1) a mineral trioxide aggregate (MTA) apical plug, (2) the regenerative endodontic protocol (blood clot scaffold), and (3) the regenerative endodontic protocol with a blood clot and an injectable scaffold impregnated with basic fibroblast growth factor. **Methods:** Immature necrotic permanent maxillary central incisors (n = 36) of patients 9–13 years old were divided into 3 groups according to the treatment protocol: the MTA group (MTA apical plug), the REG group (regenerative endodontic protocol [blood clot]), and the FGF group (regenerative endodontic protocol [blood clot + injectable scaffold]). Follow-up was done up to 18 months. Standardized radiographs were digitally evaluated for an increase in root length and thickness, a decrease in the apical diameter, and a change in periapical bone density. **Results:** After a follow-up period of 18 months, most of the cases showed radiographic evidence of periapical healing. Groups 2 and 3 showed a progressive increase in root length and width and a decrease in apical diameter. **Conclusions:** The regenerative endodontic procedure allowed the continued development of roots in teeth with necrotic pulps. The use of artificial hydrogel scaffold and basic fibroblast growth factor was not essential for repair. *(J Endod 2014;40:192–198)*

Key Words

Basic fibroblast growth factor, hydrogel scaffold, mineral trioxide aggregate, regeneration, revascularization

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The treatment of immature permanent teeth with necrotic pulp constitutes a challenging situation facing endodontists. Such conditions present difficulty in root canal debridement and obturation because of the open apex. Moreover, they are more prone to fracture because of thin weak dentinal root canal walls. Such cases were traditionally treated by apexification procedures using calcium hydroxide (1). Such management requires long-term placement of calcium hydroxide inside the root canal to induce the formation of an apical hard tissue barrier. Recently, many authors advocated the placement of an orthograde apical plug (2–4). Mineral trioxide aggregate (MTA) proved to be an excellent candidate; however, apical plugs do not solve the problem of the thin and weak dentinal root canal walls (5, 6).

Periapical tissues in immature teeth are rich in blood supply and contain stem cells that have the potentiality for tissue regeneration (7). Under suitable conditions, stem cells can be programmed for self-regeneration to restore the lost part. Hence, the concept of regeneration of immature nonvital teeth was advocated. Eradication of bacteria from the canal space is mandatory for successful regenerative endodontic procedures. Research with topical antibiotics showed that a combination of metronidazole, minocycline, and ciprofloxacin could be effective against common endodontic pathogens *in vitro* and *in vivo* (8, 9). However, a disinfected empty canal space cannot support the ingrowth of new regenerated tissues on its own so a scaffold is needed for support. Advances in tissue engineering research focused on 3 key elements for tissue regeneration (10, 11): (1) stem cells that have the ability for proliferation and differentiation; (2) scaffold, which is a 3-dimensional structure that supports the regenerated tissue integrity; and (3) growth factors, which are secreted signals governing morphogenesis and differentiation.

The regenerative endodontic protocol depends on the regenerative capacity of periradicular tissues, which act as an endogenous source of the key elements of regeneration. Several case reports and series were published (12–18) concerning revascularization procedures; however, the deficiency of prospective studies and clinical randomized trials prevents the widespread application of this promising treatment protocol. The aim of the present investigation was to assess the regenerative potential of young permanent immature teeth with necrotic pulps after the following treatment protocols: (1) an MTA apical plug, (2) the regenerative endodontic protocol (blood clot scaffold), and (3) the regenerative endodontic with a blood clot and an injectable scaffold impregnated with basic fibroblast growth factor (bFGF).

Materials and Methods

Thirty-six patients with immature, nonvital maxillary anterior teeth presenting with or without signs and/or symptoms of periapical pathology were included in this study from the outpatient clinic of the Faculty of Dentistry, Ain Shams University, Cairo, Egypt. A detailed medical and dental history was obtained from each patient's parents or guardians. Only medically free patients were included in this research. The clinical and radiographic exclusion criteria were teeth with vertical fractures, periodontally involved teeth, and nonrestorable teeth. All procedures were performed after obtaining proper institutional review board approval based on the regulations of the Ethical Committee of the Faculty of Dentistry, Ain Shams University. Intraoral periapical radiographs revealed immature apices. The age of the patients ranged between 9 and 13 years. Informed consent was signed for each case by the patient's parents or guardians including the proposed treatment and possible outcomes or complications.

78.

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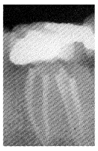

CASE REPORT

Revascularization of an immature tooth with apical periodontitis using a single visit protocol: a case report

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Abstract

McCabe P. Revascularization of an immature tooth with apical periodontitis using a single visit protocol: a case report. *International Endodontic Journal*.

Aim To discuss the clinical and radiological outcome of a revascularization procedure which was completed in a single visit (using sodium hypochlorite 5% as the sole disinfectant) in an immature tooth with a necrotic pulp and apical periodontitis.
Summary A 7-year-old girl was referred in pain following trauma to the maxillary anterior region some 6–7 weeks previously. The maxillary left central incisor tooth was diagnosed with a necrotic pulp and acute apical periodontitis. Under local anaesthesia and rubber dam isolation, an access cavity was prepared. The canal was irrigated with a 5% sodium hypochlorite solution and agitated with an ultrasonic file. A 17% EDTA solution was also used for a final rinse. Bleeding was induced into the canal space from the periapical tissues using a K-file. An MTA layer/barrier was placed directly onto the blood clot, and a further layer of GC Fuji IX cement was placed on top of the MTA to restore the access cavity. The tooth was reevaluated at 6 weeks, 3 months, 6 months, 1 year and 18 months. The tooth has remained symptom free. Radiographic examination shows progressive thickening of the root canal walls, root lengthening and apical closure.
Key learning points
- Disinfection with 5% sodium hypochlorite followed by the induction of a blood clot into the root canal space may be sufficient to promote revascularization in certain circumstances.
- A single visit revascularization procedure is a potential treatment option.

Keywords: apical periodontitis, immature tooth, open apex, revascularization, single visit, sodium hypochlorite 5%.

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Introduction

Trauma and/or carious exposure in immature permanent teeth can lead to pulp necrosis and arrested root development. The consequences of interrupted development include a

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Regenerative endodontic treatment of an immature tooth with a necrotic pulp and apical periodontitis using platelet-rich plasma (PRP) and mineral trioxide aggregate (MTA): a case report

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RUNNING TITLE: Regenerative endodontics

KEYWORDS: Incisor; mineral trioxide aggregate (MTA); platelet-rich plasma (PRP); regeneration; revascularisation

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Clinical Technique/Case Report

Reestablishing Biology, Function, and Esthetics for Fractured, Immature Incisors

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Clinical Relevance

Dental trauma is a challenge in clinical practice and often needs a multidisciplinary approach for a correct diagnosis and restorative therapy. Pulp revascularization and direct resin composite restorations successfully restored anterior teeth fractures with incompletely formed root apices.

SUMMARY

A seven-year-old boy with enamel-dentin fractures on both maxillary central incisors presented to the Piracicaba Dental School–UNICAMP seven days after the trauma. At the clinical evaluation, there were no clinical signs of pulp exposure, neither tooth was mobile, and both affected teeth presented a positive response to sensitivity tests and a negative response for percussion and palpation. The radiographic examination showed an undeveloped root and opened apex for both teeth. Indirect pulp capping was performed on the left maxillary central incisor, followed by a direct restoration. After one month, the patient complained of pain in the left central incisor, which responded negatively to sensitivity testing. Pulp revascularization was performed only on this tooth and was followed for 18 months. During this period, the left maxillary central incisor did not recover sensitivity, although radiographic examination showed apical closure, a slight increase in root length, and the formation of a mineralized barrier between the root canal and sealing material. The technique achieved its goal of restoring biological aspects,

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ARTICLE IN PRESS

Case Report/Clinical Technique

Pulp Revascularization on Permanent Teeth with Open Apices in a Middle-aged Patient

Yu Wang, DDS, Xiaofei Zhu, DDS,† and Chengfei Zhang, DDS, PhD‡§*

Abstract

Introduction: Pulp revascularization is a promising procedure for the treatment of adolescents' immature permanent teeth with necrotic pulp and/or apical periodontitis. However, the ability to successfully perform pulp revascularization in a middle-aged patient remains unclear. **Methods:** A 39-year-old woman was referred for treatment of teeth #20 and #29 with necrotic pulp, extensive periapical radiolucencies, and incomplete apices. Pulp revascularization procedures were attempted, including root canal debridement, triple antibiotic paste medication, and platelet-rich plasma transplantation to act as a scaffold. Periapical radiographic and cone-beam computed tomographic examinations were used to review the changes in the apical lesions and root apex configuration. **Results:** The patient remained asymptomatic throughout the 30-month follow-up. Periapical radiographic examination revealed no change in the apical lesions of either tooth at 8 months. The periapical radiolucency disappeared on tooth #20 and significantly decreased on tooth #29 by the 30-month follow-up, findings that were also confirmed by cone-beam computed tomographic imaging. No evidence of root lengthening or thickening was observed. **Conclusions:** Successful revascularization was achieved in a middle-aged patient's teeth. *(J Endod 2015;■:1–5)*

Key Words

Apical periodontitis, middle-aged patient, pulp revascularization

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Pulp revascularization is widely performed in the treatment of immature permanent teeth with necrotic pulp and apical periodontitis. Successful cases exhibited thickening of the canal walls, closure of root apices, continued root development, and recovery of a positive response to electric pulp testing in some cases (1, 2). Regenerative endodontic procedures have routinely been recommended for adolescent patients between 8 and 16 years old (3). Sparse reports are available on pulp revascularization in the mature permanent dentition. Although some researchers claim that the treatment outcome from regenerative endodontics does not vary according to patient age (4), the upper limit of participant age was only 28 years in a systematic review that included 51 clinical studies and case reports from January 1993 to December 2013 (5). Therefore, it is still unknown whether patient age is a critical factor in the success of pulp revascularization. Studies on bone marrow mesenchymal stem cells suggest that the total number and osteoprogenitor (CFU-F/ALP+) colonies of mesenchymal stem cells decrease with age (6, 7). Bone marrow mesenchymal stem cells and dental stem cells (including those of the dental pulp, periodontal ligament, and apical papilla) share some similarities (8); the outcome of dental pulp revascularization may also be affected by patient age.

The principles of revascularization include the use or recruitment of stem cells, growth factors, and biologic scaffolding (9). Platelet-rich plasma (PRP) has been suggested as an ideal biomaterial to improve treatment results because it releases many growth factors and acts as a scaffold (10, 11). Successful revascularization of infected immature teeth after autologous PRP transplantation has been reported (12–14).

In the present case report, we describe a PRP-supplemented revascularization of 2 nonvital permanent teeth with periapical lesions and open apices in a 39-year-old woman.

Case Report

A 39-year-old woman was referred to our department for treatment of her right and left second mandibular premolars (teeth #20 and #29). The patient visited her general dentist 1 week earlier with sharp pain of 3 days' duration in the left second premolar. She received access opening, medication with camphor phenol, and temporary seal. The dentist documented no caries but found fractured dens evaginatus in both mandibular second premolars. Her medical status was noncontributory.

Upon clinical examination at our clinic, temporary fillings were found in the center of occlusal surfaces of teeth #20 and #29, with slight tooth discoloration and no evidence of sinus tract stomata (Fig. 1*A–D*). These 2 teeth were not sensitive to percussion or palpation and did not respond to thermal or electric pulp testing (Vitality Scanner; SybronEndo, Glendora, CA). Periapical radiographic examination revealed extensive periapical radiolucencies and incomplete apices on both left and right second mandibular premolars (Figs. 2*A* and 3*A*). Cone-beam computed tomographic (CBCT) images (NewTom VG; Quantitative Radiology, Verona, Italy) further characterized the extent of the bony defects and the large diameter of roots and apices (Figs. 2*D–F* and 3*D–F*). The diagnosis of necrotic pulp and symptomatic apical periodontitis was made for both second mandibular premolars.

The patient was informed that the goal of treatment was to initiate healing of the bony defects and to stimulate further root lengthening and thickening and that the proposed treatment might not be successful. The decision was made to perform an

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Long-term follow up of revascularization using platelet-rich fibrin

CASE REPORT

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Abstract – *Introduction*: Trauma is one of the primary causes of tooth loss and pulpal injury in adolescents and children. Prior to regenerative endodontics, treatment of necrotic, immature teeth with open apices was limited to long-term calcium hydroxide ($Ca(OH)_2$) apexification and subsequent root canal therapy or extraction. Through revascularization, retention of these teeth can be achieved and the elimination of patient symptoms and the radiographic appearance of continued root development were obtained. *Case Review*: This report illustrates a revascularization protocol through a case where platelet-rich fibrin (PRF) was utilized as an autologous scaffold for traumatized, necrotic, immature teeth with incomplete root development. Through consistent follow-up reports, comprising of both clinical examination and radiographs, marked improvement in the condition of the traumatized tooth was noted. *Discussion*: This case demonstrates the feasibility of utilizing PRF as an effective treatment protocol for traumatized teeth in lieu of traditional treatment protocols, such as long-term calcium hydroxide ($Ca(OH)_2$) apexification or extraction. The choice of utilizing PRF, as opposed to other platelet concentrates, such as platelet-rich plasma (PRP) or a blood clot, lies in PRF's ability to allow for a slow, long-term release of autologous growth factors.

Key words: contact sports; dental trauma; inflammatory root resorption; permanent tooth; prognosis; treatment

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Dental caries and dental trauma are the two leading causes of injury to the dental pulp (1). Although the oral cavity comprises less than 1% of the total body area, it accounts for 5% of injuries in all ages and 17% of injuries in children (2). Dental trauma in children can lead to pulp tissue damage and is of particular concern in the underdeveloped tooth, as immature and open apices limit possible treatment options.

Historical approaches utilizing long-term calcium hydroxide therapy for apexification have resulted in increased fracture rates due to loss of flexural strength as dentinal organic matrix is weakened with extended exposure to calcium hydroxide (3, 4). The process of revascularization has allowed for enhanced survivability of the tooth, alleviation of symptoms, and a radiographic confirmation of root thickening and lengthening (5, 6). Nygaard-Ostby focused the role of blood clot in endodontic therapy (7, 8) while Myers' work encompassed dental pulp regeneration through utilization of blood and blood substitutes (9).

Unlike apexification, which mainly relies on exogenous materials to create an apical barrier within the root canal system (10, 11), revascularization utilizes a combination of antibiotic paste and a biologic scaffold (12). The antibiotic paste historically consisted of a combination of metronidazole, ciprofloxacin, and minocycline (13, 14). Its purpose was to decrease and potentially eliminate bacterial strains and the pro-inflammatory response within the root canal system in preparation for the biologic scaffold (15). Concerns over tooth discoloration have been attributed to minocycline use. The survivability of stem cell progenitors within the root canal system in the face of the antibiotic treatment may also be jeopardized (16).

In traditional revascularization cases, a blood clot within the root canal system served as a biological scaffold (17, 18). Although the induction of a blood clot within the canal space is not always possible (19), its overall clinical success paved the way for the next phase of revascularization—platelet concentrates providing an autologous scaffold (20). Torabinejad and Turman reported a case where PRP, a first generation platelet concentrate, was used for revascularization (21). Recently, Pandey (2013) reported a case of an anterior traumatized permanent tooth where platelet-rich fibrin (PRF), a second-generation platelet concentrate consisting of autologous platelets and leukocytes present in a complex fibrin matrix, was utilized as a scaffold for revitalization with a follow up of 15 months (22). PRF is composed of fibrin membranes enriched with platelets, growth factors, and cytokines (23). PRF has the capability to enhance the healing potential of soft and hard tissues and slowly release growth factors, such as PDGF and TGF-B1 over the

Regenerative Endodontic Treatment of an Immature Necrotic Molar with Arrested Root Development by Using Recombinant Human Platelet-derived Growth Factor: A Case Report

Annie Zhujiang, DMD, and Sahng G. Kim, DDS, MS

Abstract

Introduction: Regenerative endodontic treatment has provided a treatment option that aims to allow root maturation. The present report describes the regenerative endodontic treatment of a necrotic, immature molar by using recombinant human platelet-derived growth factor (rhPDGF-BB) and shows the continued root maturation in the tooth with arrested root development. **Methods:** A regenerative endodontic procedure that used a growth factor was performed for a necrotic molar with arrested root formation in a 20-year-old patient. Thorough disinfection by using mechanical instrumentation and copious irrigation of antimicrobial agents as well as intracanal medication with calcium hydroxide was performed throughout the first 2 appointments. At the third appointment, the root canals were irrigated with an antimicrobial solution and 17% EDTA, and bleeding was evoked by passing sterile paper points beyond the apex in each canal. Small pieces of a collagen membrane saturated with rhPDGF-BB solution from GEM 21S were packed into each canal. Mineral trioxide aggregate was placed, and Cavit and composite resin were used to restore the tooth. **Results:** Complete root maturation and resolution of a periapical radiolucency were observed at the 15-month follow-up. **Conclusion:** The present report presents a regenerative endodontic procedure that uses rhPDGF-BB for a necrotic molar with arrested root development. The finding of continued root development in the present case suggests that regenerative endodontic treatment may be able to resume the root maturation process in teeth with arrested root formation. Further clinical studies are required to investigate the efficacy of rhPDGF-BB in regenerative endodontic treatment. *(J Endod 2015;■:1–4)*

Key Words

GEM-21S, pulp regeneration, pulp revascularization, pulp revitalization, regenerative endodontics, rhPDGF-BB, root maturation

Treatment of necrotic teeth with immature apices presents a clinical challenge. Common treatment options such as apexification fail to allow continued root development and leave the tooth predisposed to fracture because of its thin root walls (1). Achieving an adequate apical seal is difficult in a tooth with a large apex and can be managed by long-term calcium hydroxide treatment or placement of a mineral trioxide aggregate (MTA) plug (2). Regenerative endodontic treatment has provided an additional treatment option that aims to allow both continued root formation and apical closure, while restoring immune and sensory functions within the pulpal space (3).

The advancement of pulp regeneration in endodontics has been made possible through progress in tissue engineering. Tissue engineering "integrates the fields of biology and engineering into a discipline that is focused on tissue regeneration instead of tissue repair" (3) and requires a triad of stem cells, growth factors, and scaffolds (4). The protocol for regenerative endodontic treatment has been modified continuously to achieve the optimal environment for the regeneration of the pulp-dentin complex (5). The potential to refine and improve this clinical procedure involves the ability to control the growth factors present in the pulpal space. Growth factors can modify the environment of the stem cells, thus ultimately allowing control of cell fate (6). In a prospective study Nagy et al (7) demonstrated a significant increase in root width and length by using a hydrogel containing basic fibroblast growth factor. Iohara et al (8, 9) showed *de novo* regeneration of the pulp-dentin complex by transplanting autologous stem/progenitor cells and signaling molecules such as granulocyte colony-stimulating factor or stromal derived factor-1 in the root canals of pulpectomized dog teeth.

Recombinant human platelet-derived growth factor (rhPDGF-BB) has already demonstrated its effectiveness in periodontal regeneration (10–13). Platelet-derived growth factor (PDGF) is reported to have chemotactic and mitogenic effects on mesenchymal cells and possesses angiogenic ability (6, 10). PDGF has 4 isoform homodimers (AA, BB, CC, and DD) and 1 heterodimer (AB) (6). PDGF-BB is 1 of 4 homodimers and can bind to cell receptor dimers PDGFR α/α, α/β, and β/β (6). The most effective group within the PDGF family, PDGF-BB (6), comes in the commercially available GEM 21S (Osteohealth, Shirley, NY) and is composed of highly purified rhPDGF-BB and beta-tricalcium phosphate. Using growth factors that promote chemotaxis and proliferation/differentiation of mesenchymal stem/progenitor cells can potentially provide more control of the tissue formed in pulp regeneration and may provide higher and

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Regenerative Endodontic Treatment versus Apical Plug in Immature Teeth: Three-Year Follow-Up

Saeed Asgary*/ Mahta Fazlyab**/ Ali Nosrat ***

This report compares and evaluates the treatment outcomes of regenerative endodontic treatment and apical plug as two accepted treatment protocols in a pair of necrotic immature maxillary central incisors of a 12-year old female. The patient was referred complaining of a dull pain and swelling in her upper lip area. She had a history of trauma to the anterior maxilla two years earlier. Both teeth were clinically diagnosed with pulp necrosis and periapical radiographs revealed that separate periapical radiolucent lesions surrounded the immature apices of both teeth. The left and right incisors were treated with apical plug and regenerative endodontic treatment, respectively, using calcium-enriched mixture (CEM) cement. The patient was followed-up for three years. During this period, both teeth were clinically asymptomatic and showed complete radiographic healing of the periapical lesions. The right central incisor showed root development. No tooth discoloration was evident. Apexification by apical plug placement and pulp regeneration are both reliable treatments for immature non-vital teeth. In order to choose the right treatment the advantages of either technique should be weighed against its drawbacks. CEM cement can be successfully applied for both purposes. This biomaterial causes less discoloration of the tooth.

Key words: *Calcium-Enriched Mixture, Pulp, Revascularization, Regeneration*

INTRODUCTION

Management of immature teeth with necrotic pulp and apical periodontitis is considered as a clinical challenge because of several reasons; the root canal space is infected and the thin dentinal walls cannot be adequately instrumented [1]. In addition, the patent apical foramen lowers the chances of proper sealing and due to under-developed root structure, the tooth is still vulnerable to cervical fractures [2]. These challenges necessitate specific approaches such as a chemical disinfection protocol with minimal instrumentation [3], apical sealing through apexification by means of hard tissue barrier induction with calcium hydroxide (CH) [1] or apical plug [4] and finally reinforcement of the thin dentinal walls [2].

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Although apexification with CH was claimed to be a successful procedure [5], it is time consuming and requires patient's cooperation. It has been estimated that 30% of the teeth that have undergone CH apexification fracture during/after long-term endodontic treatment [6]. Because of the special configuration of the immature apex (the apical diameter being larger than the coronal diameter), a softened filling technique is indicated in these teeth [2]. Placement of an apical plug and then backfilling of the canal during one or two visits, based on the symptoms and presence/absence of exudation/suppuration through the root canal space, has gained popularity among clinicians [7]. An ideal plug material should seal the pathway between the root canal space and the surrounding tissues. In addition, it needs to be nontoxic, biocompatible, insoluble in tissue fluids, and dimensionally stable [8]. Calcium-enriched mixture (CEM) cement is a tooth-colored water-based cement with a different chemical composition compared to mineral trioxide aggregate (MTA) and similar clinical applications [9]. It offers favorable sealing, antimicrobial properties similar to those of CH [10], hard tissue induction properties [11] and compared to MTA, it has shorter setting time, greater flowability and lower film thickness [12].

As a biologically-based treatment alternative for immature infected teeth with apical periodontitis, tissue regeneration within the root canal space is possible when the following criteria are met: proper canal disinfection, presence of a matrix into which the tissue can grow and adequate coronal seal [13,2]. There are several reports of successful regenerative endodontic procedures owing their outcome to proper infection control [14,15]. Different technique/methods of disinfection (mainly using CH or different antibiotic pastes) have been introduced. Under ideal circumstances, the successful outcome

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Julian Schmoeckel

Management of an immature, partially necrotic permanent molar by pulp revascularization: Two-year follow-up

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Root canal treatment of immature teeth is considered a great challenge for both the dentist and the patient. The thinness of the root canals walls and the lack of an apical barrier leads to unpredictable treatment results. Revascularization is a novel promising treatment option in the field of regenerative endodontics for management of immature necrotic permanent teeth. This case presents a successful treatment of an 8-year-old patient with a partially necrotic, immature permanent mandibular right first molar pulp by revascularization. The revascularization procedure was performed in two appointments using nitrous oxide sedation to reduce the child's anxiety during treatment. Following root canal disinfection with NaOCl, a triple antibiotic paste (ciprofloxacin, metronidazole, and minocycline) was applied into the canal. One week later, induction of bleeding, application of MTA, and tooth restoration using a stainless steel crown were performed. At the subsequent follow-up appointments (3, 9, and 16 months) the tooth was asymptomatic. After 2 years a radiographic assessment revealed complete periapical healing and apical closure, as well as increase in root length and dentin thickness. **Conclusion:** This case supports the evidence of revascularization as a biological treatment option for the management of necrotic young permanent teeth. *(Quintessence Int 2017;48: 309–313; doi: 10.3290/j.qi.a37801)*

Key words: antibiotic paste, children, immature permanent tooth, nitrous oxide, partially necrotic pulp, regenerative endodontic treatment

Root canal treatment of immature teeth remains a challenging task for dentists, as the instrumentation of the canal(s) with conventional techniques is difficult. Moreover, a good apical seal is impossible to obtain, because of the lack of an apical stop. Up to now, apexification has been considered as one of the suggested treatment options for nonvital immature permanent molars. This treatment aims at the creation of an apical calcified barrier by applying calcium hydroxide in multiple visits. The main disadvantages of apexification are the long treatment time and follow-up period and the remaining thinness of the root canal wall.[1]

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Second-generation Platelet Concentrate (Platelet-rich Fibrin) as a Scaffold in Regenerative Endodontics: A Case Series

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Abstract

Introduction: The purpose of this case series was to report the clinical and radiographic results of a pulp regenerative procedure using platelet-rich fibrin (PRF), a second-generation platelet concentrate, in immature teeth with necrotic pulps. **Methods:** Root canal revascularization using PRF was performed on 4 immature teeth with necrotic pulps. After access cavity preparation, the root canals were irrigated with low concentration sodium hypochlorite solution (1.5% sodium hypochlorite [20 mL/canal, 5 minutes]) and then irrigated with saline (20 mL/canal, 5 minutes). Equal proportions (167 mg) of ciprofloxacin, metronidazole, and cefaclor were mixed and diluted to a final concentration of 1 g/mL. Finally, the canal was sealed with 3–4 mm of a temporary restorative material, and patients were dismissed for 2 to 3 weeks. At the second appointment, 9 mL of the patient's whole blood was obtained and centrifuged to prepare a PRF clot. Canals were irrigated with 17% EDTA, and a sharp spreader was inserted beyond the apex. Then, the PRF clot was placed inside the root canals, and Biodentine (Septodont, Saint-Maur, France) was placed directly over the PRF. The teeth were restored permanently with glass ionomer cement and composite resin. **Results:** Clinical examinations revealed that all cases were asymptomatic at the recall appointments at 1, 3, 6, 12, and 18 months. Radiographs revealed resolution of the periapical lesions, further root development, and apical closure in all cases. **Conclusions:** On the basis of the short-term results up to 12 months, PRF clots acted as successful scaffolds for the regeneration of pulpal contents in immature teeth with necrotic pulps. *(J Endod 2016;■:1–8)*

Key Words

Open apex, platelet-rich fibrin, regenerative endodontics, revascularization, second-generation platelet concentrate

Significance

Endodontic treatment of immature teeth with necrotic pulps is challenging. Platelet-rich fibrin blood clots are suggested as a feasible alternative for regenerative endodontic protocols.

Endodontic treatment of immature teeth with necrotic pulps is challenging because of the thin dentinal walls of the roots and their susceptibility to fracture (1). Historically, calcium hydroxide was used for apexification of such teeth, but long-term calcium hydroxide treatment may disrupt the links between collagen fibers and hydroxyapatite crystals, which results in reduced micro-hardness and increased susceptibility to fracture (2–5). To overcome the drawbacks of calcium hydroxide apexification, 1-step apical closure techniques using mineral trioxide aggregate (MTA) were introduced (2). However, in this technique the root length and thickness of dentinal walls remains unchanged (6). Regenerative procedures are a feasible option for treatment of immature teeth with necrotic pulps especially since further development of the root apex and increased thickness of root walls are expected (7). In the past two decades, several reports have described clinical and radiographic evidence of successful regenerative procedures for treatment of such teeth (8). Induced bleeding and blood clots are integral parts of these procedures as they provide an appropriate scaffold and sufficient stem cells (9, 10). Platelet-rich plasma (PRP) has also been recommended as a potential scaffold for regenerative endodontic treatments (11, 12).

It has been shown that stimulated platelets release secretory granules that contain a variety of growth factors (13). PRP releases growth factors rapidly within 7 to 14 hours, which then declines (13). A second-generation platelet concentrate, platelet-rich fibrin (PRF), was developed by Choukroun et al (14). PRF has high concentrations of fibrin clots; no supplementary thrombin is needed during its preparation. PRF is formed during a gradual polymerization protocol that incorporates a higher concentration of cytokines into fibrins (13). Therefore, growth factors meshed in these fibrins are released more slowly between 7 and 14 days (13). In this case series, a regenerative procedure using PRF was performed on 4 immature single-rooted teeth

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87.

Revascularization-associated Intracanal Calcification: A Case Report with an 8-year Review

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Abstract

Revascularization-associated intracanal calcification (RAIC) is a common finding in immature teeth managed with regenerative endodontic treatment (RET). The aim of this report was to illustrate a case in which 2 mandibular premolar teeth developed complete canal obliteration and have been reviewed for 8 years. The 3 primary outcome goals as defined by the American Association of Endodontists after RET are resolution of signs and symptoms of pulp necrosis, further root maturation, and achievement of responses to pulp vitality testing. The teeth had been included in an earlier quantitative study in which an increase in root canal width of 72.1% and 39.6% and an increase in root length of 1.7% and 0% were reported for teeth #20 and #29, respectively. Qualitative assessments over the 8-year review period showed no pathosis and a response to electric pulp testing at the final review. A quantitative assessment at the 8-year review showed an increase of 100% for canal width because complete calcification had occurred and no substantive change in root length (−0.17% and 0.68% for teeth #20 and #29, respectively). In this report, complete RAIC occurred in both teeth over time. RAIC has the potential to complicate future endodontic or prosthodontic treatment if necessary. Therefore, it is recommended that the American Association of Endodontists clinical considerations for a regenerative endodontic procedure be updated to include the incidence of RAIC after RET. (*J Endod 2018;44:1792–1795*)

Key Words

American Association of Endodontists clinical considerations for a regenerative endodontic procedure, regenerative endodontic treatment, revascularization-associated intracanal calcification

Significance

Because revascularization-associated intracanal calcification is a common finding in immature teeth treated with regenerative endodontic treatment, the AAE considerations for a regenerative procedure should outline the potential for this variation of healing outcome.

Regenerative endodontic treatment (RET) of immature teeth with pulp necrosis has comparable outcomes for the resolution of signs and symptoms of apical periodontitis with other treatment approaches of calcium hydroxide apexification and mineral trioxide aggregate (MTA) apical barrier techniques (1–4). The advantage of RET is the potential for future root maturation with increased canal wall thickness, root length, and apical closure (1, 2, 5–8). Several studies have shown the extent of further root maturation is variable (1–3, 7–9). Intracanal calcification has been described as a calcific response after RET (10). However, a recent review identified a high prevalence of revascularization-associated intracanal calcification (RAIC) at 62.1% of revascularization cases and the progressive nature of this condition with time (11). Teeth were reviewed for an average of 24.9 months with a range of 12–71 months. There is a paucity of cases with long-term follow-ups, which are needed to adequately assess the efficacy of RET (1, 9, 12) and posttreatment sequelae.

The American Association of Endodontists (AAE) clinical considerations for a regenerative endodontic procedure define 3 outcome measures (13). The primary goal is the elimination of symptoms and the evidence of bony healing. The secondary goal is increased root wall thickness and/or increased root length (desirable but perhaps not essential). The tertiary goal is a positive response to vitality testing (which, if achieved, could indicate more organized vital pulp tissue). The possible adverse effects stated are staining of crown/root, lack of response to treatment, and pain/infection (13). Other posttreatment sequelae like intracanal calcification and/or ingrowth of bone are not outlined despite the significance of these phenomena on potential adverse future clinical outcomes. Long-term reports are more likely to show variations on the healing responses such as progressive intracanal calcification. The purpose of this report was to illustrate extensive RAIC of 2 mandibular premolars treated with RET at an 8-year review.

Case Report

An 11-year-old female patient was referred because of the presence of a draining sinus associated with the left mandibular second premolar (tooth #20) (Fig. 1*A*). Both mandibular premolar teeth on the left had small occlusal composite resin restorations.

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The effect of platelet-rich plasma as a scaffold in regeneration/revitalization endodontics of immature permanent teeth assessed using 2-dimensional radiographs and cone beam computed tomography: a randomized controlled trial

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Abstract

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Aim To assess the outcomes of platelet-rich plasma as a scaffold in regenerative/revitalization endodontics (RET) using cone beam computed tomography (CBCT) and 2-dimensional radiographs.

Methodology Twenty-six healthy patients with mean age of 12.66 ± 4.47, and immature permanent anterior teeth with necrotic pulps, were randomly allocated to two groups, whereby RET was performed using platelet-rich plasma (PRP, test group) and blood clot (BLC, control group). Changes in root length (RL), root dentinal thickness (RDT), apical foramen width (AFW) and radiographic root area (RRA), were assessed using both radiographic methods, whilst changes in periapical area diameter (PAD) were assessed using CBCT, over a period of 12 months. *T*-test and chi-square/Fisher's exact tests were used to compare continuous and categorical data between BLC and PRP groups, respectively. Changes in RL, RDT, AFW, RRA and PAD were examined by comparing the two groups (PRP versus BLC) using multilevel modelling, considering the clustering effect of repeated measures of several teeth originating from the same participant.

Results Changes in RL, RDT, AFW, RRA and PAD, over time, were found to be significant for both groups. There was, however, no difference between the RET techniques (PRP versus BLC), using both radiographic and CBCT methods. The results of both assessment techniques (CBCT and 2-dimensional radiographic methods) were highly consistent (overall ICC ranged between 0.80 and 0.94). In addition, a significant effect of baseline PAD was found on RL, RRA and AD at 12 months (RL effect = −0.68, $P < 0.001$; RRA effect = −1.91, $P = 0.025$; AD effect = 0.08, $P = 0.024$).

Conclusion The current study highlights successful and comparable clinical and radiographic outcomes of RET techniques using PRP and BLC. Standardized and calibrated 2-dimensional radiographic assessment was as effective as CBCT in assessing RET outcomes; therefore, the routine use of CBCT in RET is not recommended. Although an effect of baseline periapical lesion diameter on root development outcomes, at 12 months, were observed, more studies are recommended in order to assess such an effect.

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Traumatized Immature Teeth Treated with 2 Protocols of Pulp Revascularization

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Abstract

Introduction: Pulp revascularization may be considered a promising alternative for traumatized necrotic immature teeth. The aim of this study was to evaluate traumatized immature teeth treated with 2 protocols of pulp revascularization. **Methods:** Twenty-three teeth of young patients (7–17 years old) with necrotic upper incisors caused by dental trauma were divided into 2 groups; one group was treated with triple antibiotic paste (metronidazole, ciprofloxacin, and minocycline) (TAP) (n = 12), and the other was medicated with combination of calcium hydroxide and 2% chlorhexidine gel (CHP) (n = 11). Patients were treated and followed up for a period from 9–19 months in 2 dental institutions for evaluation of clinical and radiographic data. **Results:** Most of the teeth were affected by lateral luxation (47.8%). Clinical evaluation in group TAP showed significant reduction in spontaneous pain (P = .01), pain on horizontal percussion (P = .007), and pain on palpation (P = .03), whereas group CHP showed significant reduction in pain on vertical percussion (P = .03). Crown discoloration was observed significantly more in teeth of group TAP (83.3%) (P < .002). On radiographic exam, periapical repair was found in all TAP-treated teeth (P = .03). Similarly, the same findings were found for all teeth treated with CHP with exception of 1 tooth (P = .21). Apical closure was significantly observed in both groups (P < .05). Increase in root length was demonstrated in 5 teeth (41.7%) and 3 teeth (27.3%) of groups TAP and CHP, respectively. Thickening of lateral dentinal walls was observed in 5 teeth of each group. **Conclusions:** Revascularization outcomes for traumatized patients treated with the tested protocols presented similar clinical and radiographic data. However, TAP caused esthetic problem leading to tooth discoloration, which can be considered a disadvantage when compared with CHP. *(J Endod 2014;40:606–612)*

Key Words

Calcium hydroxide, intracanal medication, pulp revascularization, triple antibiotic paste

Most of the dental traumas are associated with luxation injuries, which may damage the periodontal ligament attachment as well as the apical neurovascular bundle. Depending on the severity, the tissue may be compressed or disrupted, and both the cells and intercellular structures may be damaged (1). In traumatized immature teeth, this damage to periapical region may also affect apical papilla cells and periodontal ligament cells, and any disruption of these structures may impede further root development (2). In this context, healing after a luxation injury involves reorganization and reestablishment of the continuity of the periodontal ligament fibers, including pulpal revascularization and reinnervation. When reinnervation does not occur, pulp tissue becomes necrotic (3). Pulp necrosis is the most frequent posttraumatic complication in all types of dental traumas; it is more frequent in mature than in immature teeth (4). The most traditional endodontic treatment for immature teeth is apexification through periodic changes of intracanal medication or using an apical plug of mineral trioxide aggregate (MTA) (5–8).

Recently, pulp revascularization has been studied as an alternative therapy for immature necrotic teeth, with the advantage of inducing root-end development (9, 10). Most of the pulp revascularization studies refer to case reports that use passive decontamination and antibiotic paste composed of metronidazole, ciprofloxacin, and minocycline as intracanal medication (11–13). Calcium hydroxide also has been used as intracanal dressing, although there still is controversy in the literature concerning its beneficial properties when used in contact with undifferentiated cells and dentin root walls (14–18). A recent case report demonstrated success in pulp revascularization performed with the combination of calcium hydroxide and 2% chlorhexidine gel, showing thickness in dentin walls and increase in root length and apical closure (19).

Case reports on pulp revascularization have mostly been evaluated in terms of apical periodontitis and dentoalveolar abscesses (20, 21). However, pulp necrosis in immature teeth may also occur in traumatized teeth, which present different mechanisms of complications and physical compromise of the periodontal ligament cells and apical papilla when compared with pulp necrosis caused by infection. Considering the importance of the integrity of periodontal ligament cells for revascularization repair, the clinical success of such protocols should be evaluated. In addition, dental trauma may play a different role in the apical repair of immature teeth, considering the possibility of physical destruction of stem cells. This study

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