Management of immature teeth by dentin-pulp regeneration: A recent approach

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Received: 03/07/2010
Accepted: 17/12/2010

Abstract

Treatment of the young permanent tooth with a necrotic root canal system and an incompletely developed root is very difficult and challenging. Few acceptable results have been achieved through apexification but use of long-term calcium hydroxide might alter the mechanical properties of dentin. Thus, one alternative approach is to develop and restore a functional pulp-dentin complex. Procedures attempting to preserve the potentially remaining dental pulp stem cells and mesenchymal stem cells of the apical papilla can result in canal revascularization and the completion of root maturation. There are several advantages of promoting apexogenesis in immature teeth with open apices. It encourages a longer and thicker root to develop thus decreasing the propensity of long term root fracture. So, the present article reviews the recent approach of regeneration of pulp-dentin complex in immature permanent teeth.

Key words: Immature teeth with open apices, revascularization, pulp-dentin complex regeneration.
Introduction
Pulp necrosis of an immature permanent tooth, from the trauma or caries arrests further tooth development. As the root development takes long time, an incompletely formed apex is one of the most common features seen in traumatized teeth. Loss of pulpal vitality before completion of dentin deposition leaves a weak root more prone to fracture (1) as a result of the thin dentinal walls. It will also lead to a poorer crown-to-root ratio, with possible periodontal breakdown as a result of increased mobility. So every attempt should be made to preserve the vitality of these immature teeth till maturation has occurred.

Treatment of the immature non vital anterior tooth with apical pathosis presents several treatment challenges. The mechanical cleaning and shaping of a tooth with blunderbuss apex are difficult, if not impossible. The thin, fragile lateral dentinal walls can fracture during mechanical filing (2) or during lateral condensation. Another classic option for such teeth includes endodontic surgery to seal the wide apex by retrograde means. But this invasive procedure has its own disadvantages as surgical complications. Another drawback is compromised crown-root ratio which further weakens the already fragile root. Apexification has some chances of success but it also has many limitations as multiple visits during a long period of time (6-24 months), porous barrier and inadequate seal. Even some reports have shown that long term calcium hydroxide therapy may leave the thin walls even more prone to fracture (3). An alternative to traditional apexification is to place an artificial barrier at the apex using materials as Mineral trioxide Aggregate (MTA) (4). But even MTA doesn’t strengthen the remaining root structure, so tooth stays fracture prone. Another treatment advocated for such weakened roots is root reinforcement using composite resins, but they may limit the possibility of root canal retreatment in future if need arises (5).

So, the ideal treatment to obtain further root development and thickening of dentinal walls in an immature tooth with apical periodontitis would be revascularization that is to reestablish the vitality in a non vital tooth to allow repair and regeneration of pulp dentin complex. The term “revascularization” has been used for the reestablishment of vascularity in the pulp space after traumatic injuries. However in this situation, there is generation of tissues, such as cementum, periodontal ligament, bone, and dentin, or regeneration of pulp occurs rather than just the generation of vasculature in the canal space that restores functional properties of the tooth, and fosters continued root development for immature teeth, and prevents or resolves apical periodontitis. Thus, using the term revascularization for regeneration of a pulp-dentin complex has been questioned (6) and better option would be maturation of the root. The research on regeneration of a pulp-dentin complex has a long history. It was introduced by Ostby (7) in 1961, and in 1966, Rule and Winter (8) documented root development and apical barrier formation in cases of pulpal necrosis in children. In 1971, Nygaard-Ostby & Hjortdal performed studies that can be considered the fore runner of pulpal regeneration (9). The results of these studies were variable. However, the materials and instruments available 40–50 years ago were probably not sufficient and adequate.

Instead, current research in regenerative endodontics uses greatly improved materials, instruments, and medications and applies many principles from the fields of trauma research and tissue engineering. So it could be possible to effectively disinfect an infected pulp, artificially place a scaffold, and then effectively seal the access cavity to resist subsequent infection.

Rationale of revascularization
Occasional cases of regeneration of apical tissues after traumatic avulsion and replantation led to the search for the possibility of regeneration of the whole pulp tissue in a necrotic, infected tooth. After reimplantation of an avulsed immature tooth, a unique set of circumstances exists that allows regeneration to take place. A) The young tooth has an open apex and is short, which allows new tissue to grow in to the pulp space relatively quickly. B) The pulp is necrotic but usually not infected, so it will act as a matrix into which the tissue can grow. C) 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 (10) and defects will be a slow process. Thus, the race between the new tissue and infection of the pulp space favors the new tissue. Extrapolating from this information, it is hypothesized that once the canal infection is controlled, it resembles the avulsed tooth that has a necrotic but sterile pulp space. So finally the rationale of revascularization is that if a sterile tissue matrix is provided in which new cells can grow, pulp vitality can be reestablished.

Factors affecting maturation (revascularization)
There were several factors that helped the immature teeth achieve continued root development. First, the immature tooth has a wide root canal, an open apex and short root. In the ideal case, there is pulp necrosis with an immature apex opening more than 1mm in a mesiodistal dimension radiographically (11). Murray et al. (12) have even suggested that the revascularization of necrotic pulp in a tooth with a closed apex may require instrumentation to approximately 1 to 2mm in apical diameter to allow systemic bleeding in to the root canal system. So, the new tissue has easy access to the root canal system and a relatively short distance for proliferation to reach the coronal pulp horns.
Secondly, these patients are young (8–13 years old), and so have greater healing capacity or stem cell regenerative potential. Thirdly, all root canals of diseased teeth were treated as conservatively as possible. No instrumentation was done in the root canals whereas all of the studies used sodium hypochlorite (NaOCl) as an irrigant. Minimum instrumentation and disturbance of the root canal system also preserved more viable pulp tissues. Further, both calcium hydroxide (Ca(OH)\textsubscript{2}) paste and combinations of multiple antibiotics have been used in these patients. But the results are variable. Finally, the formation of a blood clot might serve as a protein scaffold, permitting 3-dimensional ingrowth of tissue. It is suggested that the all these mentioned favorable factors finally lead to successful clinical outcomes.

Nature of the tissue formed
Nature of the tissue formed is not clear. Various possible mechanisms (13) have been documented for the origin and nature of the newly formed tissues. It is possible that a few vital pulp cells remaining at the apical end of the root canal (14) may might proliferate into the newly formed matrix and differentiate into odontoblasts to lay down dentin causing elongation and strengthening of the root. Even Stem cells from the apical papilla (SCAP) or the bone marrow can be possible mechanism of root development. This apical papilla, a very specific stem cell tissue have greater potential to regenerate the pulp tissue and continue the root development (15). The third possible mechanism could be due to presence of stem cells in the periodontal ligament, which can proliferate, grow into the canal and deposit hard tissue (cementum, bone) into the inner surface of root dentin. Another possible mechanism of continued root development could be due to multipotent dental pulp stem cells (16) which might be present in abundance in immature teeth. The blood clot itself, being a rich source of growth factors, could stimulate differentiation, growth, and maturation of fibroblasts, odontoblasts, cementoblasts, etc, and play an important role in regeneration.

Case selection
Currently there is no evidence-based guideline that can be established to help clinicians determine which condition of cases that can be treated with this conservative approach. As mentioned, the presence of radiolucency at the periradicular region can no longer be used as a determining factor, nor is the vitality test. In both situation, vital pulp tissue or apical papilla may still present in the canal and at the apex. Another obvious consideration is the duration of the infection. Hypothetically, the longer standing of an infected pulp in immature teeth there is the less survived pulp tissue and stem cells may remain. Additionally, longer infection renders the disinfection more difficult to accomplish. Clinicians may consider always choosing the conservative approach first. Other procedures are undertaken only when this attempt is failed.

Procedure
A clinical protocol to treat immature teeth is followed. An access cavity is made. A needle is placed to within 1mm of the apex, and the canal is slowly flushed with NaOCl, or Peridex. After a thorough irrigation, the canal is dried with paper points, and an antimicrobial paste \{calcium hydroxide or a mixture of ciprofloxacin, metronidazole, and minocycline paste as described by Hoshino et al. (17) was prepared into a creamy consistency and placed in the canal to a depth slightly shy of the remaining vital tissue to achieve canal disinfection. The access cavity was closed with Cavit or glass ionomer.

After 2 weeks, the patient should return for evaluation. If the tooth is asymptomatic and lack of clinical signs of pathology, the tooth is then reentered, the tissue is irritated until bleeding is started and a blood clot produced to act as scaffold and the pulp chamber is sealed with MTA cement. The accessed cavity will then be sealed with glass ionomer or resin-modified glass ionomer cement and the tooth should be followed up periodically to observe the maturation of the root.

In partially necrotic pulp, even single visit pulp revascularization protocol can be a favourable treatment option for an immature permanent tooth (18). If no signs of improvement, i.e., persistent presence of sinus tract, swelling and/or pain, other procedure should be preferred.

Disinfection
Disinfection is most important step for revascularization of a necrotic immature tooth. As discussed, mechanical instrumentation cannot be performed in these teeth. Thus, the disinfection relies solely on irrigants and intracanal medications.

Calcium Hydroxide
Traditionally, calcium hydroxide has been used as the intracanal medication in apexification procedures (19). Its effect is to create an environment conducive to the formation of a hard tissue bridge at the apex (20). Direct contact between the Ca(OH)\textsubscript{2} paste and any vital pulp tissue remaining in the canal can induce the formation of a layer of calcified tissue that will prevent the regeneration of pulp tissue in to the occupied space within the canal. Another concern is that Ca(OH)\textsubscript{2}, because of its high pH, may damage the HERS and there by losing its ability to induce the nearby undifferentiated cells to become odontoblasts.

Thus, with calcium hydroxide therapy, there is no expectation that the root canal walls will be thickened or strengthened. To the contrary, in a recent study, it was claimed that long-term calcium hydroxide treatment will in fact weaken the tooth and predispose it to frac-
It may make the tooth brittle because of its hygroscopic and proteolytic properties (21).

**Triantibiotic paste**

A mixture of ciprofloxacin, metronidazole, and minocycline has been shown to be effective in eliminating endodontic pathogens in vitro and in vivo (22). Hoshino et al. (17) showed increased efficiency of combination of different drugs whereas alone, none of the drugs resulted in complete elimination of bacteria. In addition, a study by Sato et al. (22) found that this drug combination was effective in killing bacteria in the deep layers of root canal dentine. In another animal study, Windley et al. (23) examined the effects of a triple-antibiotic paste of metronidazole, ciprofloxacin, and minocycline and found it to be effective in disinfecting immature dog teeth with apical periodontitis. Studies have shown that when an antibiotic paste is used instead of Ca(OH)₂, regenerative pulp tissue is able to occupy the remaining canal space (24,25).

Triple antibiotic paste, contains both bactericidal (metronidazole, ciprofloxacin) and bacteriostatic (minocycline) components, allowing for successful revascularization and the continued development of the root to its normal length. A wide-spectrum bactericide, metronidazole has also been shown to be effective against oral obligate anaerobes, including those isolated from infected necrotic pulp. (26) In selecting appropriate irrigants and medicaments, regenerative effects should be taken into consideration along with anti microbial properties. (12) For example, tetracycline is known to enhance the growth of host cells on dentin, not by antimicrobial action, but via the exposure of embedded collagen fibers or growth factors (27). Topical doxycycline and minocycline have shown to improve radiographic and histological evidence of revascularization in immature avulsed permanent teeth (28,29).

Other antibiotic mixtures have been evaluated in earlier studies—a mixture of penicillin, bacitracin, or chloramphenicol and streptomycin (Grossman’s polyantibiotic paste), and a mixture of neomycin, polymyxin, and nystatin. Both of these pastes were found to have limited efficacy as intracanal medicaments. A study by Molander et al. (30) found another antibiotic, clindamycin, to have no advantage over Ca(OH)₂, or other conventional root canal dressings.

**Scaffold**

An empty canal space will not support in-growth of new tissue from the periapical area on its own (31-32). Tissues are 3-dimensional structures, and an appropriate scaffold is needed to promote cell growth and differentiation. It is known that extracellular matrix molecules control the differentiation of stem cells (33), and an appropriate scaffold might selectively bind and localize cells, contain growth factors (34), and undergo biodegradation over time (35). Thus, a scaffold is far more than a simple lattice to contain cells.

Early studies on attempted revascularization used blood or blood substitutes to act as a scaffold with different growth factors to aid the in-growth of new tissue in to the empty canal space. Interestingly, many case reports include formation of a blood clot as scaffold. Hargreaves et al (36) reported that platelet-rich plasma (PRP) satisfies many of these criteria. Revascularization research has also studied collagen solutions as artificial scaffolds in the canal space.

**Seal**

Another requirement for successful results is to achieve a bacteria-tight seal coronally to inhibit bacterial invasion in to the pulp space before revascularization could take place. A double seal with MTA to a level below the CEJ covered by a bonded resin coronal seal is preferred. The use of MTA is for its excellent microleakage-proof property and biocompatibility. Additional placement with glassionomer/ resin further secures the sealing ability and the integrity of the filled access.

**Advantages of the procedure**

There are several advantages of revascularization as it can be completed in a single visit once the infection is controlled, so no need of repeated appointments as in case of calcium hydroxide apexification. It is also very cost-effective. The biggest advantage is that of restoring the tooth vitality and achieving continued root development (root lengthening) and strengthening of the root as a result of reinforcement of lateral dentinal walls with deposition of new dentin/hard tissue.

However, the benefit is so great compared with leaving a root with a thin and fracture susceptible wall that, in our opinion, it is worth attempting. If no root development can be seen within 3 months, the more traditional apexification procedures can then be started.

**Limitations**

This novel procedure produces a stronger mature root that is better able to withstand fracture but has the potential for clinical and biological complications. Amongst them, crown discoulouration (23), development of resistant bacterial strains (37) and allergic reaction to the intracanal medication. A modification (38) of the current clinical protocol (14) was established to avoid crown discoulouration. In this novel approach, the coronal dentine was sealed with flowable composite thus avoiding any contact between the tri-antibiotic paste and the dentinal walls. Furthermore, the stage and duration of pathosis that will ultimately lead to the complete destruction of the resistant apical mesenchymal cells and surviving dental pulp stem cells has not been determined. Under the circumstances of total pulpal and apical papilla necrosis, revascularization treatment may not be possible.

Additional complications such as various systemic health conditions and immunologic problems may offer
<table>
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<tr>
<th>S no</th>
<th>Tooth no</th>
<th>Age/sex of patient</th>
<th>Chief complaint c/c</th>
<th>diagnosis</th>
<th>Treatment protocol</th>
<th>Irrigant/ medicament</th>
<th>Results</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1.</td>
<td>45</td>
<td>13/F</td>
<td>Recurring swelling in buccal vestibule in relation to 45</td>
<td>Apical periodontitis with sinus tract</td>
<td>-5 weekly visits -access cavity was prepared, not mechanically cleaned. -6 weeks later: Broach Probed vital tissue in canal. -Applied Ca(OH)2 paste, glass ionomer cement, bonded Composite resin.</td>
<td>-irrigation with 5% sodium hypochlorite (NaOCl) and 3% hydrogen peroxide -medicament: Metronidazole and ciprofloxacin as intracanal medicament</td>
<td>5 months — signs of apical closure. 15 months — Formation of dentin bridge. Positive response to electric pulp testing 30 months — Complete closure of the apex and thickening of the root walls on radiographic examination</td>
<td>Iwaya S. et al. 2001 (25)</td>
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<td>2.</td>
<td>45</td>
<td>11/M</td>
<td>Lingual swelling in right mandibular area</td>
<td>Apical periodontitis with sinus tract.</td>
<td>Access cavity was done Canal irrigated and medicated -26 days later Bleeding induced by explorer Bleeding was stopped at 3mm below CEJ. - After 15 min, mta and cavit placed -after 2 weeks Bonded resin restoration</td>
<td>Irritant: 5.25% NaOCl and Periex Medicament: mixture of ciprofloxacin, metronidazole, and minocycline paste</td>
<td>-6-month complete resolution of the radiolucency -At 1-year continued development of the apex. -2-year closure of the apex and thickening of the dentinal walls, Positive response to the cold test</td>
<td>Banchs F. and Trope M. 2004 (14)</td>
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<td>3.</td>
<td>35</td>
<td>10/F</td>
<td>Chronic periradicular abscess</td>
<td>Access prepared, no instrumentation -dressings repeated -11 months: amalgam</td>
<td>Irrgiant: 2.5% NaOCl medicament:Ca(OH)2 paste.</td>
<td>-3 months: hard tissue at Ca (OH)2 site. Asymptomatic. 11 months: Thickening Of dentinal walls. 35 months: Continued Thickening of dentinal walls and apical closure</td>
<td>Chueh LH and Huang GT 2006 (6)</td>
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<tr>
<td>5.</td>
<td>11</td>
<td>8/M</td>
<td>Chronic periradicular abscess</td>
<td>Access prepared. No instrumentation. Deep irrigated and medicated and sealed -induce bleeding with endoexporler till 3mm below cementoenamel junction MTA and Cavit. -Remove Cavit and place bonded composite.</td>
<td>Irrigation with 5.25% NaOCl and 0.12% chlorhexidine. Interappointment medicament: metronidazole, minocycline, and ciprofloxacin.</td>
<td>8 months: Asymptomatic. Apical Closure with thickening of dentinal walls</td>
<td>Petrino JA 2007 (39)</td>
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<td>6.</td>
<td>35</td>
<td>10/f</td>
<td>Pain in relation to 35</td>
<td>Apical periodontitis</td>
<td>Access cavity was made. After 1 week, blood clot formed Mta and caviton. After 2 weeks, bonded resin restoration</td>
<td>Irrigant: 2.5% NaOCl medicament mixture of ciprofloxacin, metronidazole, and minocycline paste</td>
<td>At 12-months asymptomatic complete resolution of radiolucency, and canal narrowed. After 24-months: continued thickening of the dentinal walls</td>
<td>Jung IY et al. (2008) (40)</td>
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<tr>
<td>No.</td>
<td>Age</td>
<td>Sex</td>
<td>Clinical Characteristics</td>
<td>Radiographic Findings</td>
<td>Treatment</td>
<td>Outcome</td>
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<td>7</td>
<td>35</td>
<td>9/f</td>
<td>Lingual swelling of left mandibular area</td>
<td>Apical periodontitis</td>
<td>Access cavity was made. After 1 week, blood clot formed MTA and caviton After 2 weeks, bonded resin restoration</td>
<td>Irrigant: 2.5% NaOCl medicament mixture of ciprofloxacin, metronidazole, and minocycline paste</td>
<td>At 6-months: asymptomatic, Complete resolution of radiolucency, with continued development of apex At 24-months: continued thickening with closure of apex</td>
<td>Jung Y et al. (2008) (40)</td>
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<td>8</td>
<td>45</td>
<td>14/f</td>
<td>Swelling of right mandibular area</td>
<td>Chronic suppurative periodontitis</td>
<td>Access cavity was made. After 1 week, blood clot formed MTA and caviton After 3 weeks, bonded resin restoration</td>
<td>Irrigant: 2.5% NaOCl Medicament: Ca(OH)₂ paste</td>
<td>At 12-months: asymptomatic, Greatly resolved radiolucency,</td>
<td>Jung Y et al. (2008) (40)</td>
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<td>9</td>
<td>15</td>
<td>10/f</td>
<td>Pain in relation to 15</td>
<td>Apical periodontitis</td>
<td>Access cavity was made. After 3 weeks, blood clot formed was insufficient So coltapate used as matrix for growth of new tissue into pulp space MTA and caviton placed After month, bonded resin restoration</td>
<td>Irrigant: 2.5% NaOCl Medicament: Ca(OH)₂ paste</td>
<td>At 17-months: asymptomatic, Complete resolution of radiolucency with continued apical closure</td>
<td>Jung Y et al. (2008) (40)</td>
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<tr>
<td>10</td>
<td>35</td>
<td>11/F</td>
<td>Swelling and pain in the mandibular left premolar region</td>
<td>Pulpal necrosis with chronic suppurative periradicular periodontitis in relation to 35 (dens evaginatus)</td>
<td>Access prepared. No instrumentation Coronal dentine sealed with flowable composite medicated and temporarily sealed with caviton After 1 month: bleeding stimulated to create a biological scaffold sealed with MTA and caviton After two weeks restored with resin bonded composite</td>
<td>Irrigants: 6% sodium hypochlorite, followed by saline and then a final irrigation 2.0% chlorhexidine gluconate tri-antibiotic paste prepared by mixing 250mg of Ciprofloxacin, 250 mg of Metronidazole and 250mg of Minocycline with sterile water</td>
<td>18-month follow-up: asymptomatic. Clinically, -responded to cold test Radiographic evidence of periradicular bone healing and significant root development with root maturation</td>
<td>Reynolds K et al. (2009) (38)</td>
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<tr>
<td>11</td>
<td>45</td>
<td>11/F</td>
<td>Swelling in the mandibular right premolar region</td>
<td>Pulpal necrosis with chronic suppurative periradicular periodontitis in relation to 45 (dens evaginatus)</td>
<td>Premolar was accessed. Coronal dentine sealed with flowable composite medicated and temporarily sealed with caviton After 1 month: bleeding stimulated to create a biological scaffold sealed with MTA and caviton After two weeks restored with resin bonded composite</td>
<td>Irrigants: 6% sodium hypochlorite, followed by saline and then a final irrigation 2.0% chlorhexidine gluconate tri-antibiotic paste prepared by mixing 250mg of Ciprofloxacin, 250 mg of Metronidazole and 250mg of Minocycline with sterile water</td>
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<td>Reynolds K et al. (2009) (38)</td>
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<td>12</td>
<td>14</td>
<td>11/F</td>
<td>Fracture or Discolored anterior teeth</td>
<td>Acute or chronic apical infection</td>
<td>Canal was accessed Blood clot induced when teeth are asymptomatic Sealed with glass ionomer cement</td>
<td>Irrigants: 3% hydrogen peroxide 2.5% sodium hypochlorite, paste prepared by mixing 250mg of Ciprofloxacin, 250 mg of Metronidazole and 250mg of Minocycline with sterile water</td>
<td>Complete Resolution of clinical signs and symptoms and healing of periapical lesions Thickening of lateral dentinal walls in B of 14 cases, and increased root length in 10 out of 14 cases.</td>
<td>Shah N et al. (2008) (13)</td>
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other obstacles in achieving adequate root maturation in the presence of a periapical infection. Long term studies are not available to tell whether the canal obturates or whether apical periodontitis will develop at a later stage. Even if these non desirable outcomes do occur, the tooth is still likely to last for long time, which would not have been the case if it had been treated endodontically at the time of presentation as tooth can fracture because of weakened roots.

Case reports

The growing body of case reports (Table 1) provides impetus for developing prospective randomized controlled trials evaluating these methods.

Conclusion

Clinical experience on the outcome of those teeth that inherit a thin and weak root after successful apexification is that they are susceptible to fracture. Shifting apexification to apexogenesis even for teeth that show a negative response to pulp vitality test and with periapical periodontitis or abscess is a clinically beneficial approach for the patients. So as well stated by Windley et al. (23), revascularization of immature teeth with apical periodontitis depends mainly on: (a) disinfection of the canal; (b) placement of a matrix in the canal for tissue in-growth; and (c) a bacterial tight seal of the access opening.

References


Dentin-pulp regeneration

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