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REVIEW ARTICLE

Recent considerations in regenerative endodontic treatment approaches

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Abstract *Background/purpose:* Regenerative approaches in endodontics are considered in two concepts. One is a revascularization approach to achieve continued apical formation while the other involves the pulp/dentin regeneration via tissue engineering technology. Recently, some case reports have shown that infected immature teeth can be treated by revascularization approach. However, there is still no standardized treatment protocol for this procedure. The purpose of this review article was to evaluate the effects of regenerative endodontic treatment for necrotic immature permanent teeth and to discuss recent treatment approaches.
Materials and methods: Articles published in dental journals from January 2001 to August 2013 were searched using the following keywords: immature permanent teeth OR immature teeth OR pulp revascularization OR pulp revitalization OR regenerative endodontics by using electronic databases (MEDLINE using the PubMed search engine, Embase, Scopus, and Cochrane Central Register of Controlled Trials).
Results: The regenerative endodontic treatments with various methods and materials result in a significant increase in root length and dentinal wall thickness. Stimulation of stem cells in apical root canal system is required to induce tissue formation and continued root development. Alternative disinfection materials and protocols are required.
Conclusion: Although the regenerative treatment approaches have good clinical outcomes in the majority of case reports, the outcomes are unpredictable. Since the current clinical protocols for regenerative endodontics do not fully fulfill the triad of tissue engineering ((growth factors, scaffold and stem cells), further translational studies are required to achieve more pulp- and dentin-like tissue in the root canal system to achieve pulp regeneration.
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Introduction

The main goal of root canal treatment is the prevention or treatment of apical periodontitis.[1] For the treatment of immature permanent teeth, the goal is to restore the original physiologic structures and functions of the pulp–dentin complex. However, treating necrotic immature teeth has always been a clinical challenge for several reasons.[2] It is difficult to achieve an appropriate apical seal with an open apex by using conventional root canal treatment. In addition, the discontinued development of dentinal walls after pulp necrosis can cause thin dentinal walls that make the tooth more prone to fracture.

One-visit apexification that is performed by placing an apical barrier by using mineral trioxide aggregate (MTA) is an alternative to conventional long-term calcium hydroxide [$Ca(OH)_2$] therapy and may shorten the treatment time between the patient's first appointment and the final restoration.[3] The survival rate of MTA apexification is greater than that of $Ca(OH)_2$ apexification.[4] Mineral trioxide aggregate is effective in supporting the formation of new hard tissue in the apical area of the affected immature necrotic teeth; however, the risk of future fracture may remain because the root width will not increase in MTA apexification-treated teeth.[4]

Regenerative approaches in endodontics comprise two clinical concepts. One concept involves a revitalization approach to achieve tissue regeneration. In this method, new living tissue is expected to form in the cleaned canal space, thereby allowing continued root length and thickness. The other concept is the active pursuit of pulp and dentin regeneration via tissue engineering technology to implant or re-grow pulp tissue. The technology is in its infancy, but it potentially allows immature pulpless teeth to continue growing and maturing. With this understanding, it may be that apexification will become less needed in years to come.

Revascularization is a valuable treatment in immature necrotic teeth. Procedures attempting to preserve the remaining dental pulp stem cells and the mesenchymal stem cells of the apical papilla (SCAPs) can result in root canal revascularization and the completion of root maturation.[5] Stem cells generally remain in a quiescent state to protect their proliferative potentials *in vivo*.[6] Quiescent stem cells may be activated by microenvironmental changes such as tissue injury or disease.[7] In the presence of apical periodontitis, the root canal lumen is probably devoid of vital tissues. However, traces of pulpal tissue may survive apically, even in the presence of a large periapical lesion.[8,9]

The key procedures of the regenerative protocol are minimal or no instrumentation of the canal while relying on a gentle but thorough irrigation of the root canal system. The disinfection is augmented with intracanal medication, and the treated tooth is sealed with MTA and glass ionomer/resin cement at the completion of the treatment. Periodical follow-ups will take place to observe any continued maturation of the root.[10]

To date, pulp revascularization is reported as a promising approach for treating immature permanent teeth. By contrast, there are some drawbacks and variables in relation to this treatment approach.[11,12] The purpose of this article was to review the recent literature and to evaluate recent treatment approaches to guide clinicians in using regenerative endodontic procedures in clinical endodontics.

Materials and methods

Articles concerning pulp revascularization published in dental journals from January 2001 to October 2013 were searched using the following keywords: "immature permanent teeth", "immature teeth", "pulp revascularization", "pulp revitalization", or "regenerative endodontics". The electronic databases that use MEDLINE were the PubMed search engine (http://www.ncbi.nlm.nih.gov/sites/pubmed), Embase (http://www.embase.com), Scopus (http://www.scopus.com), and Cochrane Central Register of Controlled Trials (http://www.cochrane.org). The data obtained from the clinical case series and case reports are summarized in Table 1.[4,9,11,12,19,20,23–27,30,31,49] The percentages of the treatment factors are evaluated in Table 2.

Results

Dental caries (12.9%), trauma (33.8%), and dens evaginatus (25.9%) were the potential causes of necrotic pulp of immature teeth, that lead to the cessation of root formation. Premolars were the most affected and treated teeth (59.6%). Sodium hypochlorite (2.5–6%) has been used for irrigation and disinfection during regenerative endodontic therapy. Calcium hydroxide and triple antibiotic paste are mostly used as intracanal medicaments. Regenerative endodontic procedures potentially allow the thickening of the dentinal walls (76%) and lengthening of the root canals (54%).

Discussion

Factors causing pulp necrosis in immature teeth

Dental caries, trauma, and anomalous tooth morphology (i.e., dens evaginatus) cause pulp necrosis of immature teeth and thereby cause the cessation of root formation. Eradication of bacteria from the pulp canal has a key role in successful revascularization because revascularization halts in the presence of infection.[13]

Premolars are the most affected and treated teeth. This may be related to the prevalence of dens evaginatus in this tooth type. Dens evaginatus is an uncommon dental anomaly that presents by protrusion of a tubercle from the occlusal surfaces of the posterior teeth or lingual surfaces of the anterior teeth. It occurs primarily in Asian people.[14] It is also called central cusp in premolars.[14] The greatest disadvantage of dens evaginatus is that it makes the tooth more susceptible to pulp exposure caused by wear or fracture, and therefore leads to pulpal complications soon after eruption. In addition, because dental trauma mostly occurs in the anterior dental region and the upper incisors are more protracted than the other teeth, these tooth types most often have necrotic dental pulp.[15]

Table 1 The case series and case reports summarized in this review.

Authors	Year	Sex	Tooth type (no.)	Cause (no. of cases)	Change in length	Change in thickness	Follow-up duration (mo)
Jung et al[49]	2008	5 Females 3 Males	PM (9)	Fracture of the occlusal tubercule (2) Previously initiated therapy (4)	+ (5 cases)	+	24
Shin et al[19]	2009	1 Female	PM	Caries	−	+	19
Ding et al[9]	2009	2 Females 1 Male	I (2) PM (1)	Caries (2) Dens invaginatus (1)	+ (1 case)	+	17
Chueh et al[25]	2009	12 Females 11 Males	I (1) PM (21) M (1)	Caries (1) Trauma (1) Central cusp fracture (21)	+	+	12
Petrino et al[12]	2010	1 Female 2 Males	I (4) PM (2)	Caries (1) Trauma (4)	+ (4 cases)	+ (4 cases)	10
Kim et al[30]	2010	1 Female	I (2)	Trauma (2)	+	+	8
Nosrat et al[23]	2011	1 Female 1 Male	M (2)	Caries	+	+	
Cehreli et al[26]	2011	5 Females 1 Male	M (6)	Previously initiated therapy	+	+	9.5
Kim et al[31]	2012	2 Males	PM (3)		+ (1 case)	+	38
Jeeruphan et al[4]	2012	10 Females 10 Males	I (7) PM (13)	Caries (1) Dens invaginatus (12) Trauma (7)	+ (6 cases)	+ (3 cases)	21.2
Chen et al[24]	2012	11 Females 9 Males	I (10) PM (10)	Caries (3) Dens invaginatus (7) Trauma (10)	+ (15 cases)	+	11.5
Nosrat et al[11]	2012	1 Female	I (2)	Trauma	+	−	72
Soares et al[27]	2013	1 Female	I	Trauma	+	+	24
Chen et al[20]	2013	1 Female	PM	Dens evaginatus	+	+	12

I = incisor; M = molar; PM = premolar.

Disinfection of the root canal system

Decalcification of the dentin surface, removal of the smear layer, exposure of dentinal tubules and collagen fibrils, and release of growth factors from the dentin matrix are required for cellular differentiation at the dentin interface.[16] Sodium hypochlorite (2.5–6%) has been used for irrigation and disinfection during regenerative endodontic therapy. Modification to this protocol—a final step or irrigation with EDTA—has been recommended to optimize the conditions for cellular differentiation, tissue formation, and regeneration.[17,18] The additional use of chlorhexidine (CHX) has been described in some case reports,[12,19,20] although it can be detrimental to stem cells.[21] In addition, the use of the EndoVac system (Discus Dental, Culver City, CA) reportedly provides similar bacterial reduction as that of apical positive pressure irrigation (i.e., conventional irrigation) plus intracanal dressing with a triantibiotic paste, and it has been concluded that using intracanal antibiotics may be unnecessary.[22]

In addition to irrigation, the use of intracanal medicaments contributes to the decontamination of root canals. Triple antibiotic paste (TAP),[11,20,23] calcium hydroxide [$Ca(OH)_2$],[24–26] and CHX gel[27] have been used for 1–4 weeks for this purpose. For teeth with a persistent infection or in which the canal can not be dried, the medicament dressing can be repeated until no symptoms or exudation is present.[9] Redressing the tooth with medicaments may control the infection and eliminate symptoms in time, but this requires stringent follow up. Therefore, noncompliance of the patient may be a contraindication for these procedures. Triple antibiotic pastes are mainly composed of metronidazole, ciprofloxacin, and minocycline. These pastes show antimicrobial activity against endodontic pathogens and satisfactory results with root development in pulp revascularization.[28] The pastes nevertheless cause some side effects such as coronal discoloration, bacterial resistance, and allergic reaction.[29,30] Sealing the dentinal walls of the access cavity by using a dentin bonding agent and a composite resin prior to placing TAP inside the canal has been recommended.[29] By contrast, Kim et al[30] showed that using dentin bonding agents prior to the placement of TAP does not completely prevent tooth discoloration. Cefaclor is instead recommended to prevent tooth discoloration.[31]

Calcium hydroxide is reportedly a promising alternative for intracanal medications because of its antimicrobial properties,[32] the unlikelihood of crown discoloration,[25] the possible release of growth factors and biomolecules from dentin,[16] and the availability of this medication in routine clinical practice. Better results are achieved when the placement of $Ca(OH)_2$ is restricted to the coronal half of the

Table 2 Summary data of the studies included in this review.

Variable	%
Sex	
Female	56.5
Male	43.5
Tooth type	
Incisor	31.3
Premolar	59.6
Molar	9.1
Tooth location	
Maxilla	31.3
Mandible	68.7
Cause	
Caries	12.9
Dens evaginatus	25.9
Trauma	33.8
Fracture	27.4
Intracanal medicament	
Triple antibiotic paste	47
Calcium hydroxide	51
Chlorhexidine gel	2
Change in root length	54
Change in dentin thickness	76
Age (y), mean ± SD	10.17 ± 1.96
Follow-up time (mo), mean ± SD	17.45 ± 8.58

tooth (53.8% versus 3.3% increase in dentinal wall thickness).[33] However, because of its high pH, $Ca(OH)_2$ can cause tissue necrosis immediately on contact and thereby destroy tissue differentiation into new pulp.[10] Another disadvantage is associated with the increased risk of root fracture in immature teeth with $Ca(OH)_2$ dressing because of its reaction with dentin.[2] A recent study by Ruparel et al[34] evaluated the effects of TAP, double antibiotic paste, and $Ca(OH)_2$ on human SCAPs. Ruparel showed that medicaments used in regenerative procedures have a detrimental effect on the survival of SCAPs, except for $Ca(OH)_2$. For this reason, it is important to use these medicaments at an adequate concentration to have antibacterial efficacy while not evoking toxicity in the host stem cells. Another recent study reports that mechanical instrumentation of the cervical third and middle third of the root canal with an intracanal dressing composed of $Ca(OH)_2$ and 2% CHX gel leads to satisfactory root development in necrotic immature teeth.[27] Nosrat et al[35] recommend using amoxicillin plus clavulanate (i.e., Augmentin, GlaxoSmithKline, Research Triangle Park, NC) as an intracanal medicament for healing periradicular disease. Furthermore, Shin et al[19] present a successful single-visit regenerative endodontic treatment technique to discard the intracanal medication process.

Blood clot formation

Research shows that the inclusion of a blood clot in the root canal tends to improve the revascularization outcome,[36] and that the induction of bleeding into the canal may provide stem cells that can induce dentin formation.[37] Besides acting as a scaffold, the blood clot may also contain growth and differentiation factors that may be important for successful revascularization of the empty pulp canal.[38] Ding et al[9] suggested that failed regenerative procedures were attributed to the inability to evoke bleeding into the root canal. It is important to consider that if bleeding after canal disinfection is not achieved, clinicians should consider using an anesthetic without a vasoconstrictor when trying to induce bleeding because bleeding is easier when an anesthetic solution does not contain a vasoconstrictor.[12] The bleeding should be allowed to reach a level of 3 mm below the cementoenamel junction, and the tooth is left for 15 minutes so that a blood clot forms.[9] However, cases of successful revascularization using $Ca(OH)_2$ without inducing bleeding have also been reported.[25] This could be related to the presence of SCAPs in immature teeth, although induced bleeding will increase the chance of stem/progenitor cell migration.

MTA placement

Mineral trioxide aggregate is carefully placed over the blood clot, followed by a wet cotton pellet. A coronal seal with MTA is used because the material possesses an excellent sealing ability.[39,40] To allow more root development, the coronal edge of the MTA should be placed 1–2 mm apical to the cementoenamel junction rather than 3–4 mm as described by Banchs and Trope.[10] A resorbable barrier is also used to serve as a matrix for the MTA.[12]

The size of the apical foramen

The size of the apical foramen is of great interest, especially for the regenerative endodontic treatment of permanent teeth after the completion of root development. In a radiographic study, Andreasen et al[41] showed that revascularization of the pulp tissue is unpredictable if the apical foramen of the tooth is smaller than 1 mm. However, in a recent animal study, Laureys et al[42] reported that an apical foramen of 0.32 mm did not prevent the ingrowth of new tissue in two-thirds of the pulp chamber at 90 days after transplantation. With this finding, they concluded that the size of the apical foramen seems not to be the only decisive factor for successful revascularization and ingrowth of new tissue after transplantation. In addition, similar results were described in a case report in which the apical foramen was enlarged only up to 0.6 mm.[43]

Regenerated tissue properties and tooth vitality

The nature and histologic appearance of the new tissue occurring in the root canal space are also unknown, although a positive response to the pulp sensibility test has been achieved in some patients.[9] In fact, the lack of response may not even be related to the presence or absence of regenerated nerve tissue. According to Torabinejad and Turman,[44] the coronal level of regenerated tissue and the thickness of filling materials placed over this tissue both affect the presence or absence of responses to the electric pulp test and cold.

According to a series of clinical and histologic studies, regenerative tissues in root canals are primarily of the following four types: (1) revascularization of the pulp with accelerated dentin formation, which leads to pulp canal obliteration; (2) ingrowth of the cementum and periodontal ligament; (3) ingrowth of the cementum, periodontal ligament, and bone; and (4) ingrowth of bone and bone marrow.[45] The first type is believed to have the best prognosis. Human teeth with an immature apex are an effective source of cells for hard tissue regeneration.[46] Hard tissues newly formed on dentinal walls are reportedly distinct from dentin, bone, or bone-like tissue in the root canal space; they resemble cementum, but with significantly different organization and maturation of the collagen matrix.[47] However, whether the root thickened by cementum-like tissue provides needed physical strength is unknown. Besides the hard tissues, the type of soft tissues newly formed in the root canal is also of interest. In a clinical study,[48] one patient received a regenerative endodontic treatment by using platelet-rich plasma. Because of the patient's complaint, the root canal treatment was performed. Histologic examination of the tissue removed from the root canal during root canal treatment revealed that it is a pulp-like connective tissue without odontoblasts.[48]

To date, clinical cases have been reported showing that immature teeth healing via revitalization have the characteristics of gaining root thickness and length that resembles the normal maturation of the root.[4,8,10,19,20,23,24,26,27,29,43,44,49–51] This leads to the speculation that some surviving pulp tissue, and likely the apical papilla, must have been present after disinfection.[52] Hertwig's epithelial root sheath and the apical papilla have been observed in an immature permanent tooth clinically diagnosed as having irreversible pulpitis.[52] These vital tissues contribute to the maturation of root development.[53,54] Inflammation is believed to provide factors that guide the differentiation of stem/progenitor cells in the healing soft tissue into cementoblasts.[54] However, infection and/or inflammation can hinder the potential of tissue regeneration and stem cell function.[55]

Based on the studies and case reports, Table 3[8,9,12,17,21,22,30] summarizes some important findings that can guide clinicians to achieve more successful treatment for infected immature permanent teeth. The success rate of regenerative endodontic treatments can be evaluated in accordance with the clinical outcomes. A retrospective outcome study by Jeeruphan et al[4] showed a great survival rate (100%) with healed cases (80%) and healing cases (20%) after pulp revascularization. This survival rate was based on an approximately 21-month mean follow-up evaluation. Successful outcomes have been reported by clinical cases, histologic analysis revealed tissue repair, instead of regeneration, of the pulp-dentin complex.[54] In a recent case report, Nosrat et al[35] showed the maturation of the root apex in the absence of the regenerated pulp tissue in the root canal by observing the root canal with an operation microscope after patients had undergone 17 months of initial endodontic therapy. Therefore, it could be concluded that the protocol for regenerative endodontic treatment is not predictable for pulp–dentin regeneration. To achieve the regeneration of anatomic pulp–dentin

Table 3 Recommendations for successful regenerative endodontic treatment, based on the reviewed articles.

Authors	Year	Important findings
Ding et al[9]	2009	Case selection is the most important part of this treatment approach because it requires stringent follow-up.
Cotti et al[8]	2008	Concentrations of NaOCl
Kim et al[30]	2010	ranging from 2.5% to 6% have been used for irrigation and provides favorable results.
Galler et al[17]	2011	A final irrigation with EDTA provides more optimal conditions for cellular differentiation, tissue formation, and regeneration.
Trevino et al[21]	2011	CHX is detrimental to stem cells of the apical papilla. It is not recommended.
Cohenca et al[22]	2010	EndoVac system (Discus Dental, Culver City, CA) can be used without the necessity of intracanal medication (especially triantibiotic paste) to prevent further discoloration.
Ding et al[9]	2009	If $Ca(OH)_2$ is used as an intracanal medicament, its placement should be restricted to the coronal half of the tooth to achieve greater increase in the dentinal wall thickness.
Petrino et al[12]	2010	If bleeding after root canal disinfection fails, clinicians should consider using an anesthetic without a vasoconstrictor when trying to induce bleeding.
Ding et al[9]	2009	For the formation of a blood clot, the tooth should be left for 15 minutes. The blood clot acts as a suitable barrier for the placement of MTA (ProRoot MTA, Dentsply Maillefer, Tulsa, OK, USA).
Petrino et al[12]	2010	It is recommended to place the coronal edge of the MTA (ProRoot MTA, Dentsply Maillefer, Tulsa, OK, USA) 1–2 mm apical to the CEJ to allow new tissue growth into the root canal.

$Ca(OH)_2$ = calcium hydroxide; CEJ = cementoenamel junction; CHX = chlorhexidine; MTA = mineral triaggregate; NaOCl = sodium hypochlorite.

complex, it is necessary to add growth factors and scaffolds. Utilizing of growth factors to regenerate pulp tissue without the inclusion of cells yielded the formation of regenerated dental—pulp-like tissue in endodontically treated root canals.[56] This approach, called "cell homing", contains active recruitment of endogenous cells such as stem/progenitor cells into an anatomic compartment.[57] The regeneration of dental pulp by cell homing, rather than by cell delivery, can accelerate clinical translation, although stem cell-mediated tissue regeneration has more optimal results and has the capability of *de novo* regeneration of pulp and new dentin.[58,59] The success of applying cell homing and cell-based tissue engineering to regenerate pulp and dentin in the root canal system is based on preclinical studies. For this reason, more translational research is required to evaluate the effects of growth factors, stem cells, and scaffolds in treating immature permanent tooth with pulp necrosis.

Conclusion

Clinical literature indicates that regenerative endodontic treatments using various methods and materials result in a significant increase in root length and dentinal wall thickness. This clinical approach is technically simple and can be completed without expensive biotechnology by using currently available instruments and medicaments. However, for regenerative endodontic procedures to be widely available and predictable, it seems essential to focus on tissue engineering therapies to regenerate pulp—dentin tissue. The proposed therapy can provide a tissue-engineered pulp construct to implant into the root canal. However, this approach is still in its infancy and more translational research is needed to continue to improve this method.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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