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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.

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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) or multiple monthly appointments to place calcium hydroxide inside the root canal to eliminate the intra-radicular infection and to stimulate calcification to close the apex. After monthly appointments, the tooth should be stronger to allow the root canals to be obturated with gutta-percha (38, 39). A problem with calcium hydroxide is that it can alter the mechanical properties of dentin and render these teeth more susceptible to root fracture (40). The traditional use of calcium hydroxide to accomplish apexification is gradually being replaced by MTA as a one-step technique (41). The MTA can be placed as an apical plug with calcium hydroxide (42, 43) or even as a root canal obturation material (44). Although effective, a drawback is that the obturation of whole root canals with MTA is expensive. The developing consensus approach to accomplish apexification is to instrument root canals to remove the necrotic tissue and to place MTA in the root canal apex, with the remainder of the canal obturated with gutta-percha (29, 45–47).

There have been few long-term studies of apexification with MTA, but the few studies that have been published have reported root apexification and periapical healing (29, 45–47). Over a 2-year period, the apexogenesis failure rate of an MTA apical plug with a single placement of calcium hydroxide for immature permanent teeth was 7.1% (37). Over the longer term, the failure rate may be expected to increase, but the longer-term rate of apexogenesis failures is difficult to predict. Among two hundred case reports, some variable success rates have been reported for apexification with calcium hydroxide with evidence lacking about its adverse events or long-term effects (38). Apexification with calcium hydroxide has not always been successful in completing the root formation of immature teeth or for healing persistent periradicular inflammation and tenderness to percussion (41), avoiding root resorption (42), and there is no clinical evidence that its use may help avoid root fracture. Apical MTA plugs are more effective for accomplishing apexogenesis (46) and for strengthening the roots of traumatized immature permanent anterior teeth (48), but it is not clear whether these MTA apexification procedures are as effective as regenerative endodontic procedures.

Regenerative endodontic treatment recommendations

Guidelines are necessary to ensure that regenerative endodontic procedures are used on teeth, which are most likely to benefit patients. Prolonging patient suffering by providing them with a regenerative endodontic procedure, which will ultimately fail, must be avoided. The American Association of Endodontists (AAE) established a database in 1996 to collect regenerative endodontic cases submitted by endodontists (49); more than 100 cases have been collected to date, but the AAE has not yet established guidelines from the database. In 2010, the American Dental Association (ADA) provided the first treatment codes for regenerative endodontic procedures. The codes for apexification/recalcification are D3351–D3354. The formal acknowledgement by the AAE and ADA and collection of cases to analyze success rates marks a milestone in the growing acceptance of regenerative endodontic procedures into more mainstream dental practice. The terminology used to describe regenerative endodontic procedures includes direct pulp capping, revascularization, apexogenesis, apexification, and even dental pulp constructs (50). The first review article to group these terms into the field of regenerative endodontics was published in 2007 (12).

Regenerative endodontic procedures could have the potential to increase the health risk to patients if they are not used correctly and fail to disinfect and remove necrotic tooth tissues. Most of the evidence for regenerative endodontic procedures involves successful case reports and case series (10, 13–23). Because no failures have been reported, it is not yet possible to predict its failure rate. To help avoid treatment failure by using regenerative endodontic procedures incorrectly, standardized guidelines are needed. Nevertheless, no established standardized treatment guidelines for endodontic regeneration are available. Guidelines are needed to ensure that regenerative endodontic procedures are used appropriately. The existing treatment guidelines for traumatized teeth established by the International Association of Dental Traumatology (51–53) advocate root canal obturation, pulp capping, and partial pulpotomy. These beneficial and practical guidelines contain little or no information concerning when revascularization, apexogenesis, or apexification procedures are indicated, likely because so little evidence was available at the time of their discussion. A flow chart of the regenerative treatment recommendations for traumatized teeth is shown in Fig. 1. The evidence base for the recommendations will be discussed in the following paragraphs.

Revascularization of avulsed and replanted teeth

The common age-group for avulsion injuries is children between the age of 7 and 10 years, when the permanent

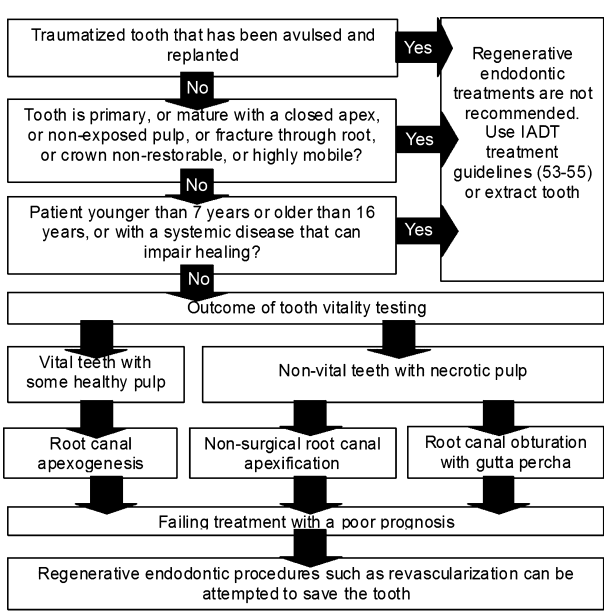

Fig. 1. Regenerative treatment guidelines for traumatized teeth.

incisors are erupting (54). Avulsed intact teeth with no fracture through the root should be washed with water, saline, or chlorhexidine to remove any contamination and be replanted immediately (55). The removal of coagulum and cleaning of the socket is not regarded as being beneficial (56). If the tooth has an extra-oral dry time of 60 min or more, replantation is usually not recommended (55). The most severe pulp damage is seen in the coronal pulp in mature replanted teeth with a closed apex, whereas teeth with an open apex healed more rapidly (57). The likelihood of natural revascularization after replantation of an avulsed tooth is influenced by the extra-alveolar time and the stage of root development, which is reflected by the diameter of the apical foramen. An open foramen >1.1 mm is beneficial, with natural revascularization occurring in approximately 18% (58) to 34% (59) of teeth with immature roots. Successful periodontal healing can be improved if the pulp is extirpated within 14 days (60). There is little evidence to support the use of an endodontic revascularization procedure on an avulsed and replanted tooth. A rare case report is shown in Fig. 3 of new tissue formation in the root canal after performing the endodontic revascularization procedure on an avulsed and replanted tooth. This case suggests that avulsed and replanted immature permanent teeth with an open apex that develop symptoms of necrosis can benefit from using a regenerative revascularization procedure. It is not recommended to attempt the revascularization procedure on replanted deciduous teeth, or teeth with a closed apex, as these are unlikely to revitalize or benefit.

The outcomes of traumatic fracture types on tooth survival

The survival of permanent teeth following non-surgical endodontic treatment, which have no root fractures, can be 97.1% after 8 years (61). Teeth that suffer a small coronal fracture are routinely repaired using common dental restoration materials. Teeth that suffer an extensive coronal tooth structure loss will require the placement of crowns. When artificial complete crowns are required, the incorporation of 1.5- to 2.0-mm-high circumferential coronal ferrules might reduce substantially the occurrence of subsequent root fractures (62). Teeth that suffer a radicular root fracture are most often not restorable and will have to be extracted (63). Teeth that have a coronal fracture that is not detected prior to non-surgical endodontic treatment, or suffer a fracture that develops following root canal obturation, can account for 28.1% of teeth that are extracted within 5 years of non-surgical root canal treatment (64). Relatively few large clinical studies from general practices have examined the prevalence and incidence of complete and incomplete coronal and/or radicular fractures in restored and non-restored teeth with either vital pulps or endodontically treated non-vital pulps and with either complete or incomplete root formation (65). It is too soon to know how the type of traumatic fractures will guide the delivery of regenerative endodontic procedures, because the evidence is scarce and comprises case reports. However, it is essential that regenerative endodontic procedures are not provided to teeth with a radicular root fracture. This is because of the high risk of microleakage and failure of treatment caused by the infection of regenerated tissues within the root canal. Saving the roots of immature permanent maxillary teeth that suffered a complicated crown fracture can be beneficial for 90% of children over a 2-year period (66). Cvek or partial pulpotomy (9, 30–34) can be an excellent choice for the regenerative treatment of vital teeth with a fractured crown, provided that no caries or infection extends into the pulp tissues (31, 53). If the tooth is not vital, has a necrotic pulp, periapical lesion, or has a caries lesion extending into the root canal, other types of regenerative endodontic procedures should be considered (Fig. 1).

Tooth vitality, necrosis, and resorption

Following trauma, a patient will benefit from keeping a healthy tooth if the pulp vitality can be preserved. An acute dental trauma can impact to the hard dental tissues and damage the pulp, periodontium, and surrounding alveolar bone. In the case of luxation injuries, the trauma can rupture the neurovascular supply at the level of the apical foramen, whereas in a root fracture, the rupture can occur at the level of the fracture (67). A disruption to the blood supply of the tooth can cause tissue asphyxia, which will lead to necrosis and a loss of pulp vitality. The traumatized pulp can suffer inflammation and irreversible pulpitis, which will eventually lead to liquefaction necrosis (68). Pulp necrosis is often asymptomatic but may be associated with episodes of pain and discomfort. Teeth with a necrotic pulp would be expected to be non-responsive to vitality testing, but the presence of varying degrees of inflammatory responses in teeth with multiple canals can sometimes give positive responses to vitality tests. Pulpitis following trauma can initiate the resorption of adjacent hard tissues. Normally, an intact tooth is resistant to resorption, even if inflammation is present. However, when an injury damages the protective layer of precementum, inflammation of the pulp or periodontium will induce root resorption (69). Resorption of the dentin walls by osteoclast activity from the center to the periphery can occur. Most of these cases are asymptomatic, but over the long-term, pink spots can be observed where advanced internal resorption has taken place. To prevent resorption and necrosis, the acutely traumatized teeth should have a non-surgical root canal procedure or more ideally have an apexification procedure (70). It is not clear whether any of the regenerative endodontic procedures can prevent resorption; this is unlikely, but little or no evidence is available. Until more evidence is available, endodontic revascularization procedures should not be performed on severely traumatized teeth where resorption is expected. Some recent limited evidence suggests that MTA apexification may be more beneficial for severely injured teeth than a regenerative endodontic procedure (70). The problem is the lack of long-term studies to

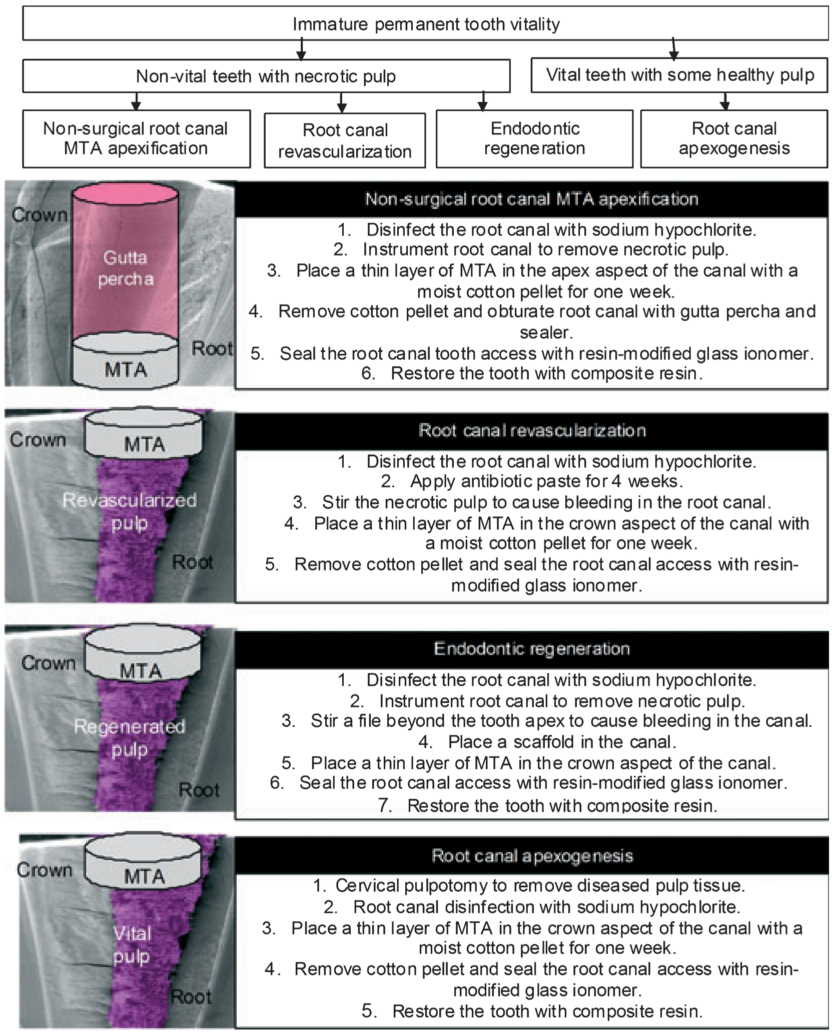

Fig. 2. Steps to accomplish four types of regenerative endodontic treatments.

support the use of MTA apexification. The suggested protocols for endodontic regeneration treatments are shown in Fig. 2.

Apex size and stage of tooth maturity

When the pulp of adult teeth with a closed apex becomes exposed because of trauma or caries decay, its debridement followed by root canal obturation with gutta-percha is an extremely successful procedure (61). In the immature traumatized teeth of children, apexification can help avoid the risk of pain and necrotic pulp complications from developing (70). Non-surgical endodontic treatment is not ideal, because instrumentation could further weaken the thin walls of immature teeth and make them more prone to fracture. Traumatized immature teeth could benefit from regenerative endodontic procedures such as revascularization (10, 13–23), apexogenesis (24–29), and partial pulpotomy (9, 30–34). A critical aspect prior to consider providing a regenerative endodontic treatment to immature permanent teeth is the size of the apical foramen. A small apical foramen can limit blood flow into teeth. Teeth with restricted blood flow are not likely to revascularize and regenerate in response to regenerative endodontic procedures. Traumatized immature permanent teeth with an apex that is open to a diameter of 1.1 mm or larger are the best candidates for regenerative endodontic procedures (58). Revascularization through an open apex allows the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after a clinical regenerative endodontic procedure (71). This could allow host cell homing to form new tissues in the root canal space (72), as shown in Fig. 3.

Age and health status of patient

Several case reports of revascularization procedures have been generally limited to patients who are reaching adolescence, mostly aged 8–16 years (10, 13–23), although one patient was 41 years of age (9). There have been no studies of children who have genetic diseases, or severe medical conditions, and a compromised immune system, which could impair the dental healing responses. We can expect that children with a compromised ability to heal will not be good candidates for regenerative endodontic

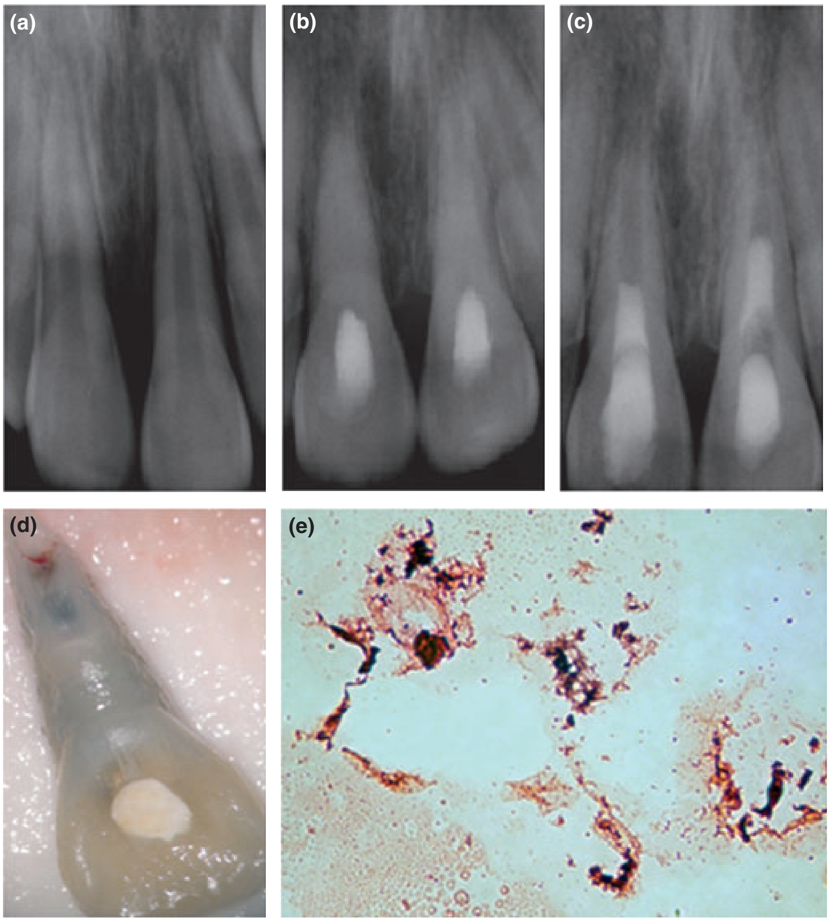

Fig. 3. Case of an avulsed replanted tooth and tissue formation in the root canal following a regenerative endodontic procedure. A 9-year-old girl avulsed her #8 and #9 teeth in an accident; they were replaced after 2 h and had a poor prognosis because of replacement resorption. Revascularization was attempted after apexification with calcium hydroxide had been attempted, but had a poor prognosis. The calcium hydroxide was changed every 3 months and was inside the canal for 9 months. No antibiotic paste was used. A blood clot was initiated by stirpating the apex to cause bleeding into the root canal. The canal was irrigated with saline and flushed with EDTA. Amoxicillin was prescribed. Unfortunately, the patient fell again and avulsed the #9 tooth, but it was fractured through the root and could not be replaced. The avulsed tooth was collected for histology. The histology shows little tissue regeneration, and there are mostly red blood cells. Courtesy of Dr. Shiju Cherian.

procedures. The success of regenerative endodontic procedures is dependent on the ability of the patient to heal the dental pulp tissues (73). The provision of regenerative procedures should be restricted to healthy patients who can heal and benefit from the procedure. Regenerative endodontic procedures should not be delivered to deciduous (baby) teeth as it risks retaining teeth, which could impair the eruption pattern of adult teeth. Given the limited data available, it would not be advisable to give regenerative endodontic procedures to children younger than 8 years or older than 16 years, unless the tooth has an open apex with thin walls that are at risk from subsequent fracture. Endodontic revascularization regeneration treatments can be successful if they are used in cases where the age and health status of the patient are restricted (Table 1). In a retrospective study of 30 cases, followed for up to 1 year, only two cases (6.7%) had complications. The complications were minor and restricted to discomfort or discoloration.

Disinfection treatments

The most common disinfectant and irrigant used in endodontics is sodium hypochlorite (74). Sodium hypochlorite is not biocompatible, and it can kill dental pulp stem cells and prevent them from attaching to the surfaces of root canals (75). If sodium hypochlorite is used in conjunction with regenerative endodontic treatments, it should be flushed from the root canals with saline in an attempt to reduce any lingering toxicity that can reduce the regeneration responses (64). Some alternative irrigants to sodium hypochlorite have been developed which are more biocompatible, including noni juice (76) and Aquatine EC (77), among others. So far, the biocompatible irrigants have not been used in conjunction with regenerative endodontic procedures; further research is necessary to determine whether biocompatible irrigants can help improve tissue healing following regenerative endodontic procedures.

Table 1. Summary of revascularization outcomes from our archive of 30 cases

Patient#	Health	Age	Oral health	Etiology	Periapical lesion	Did lesion heal?	Sensation restored?	Pain or type of complications
1	Normal	11	Good	Trauma	Yes	Yes	No	No
2	Normal	11	High	Caries	No	NA	TBA	No
3	Normal	11	High	Caries	No	NA	No	No
4	Normal	11	High	Caries	No	NA	NA	No
5	Prolactinoma	11	Good	Trauma	Yes	Yes	No	Discoloration
6	Normal	10	High	Trauma	Yes	Yes	No	No
7	Normal	10	High	Trauma	Yes	Yes	No	No
8	Normal	11	High	Caries	Yes	Yes	NA	No
9	Pen Allergy	11	High	Caries	Yes	Yes	No	No
10	Guillaume Barre	12	High	Trauma	No	NA	No	No
11	Guillaume Barre	12	High	Trauma	No	NA	No	No
12	Guillaume Barre	12	High	Trauma	No	NA	No	No
13	Pen Allergy	17	High	Caries	No	NA	NA	No
14	Pen Allergy	17	High	Caries	No	Yes	NA	No
15	Normal	16	Good	Trauma	Yes	Yes	No	No
16	Normal	16	Good	Trauma	Yes	Yes	No	No
17	Normal	10	High	Caries	No	NA	NA	No
18	Normal	9	Good	Trauma	Yes	Yes	No	No
19	Normal	15	High	Caries	No	NA	NA	No
20	Anemia	12	Good	Trauma	Yes	Yes	No	Yes, discomfort
21	Normal	9	High	Caries	No	NA	NA	No
22	Normal	9	High	Caries	No	NA	NA	No
23	Normal	11	High	Caries	No	NA	NA	No
24	Normal	11	High	Caries	No	NA	NA	No
25	Normal	9	Good	Trauma	No	NA	NA	No
26	Normal	9	Good	Trauma	No	NA	NA	No
27	Normal	9	High	Caries	No	NA	NA	No
28	Normal	12	Good	Unknown	Yes	Improvement	No	No
29	Normal	10	Good	Trauma	No	NA	NA	No
30	Normal	9	Good	Caries	No	NA	NA	No

Most of the revitalization regeneration procedures use a triple antibiotic paste, sometimes called Hoshino's paste (78). The paste contains 200 mg cirofloxacin, 500 mg metronidazole, and 100 mg minocycline, which must be mixed by a compounding pharmacist (78). The triple antibiotic paste is placed in contact with the necrotic pulp inside the root canal for up to 1 month prior to the revascularization procedure (17). If the triple antibiotic paste is used, it is important to warn the patient and their parents/guardians that it is not FDA approved. Although the triple antibiotic paste was used in most studies, some teeth did not get any antibiotic and reported success (79). In other teeth, calcium hydroxide proved to be equally as effective as the antibiotics at promoting root lengthening and thickening (79). The case reports and case series (79) raise questions about the need to use any antibiotic for disinfection. Given the lack of evidence, the risk of not accomplishing root canal disinfection without antibiotics would be higher. Therefore, antibiotics, calcium hydroxide, or another disinfectant should be used in revascularization cases unless it can be established that sodium hypochlorite is sufficient to disinfect necrotic pulp tissues.

Dental materials and sealers

Regenerative endodontic procedures evoke bleeding into the root canal, which delivers undifferentiated mesenchymal stem cells in the root canal space (71). The mesenchymal stem cells are multipotent, meaning that they can differentiate into a variety of cell types, including osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells) (80). In the dog root canal, the mesenchymal cells are responsible for new tissue formation that has been described as fibrous connective tissue and intracanal cementum (81). It has a similar appearance to the tissues regenerated in the root canal of a human tooth (Fig. 3). The survival of cells and regeneration of tissues is sensitive to the conditions within the intracanal environment (75). The intracanal environment must be biocompatible to allow new tissue formation. The placement of restorative materials including amalgam, composite resins, or glass ionomers, in contact with the pulp tissue, root canal blood clot, or regenerating tissues is not recommended. These materials are not biocompatible to exposed pulp tissues because they can cause cell death and pulpitis and allow bacterial leakage (82). A 'total etch' adhesive technique must never be used to place composite resin in contact with exposed pulp tissues (83). Restorative materials should be used only after a thin protective liner of MTA (14) or calcium hydroxide (84) has been placed in contact with the coronal pulp tissue, root canal blood clot, or regenerating tissues (14). Endodontic sealers were never designed to be biocompatible with soft tissues and are highly toxic to cells (84). The toxicity of endodontic sealers will impede cell survival and tissue regeneration in

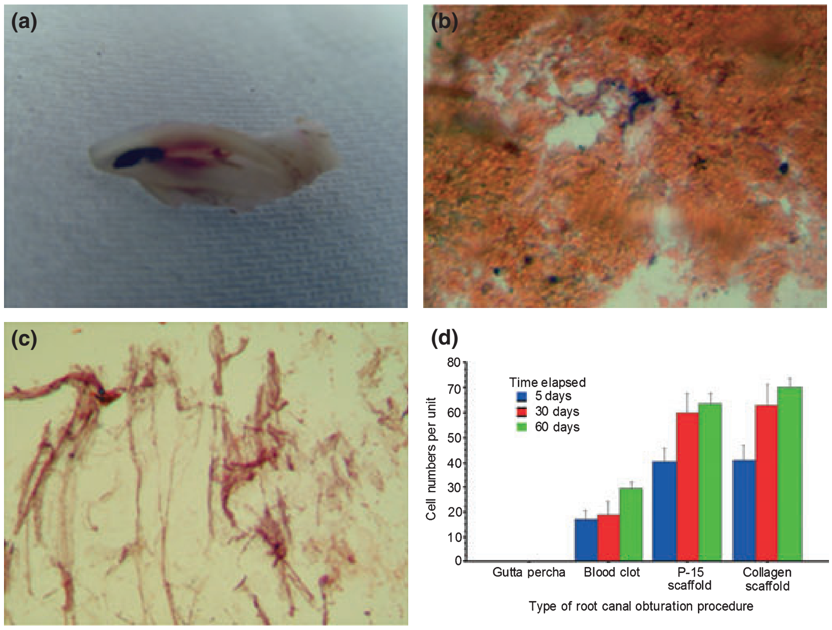

Fig. 4. Tissue formation in the root canals of primate teeth following regenerative endodontic procedures. The bar chart shows the numbers of cells per microscope field following endodontic revascularization or regeneration procedures.

the root canals and is not suitable to be used in conjunction with regenerative endodontic procedures.

Future regenerative endodontic therapies

Endodontic regeneration can be accomplished through the activity of the cells from the pulp, periodontium, vascular, and immune system (71). Most therapies use the host's own pulp or vascular cells for regeneration, but other types of dental stem cell therapies are under development. Because of the increasing activity of dental stem cell banks, we can expect stem cells from baby teeth to be implanted into mature teeth to accomplish regeneration (85). We performed an endodontic regeneration study on non-human primates by cleaning and shaping root canals and then stirpating the periapical tissues to cause bleeding into the root canals. We then placed a spongy collagen scaffold in some teeth and a PepGen P15 injectable scaffold in other teeth. After 5, 30, and 60 days, the teeth were extracted for histologic analysis (Fig. 4). The results showed that the basic revascularization procedure can be improved by inserting a scaffold into the root canal (Fig. 4). FDA approval and clinical trials of these regenerative endodontic procedures are in progress. The outcomes of using scaffolds, tissue engineering, and growth factors are expected to benefit patients and dentists by improving the effectiveness of regenerative endodontic treatments.

Conclusions

If a clinician does decide to give a tooth a revascularization procedure, the following recommendations need to be considered:

1 The traumatized tooth must be non-vital and not be suitable for apexogenesis, apexification, partial pulpotomy, or root canal obturation treatments.

2 The tooth must be permanent and very immature with a wide-open apex and exposed pulp. The tooth must have thin walls that will benefit from a continued development of the root, so that it can become stronger and less prone to failure in later life.

3 The patient must be aged 7–16 years, in good health, and have parents/guardians willing to take them to attend multiple appointments.

4 The patient/parents/guardians must be told that the endodontic regeneration treatment is experimental and that no standardized guidelines have yet been created.

5 Antibiotic paste can be used as an additional disinfectant to sodium hypochlorite, and the patient needs to be warned about the potential for discoloration.

6 An anesthetic without a vasoconstrictor should be used when attempting to induce revascularization (bleeding) into the root canal.

7 A thin liner of white MTA or calcium hydroxide should be placed above the blood clot.

8 An endodontic sealer is not biocompatible for regeneration and cannot be used.

9 The tooth should be restored with a resin-modified glass ionomer to help prevent microleakage, with a composite resin overlay restoration, or full-crown replacement depending on the severity of crown damage.

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