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REVIEW

Apexification: the beginning of its end

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Abstract

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Apexification is a procedure for treating and preserving immature permanent teeth that have lost pulp vitality. It contrasts apexogenesis in terms of its outcome in that apical maturation and normal root thickness cannot be obtained. Apexification has been a routine practice for such teeth for many decades, and despite a literature replete with discussion, including recent artificial barrier methods with mineral trioxide aggregate, ultimately there has been no major breakthrough to improve this treatment. Recently, two new clinical concepts have emerged. One involves a revitalization approach to achieve tissue generation and regeneration. In this method, new living tissue is expected to form in the cleaned canal space, allowing continued root development in terms of both length and thickness. The other is the active pursuit of pulp/dentine regeneration via tissue engineering technology to implant or re-grow pulps. Although the technology is still at its infancy, it has the potential to benefit immature pulpless teeth by allowing continued growth and maturation. With this understanding, it may be predicted that apexification will become less needed in years to come. This study will overview the recent concept of pulp revitalization in the treatment of immature teeth with nonvital pulps and the emerging research on pulp tissue engineering and regeneration.

Keywords: apexification, calcification, pulp/dentine tissue regeneration, stem cells.

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Introduction

Apexification is a procedure to promote the formation of an apical barrier to close the open apex of an immature tooth with a nonvital pulp such that the filling materials can be contained within the root canal space (Rafter 2005). The capacity of materials such as calcium hydroxide [$Ca(OH)_2$] to induce the formation of this calcific barrier at the apex made apexification possible and allowed the preservation of many compromised, immature teeth with nonvital pulps by endodontic and restorative means. Clinically, when the pulpal diagnosis of an immature tooth is nonvital, apexification is undertaken to close the root-end, but with an understanding that there will be no more development of the root in terms of apical maturation and thickening of its dentine walls.

The clinical decision as to whether to perform apexogenesis or apexification for immature teeth appears to be clear cut with the teeth deemed to contain vital pulp tissue being subject to apexogenesis and teeth deemed to have nonvital pulp tissue receiving apexification. However, certain clinical observations reported recently have broken this clear-cut guideline by showing that apexogenesis may occur in teeth which have nonvital pulps (Iwaya *et al.* 2001, Banchs & Trope 2004, Chueh & Huang 2006). Moreover, it is

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likely that many clinicians had been treating some cases by an apexogenesis approach despite apparent pulp necrosis, but never reporting the outcome. A new protocol has been suggested in which a haemorrhage is induced to fill the canal with blood clot as a scaffold to allow generation of live tissues in the canal space and continued root formation (length and wall thickness) (Banchs & Trope 2004, Thibodeau & Trope 2007, Thibodeau *et al.* 2007). Instead of using $Ca(OH)_2$ as the intracanal medicament between visits to disinfect and to induce apical barrier formation, an antibiotic paste is used for the purpose of disinfection only (Iwaya *et al.* 2001, Banchs & Trope 2004). This new protocol of treatment coincides with the recent concept of regenerative medicine which promotes the research and practice of tissue regeneration (National Institutes of Health 2006).

On another front, pulp/dentine tissue may be regenerated using tissue engineering technologies. Attempts to regenerate pulp tissue have been considered impossible until recently and major developments in two basic research, namely tissue engineering and stem cell biology. Investigations on dental pulp tissue engineering began in the late 1990s (Mooney *et al.* 1996, Bohl *et al.* 1998, Buurma *et al.* 1999). The isolation and characterization of dental pulp stem cells (DPSCs) (Gronthos *et al.* 2000), stem cells from exfoliated deciduous teeth (SHED; Miura *et al.* 2003) and stem cells from apical papilla (SCAP) (Sonoyama *et al.* 2006) has capitalized the possibility for pulp/dentine regeneration (Huang *et al.* 2006, 2008, Murray *et al.* 2007a, Cordeiro *et al.* 2008, Prescott *et al.* 2008). Because of the wide-open apex of the immature tooth, vascularization via apical ingrowth of blood vessels into an engineered construct containing stem cells may facilitate a successful regeneration of pulp/dentine within the canal space (Huang *et al.* 2008).

This study will overview the shifting concept of treating immature teeth using revitalization rather than apexification and the current status of pulp tissue engineering and regeneration. The review will analyse the fate of apexification as a first-line treatment for immature teeth with nonvital pulps and how this is affected by the shifting paradigm of the management and the coming era of pulp/dentine tissue regeneration. Again, apexification does not allow generation or regeneration of vital tissues in the canal space whereas the revitalization or tissue regeneration approaches provide a new chance for those affected teeth to regain biological tissue recovery and growth. From this point of view, it seems inevitable that in the interest of patients, apexification may become a less-desirable and less needed clinical treatment in the foreseeable future.

Apexification

Immature teeth undergoing apexification are usually disinfected with irrigants including NaOCl, chlorhexidine, EDTA and iodine–potassium iodide (Rafter 2005). The canal is then filled with $Ca(OH)_2$ paste for the purpose of further disinfection and induction of an apical calcific barrier. $Ca(OH)_2$ is antimicrobial because of its release of hydroxyl ions which can cause damage to the bacterial cellular components. The best example is the demonstration of its effect on lipopolysaccharide (LPS). $Ca(OH)_2$ chemically alters LPS which affects its various biological properties (Safavi & Nichols 1993, 1994, Barthel *et al.* 1997, Nelson-Filho *et al.* 2002, Jiang *et al.* 2003).

Filling the root canal is undertaken normally when the apical calcific barrier is formed. Without the barrier, there is nothing against which the traditional gutta-percha filling material can be condensed. Besides the fact that $Ca(OH)_2$ functions as a potent disinfectant, early evidence has suggested osteo-inductive properties (Mitchell & Shankwalker 1958), although it has been difficult to demonstrate this effect *in vitro* (Raquel Assed Bezerra da *et al.* 2008). It was considered that the high pH may be a contributing factor for the induction of hard tissue formation (Javelet *et al.* 1985). The time required for apical barrier formation in apexification using $Ca(OH)_2$ may be considerable, often as long as 20 months and other conditions such as age and presence of symptoms or periradicular radiolucencies may affect the time needed to form an apical barrier. Refreshing the $Ca(OH)_2$ paste usually takes place every 3 months (Rafter 2005). A number of shortcomings can be summarized for $Ca(OH)_2$ apexification: (i) long time-span of the entire treatment; (ii) multiple visits with heavy demands on patients and carers and inevitable clinical costs; (iii) increased risk of tooth fracture using $Ca(OH)_2$ as a long-term root canal dressing (Cvek 1992, Andreasen *et al.* 2002). These drawbacks led to the use of mineral trioxide aggregate (MTA) to fill the apical end without the need for calcific barrier formation. In comparison to $Ca(OH)_2$, some data suggest that MTA appears to be more predictable with consistent hard-tissue formation based on *in vivo* studies in dogs (Shabahang *et al.* 1999). Using MTA for apexification may shorten the treatment period with

more favourable results and improved patient compliance (Maroto *et al.* 2003, El-Meligy & Avery 2006, Pace *et al.* 2007). Many authors and clinicians propose a one-visit apexification protocol with MTA, which presents a major advantages over traditional $Ca(OH)_2$ methods (Witherspoon & Ham 2001, Steinig *et al.* 2003). This expedient cleaning and shaping of the root canal system followed by its apical seal with MTA makes the rapid placement of a bonded restoration within the root canal possible, which may prevent potential fractures of immature teeth.

While advances with MTA and bonded restorations go some way towards a better outcome, ultimately no apexification method can produce the outcome that apexogenesis can achieve, i.e. apical maturation with increased thickness of the root. As noted above, clinical experience on the outcome of apexified teeth with thin and weak roots after successful treatment is that they are highly susceptible to fracture (Cvek 1992, Katebzadeh *et al.* 1998). Therefore, alternative approaches that allow the increase of root thickness and/or length should be pursued.

A paradigm shift in the management of immature teeth

Although the standardized clinical approach for apexogenesis or apexification has been widely practiced, some clinicians inevitably modify their treatment procedures based on their clinical judgement. Some reported their cases using alternative approaches, with three appearing to capture great interest from the endodontic community. The first, reported by Iwaya *et al.* (2001) presented an immature mandibular premolar with a sinus tract and periradicular radiolucency. During canal preparation, they did not instrument to full working length because the patient felt discomfort on the insertion of instruments. The canal was mainly irrigated with NaOCl and hydrogen peroxide and further disinfected with antibiotic agents (metronidazole and ciprofloxacin). Thirty-five months after the completion of these procedures, they observed complete maturation of the root apex with thickened root structure. The tooth also responded positively to electronic pulp testing. After observing the success of this alternative approach, the same idea was applied to treatment of a mandibular premolar having a similar condition but with more extensive periradicular bone loss. During careful follow-up to 2 years after the treatment, complete maturation of the root was observed with a positive response to cold testing (Banchs & Trope 2004). Chueh & Huang (2006) later reported four mandibular premolars in a similar clinical condition that were treated between 1988 and 2000, all again demonstrating healing and apical maturation. These reports raised a great response and encouraged further reports (Thibodeau & Trope 2007, Hargreaves *et al.* 2008, Jung *et al.* 2008). A more conservative approach and a shifting paradigm for the treatment of nonvital immature teeth has thus been proposed (Huang 2008). Furthermore, the Regenerative Endodontics Committee of the American Association of Endodontists has initiated a pilot study by encouraging endodontists to submit their cases to a data base (http://www.aae.org/members/revascularizationsurvey.htm). The study is designed to determine the incidence and predictors of healing of apical periodontitis in cases considered to have nonvital pulps when treated by nonconventional, biologically based revitalization methods. Currently, the success rate of this type of approach is only available from an animal study model (Thibodeau *et al.* 2007) and a pilot clinical study in humans (Shah *et al.* 2008). In the animal model, it was found that after disinfection of the root canals, 43.9% of the cases had thickened canal walls, 54.9% had apical closure and 64.6% had no radiographic evidence of periapical radiolucency or showed improvement/healing of previous periapical radiolucencies (Thibodeau *et al.* 2007). The clinical pilot study involving teeth in 14 patients demonstrated 93% resolution of periradicular radiolucencies, thickening of lateral dentinal walls in 57%, and increased root length in 71%. None of the cases presented with pain, reinfection or radiographic enlargement of pre-existing periapical lesions (Shah *et al.* 2008). However, due the preliminary nature of the study, the clinical success rates should be interpreted with caution (Messer 2008).

Regarding the use of $Ca(OH)_2$ versus antimicrobial paste, it was suggested that the former may not be suitable if there is remaining vital pulp tissue in the canal. The direct contact of $Ca(OH)_2$ paste with the tissue will induce the formation of a layer of calcific tissue which may occlude the pulp space, therefore preventing pulp tissue from regeneration (Huang 2008). Another concern is that $Ca(OH)_2$ may damage the Hertwig's epithelial root sheath (HERS) and thereby destroy its ability to induce the nearby undifferentiated cells to become ododontoblasts (Banchs & Trope 2004). The effectiveness of a triple-antibiotic regimen to disinfect root canal space was first tested and verified by Sato *et al.* (1996) and the clinical use of the mixture has shown success in terms of clinical outcome (Sato

et al. 1996, Banchs & Trope 2004, Jung *et al.* 2008). Whether the three antibiotics originally described (i.e. metronidazole, minocycline and ciprofloxacin) must be used for this purpose or if other choices may serve this purpose requires further investigation.

These clinical case reports demonstrate that despite the formation of periapical abscesses with extensive periradicular bone resorption as the result of root canal infection in immature teeth, conservative treatment may allow roots to increase in length and thickness or even reach mature form. One explanation is that the clinical diagnosis of pulp status is inaccurate and that some of those teeth must have contained vital tissues in the apical pulp space despite negative pulp testing and periapical lucencies. It is also acknowledged that there is a lack of scientific studies on the diagnosis of pulpal pathology in permanent teeth with open apices (Camp 2008). It has been considered that, to have continued root development, HERS and the recently identified tissue, apical papilla, must be functional (Huang *et al.* 2008). On the other hand, if the pulp, HERS and apical papilla are completely lost, the root may still gain some level of thickness by the ingrowth of cementum from the periapical areas onto the internal root canal dentine walls. Additionally, this cementum ingrowth is accompanied by periodontal ligament (PDL) and bone tissue (Kling *et al.* 1986, Andreasen *et al.* 1995a,b).

The outcome of guided generation and regeneration approach

The use of the term 'revascularization' was adapted by Iwaya *et al.* (2001) to describe the clinical healing of periapical abscesses and continued root formation in immature teeth with nonvital pulps. Other authors adapted the term without questioning until Huang & Lin (2008) considered that 'revascularization' did not encompass the actual healing and repair process that takes place in these clinical cases (Huang & Lin 2008). The term 'revitalization' used by earlier studies attempting to revive tissues in the pulp space would perhaps describe the phenomenon more accurately (Nevins *et al.* 1976).

Pulp space filled with regenerated pulp

The ideal situation is that there is surviving pulp and apical papilla tissue after root canal disinfection. Continued root formation to its maturity and an increased thickness of root dentine may then be anticipated. The dental papilla at the apex contains stem cells, 'SCAP' that have been recently described to be more robust stem cells than DPSCs (Sonoyama *et al.* 2006). The SCAP may survive the infection and retain the capacity to give rise to new odontoblasts influenced by HERS, allowing new root dentine to form and root maturation to proceed to completion. It was speculated that the surviving DPSCs in the remaining vital pulp may rebuild the lost pulp tissue in the canal and differentiate into replacement odontoblasts to substitute for the damaged primary odontoblasts (Sonoyama *et al.* 2008). Under this circumstance, one may anticipate the newly formed odontoblasts from SCAP to produce root dentine that leads to the apical extension of the root. Additionally, the existing primary odontoblasts that survived in the residual pulp tissue and perhaps some new replacement odontoblasts may continue to lay down dentine on the dentinal walls, causing the root to increase its thickness (Fig. 1). Whilst this explanation is conjecture and requires further basic and clinical investigation, some data on the recovery of pulp tissue after tooth replantation appear to support this speculation (Kling *et al.* 1986, Ritter *et al.* 2004).

Pulp space filled with periodontal tissues

In cases where the entire pulp, apical papilla tissues and the HERS are lost, current understanding is that self-regeneration of pulp and new dentine formation is unlikely to occur. There is abundant evidence in the literature demonstrating that when the pulp tissue of

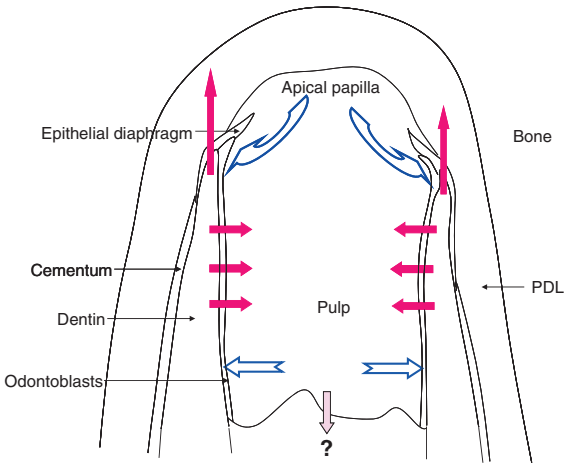

Figure 1 Hypothetical pulp regeneration from the remaining recovered pulp. The question mark indicates that the regeneration of pulp into the empty pulp space is uncertain at present.

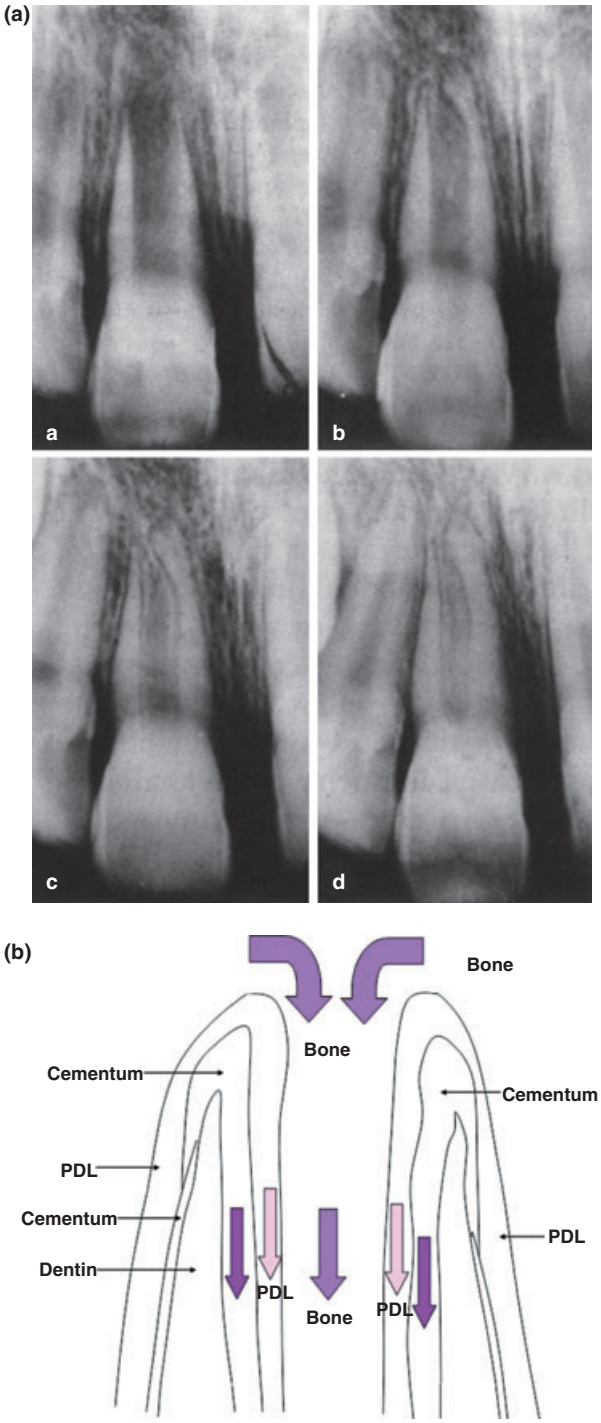

Figure 2 Ingrowth of periodontal tissue into pulp space. (a) Radiographs showing an immature tooth 11 (FDI notation) with an open apex which was re-implanted and healed. At recalls, the ingrowth of bone tissue with the PDL space and lamina dura is evident [adapted from Kling et al. (1986) with permission). (b) Illustration depicting the ingrown tissues of bone, PDL and cementum into the canal space.

immature teeth with wide-open apices undergoes complete necrosis but in a sterile environment, other tissues are capable of filling the canal space. As shown in the radiographic images (Fig. 2), the replanted avulsed immature tooth lost pulp vitality but the pulp space became occupied by the ingrowth of alveolar bone from the periapex (Kling *et al.* 1986). There is a space separating the ingrown bone and the canal dentinal walls. If one traces this space, it is apparent that it is continuous with the PDL space on the external

root surfaces. Lamina dura also appears to have been established in the ingrown bone occupying the pulp space. The contents of the pulp space were described by Holan (1998) as 'tube-like mineralization' and following histological examination it was interpreted that secondary dentine and the pulp tissue existed in the canal space. In fact, this 'secondary dentine' was actually cementum and the 'pulp tissue' was PDL. Careful examination of the characteristics of the ectopic cementum and PDL in the canal space should be the basis of further research.

There also seems to have been some degree of vertical and horizontal extension of the root over time (Fig. 2). Since the pulp tissue has been entirely lost, it has not been possible to deposit new dentine, and the newly acquired calcified tissue has to come from a tissue source where the cellular components are capable of proliferating and producing new tissues. Cementum has the capacity to fulfill this purpose. Histologically, the hard tissues, bone, cementum and dentine can usually be distinguished unambiguously merely for their anatomical location. However, when ectopic formation of these tissues occurs, discerning them without specific markers may be difficult. Nonetheless, the ingrown hard tissues within the pulp space have been verified by histological examination, revealing the deposition of cementum onto the dentine surface in the canal, extending from the outside surface of the apex (Nevins *et al.* 1977, Lieberman & Trowbridge 1983). The apical extension of roots resulting from the apposition of cementum is a normal physiological process. The apposition of calcified cemental tissue on the internal canal wall also increases the thickness of the root. A distinct feature of cementum is its connection with the PDL by Sharpey's fibres, which can also be observed in the ingrown tissues in the pulp space. The ingrowth of periodontal tissue may reach all the way to the coronal pulp chamber (Nevins *et al.* 1977, 1978, Ellis *et al.* 1985, Hitchcock *et al.* 1985). Similar results were observed in a dog as a study model (Thibodeau *et al.* 2007).

When the pulp space is filled with periodontal tissues, the situation is totally different from normal because the pulp space is no longer part of the root canal system, but part of periapical tissues. If the tooth becomes reinfected causing destruction of the periodontal tissue in the canal space, the understanding of a root canal infection to this type of infection cannot be applied, but perhaps more appropriately that of a periapical tissue pathosis. It is known that periapical tissue loss will recover if the source of infection from the root canal space is eliminated though the establishment of a biofilm by the invading microbes may complicate management (de Paz 2007). From this perspective, disinfection does not have to involve with the aggressive entrance into the canal space, but rather dealing mainly with the source of infection in the crown. Currently, there is no case report showing the management and the outcome of infected canal space that has been filled with periodontal tissues.

Severe disorganized calcification of the pulp space

Whether the pulp space is filled with regenerated pulp or periodontal tissues, long-term radiographic observations demonstrate that the pulp space becomes severely narrowed or filled with radio-opaque mineralized tissue over time. Histologically, the mineralizing tissues are either bone-like or dentine-like (Robertson *et al.* 1997). The hard tissues may begin as calcific particles that have been observed to originate or are closely associated with blood vessels and perineurium sheaths (Pashley *et al.* 2002). Interestingly, these are also the locations where pulp stem cells are believed to exist (Shi & Gronthos 2003). Whether these stem cells are activated by the low-grade inflammation to undergo osteogenic differentiation is unclear at present. Over time, these particles merge into larger calcific masses and obliterate the pulp space (Fig. 3). Although this calcifying phenomenon within the pulp has been well-documented, the mechanisms underlying this process are still elusive.

Prolonged inflammation causes calcification in many parts of the body, e.g. calcifying tendonitis (Uhthoff 1996). Arthritic joints tend to build osteophytes as a result of the expanding bone tissue over the damaged cartilaginous tissues (van der Kraan & van den Berg 2007). Another phenomenon named heterotropic ossification is characterized by the formation of mineralized inclusions within the soft tissues (McCarthy & Sundaram 2005), e.g. muscles of patients who suffered from severe trauma to their extremities including soldiers injured by bomb explosions (Owens *et al.* 2006). It has been speculated that the causes of such phenomena include systemic factors and/or local inflammatory conditions. Stem cells in the muscle have been investigated for their potential contributory role in this disease. Deficiencies in osteopontin may lead to vascular calcification (Giachelli 2005).

There has been an ongoing debate on the relative benefits of calcified material or gutta-percha filled canals. From a physiological point of view, calcific

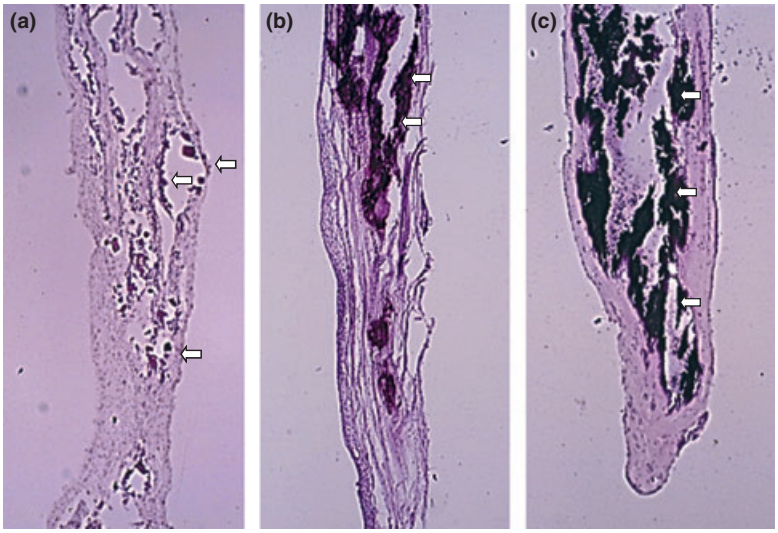

Figure 3 Common feature of pulp undergoing calcific metamorphosis. (a) Pulp tissue from a tooth which had been previously restored with old fillings and a clinical diagnosis of normal pulp (arrows indicate mineral deposits that appear to have been associated with vascular structures) (b, c) Pulp tissues from teeth diagnosed with irreversible pulpitis. Arrows indicate heavy mineral deposits.

metamorphosis is a degenerative disease. Moreover, from a technical perspective, calcified canals pose a challenge if they need treatment. Most of the literature does not support endodontic intervention in the case of mineralized obliteration unless periradicular pathoses is detected or the involved tooth becomes symptomatic (Robertson *et al.* 1997, Gopikrishna *et al.* 2004). Surgical intervention may be the only option to contain the infection from the periradicular tissues if calcified canals are not accessible for nonsurgical root canal treatment.

Progress on pulp/dentine tissue engineering and regeneration

The potential of pulp tissue to regenerate lost dentine is well-known. Direct pulp capping therapy to induce dentinal bridge formation is practiced on the basis of this understanding. The use of various cement-based materials such as $Ca(OH)_2$ and MTA is believed to promote such activity. Long-term success using MTA for direct pulp capping has been reported recently (Bogen *et al.* 2008). The application of recombinant growth factors to the injured site to enhance the regeneration of dentine has also been investigated (Rutherford & Gu 2000). Cell-based therapy using isolated pulp cells or DPSCs, with genetic manipulation to express bone morphogenic proteins, to augment the generation of new dentine bridge formation is an additional area of exploration (Rutherford 2001, Iohara *et al.* 2004).

When dealing with the initial phases of dentine destruction where there is minimal damage, applying a complicated biotechnological approach appears impractical. When the tooth is further damaged, regeneration of dentine becomes difficult as it needs a healthy pulp which may be compromised by the disease. Ideally, the regenerated dentine should not replace the pulp space. Two types of pulp regeneration can be considered based on the clinical situations: (i) partial pulp regeneration and (ii) *de novo* synthesis of pulp.

It has been observed that pulpal infection and inflammation is compartmentalized until the entire pulp tissue undergoes necrosis (Seltzer *et al.* 1963, Trowbridge 2002). Before the end stage, the remaining pulp tissue may be recoverable and help regenerate the lost portion. To enhance the regeneration, engineered pulp tissues may be inserted into the pulp space to facilitate the entire recovery of pulp tissue and the generation of new dentine. When the entire pulp tissue is lost, *de novo* synthesis of pulp must take place to regenerate the tissue.

Early efforts on pulp regeneration

Regenerating pulp tissue has been a long quest. Ostby (1961) studied the tissue re-organization in the canal space filled with blood clot. It was observed that the tissue formed in the canal was not pulp but granulation or fibrous tissues and in some cases the ingrowth of cementum and bone occurred. Similar findings were observed by Myers & Fountain (1974) in a primate study using blood clot as a scaffold. The average generation of soft connective tissue into the canal was only 0.1–1.0 mm, although the authors mentioned

that teeth with open apices had a few more millimetres of ingrowth than those with mature apicies (Myers & Fountain 1974).

It appears that in a natural situation, regeneration of pulp cannot occur following total loss of pulp tissue. Pulp cells have been isolated for various studies for many decades and they have been shown to have the capacities to differentiate into mineral forming odontoblast-like cells *in vitro* (Tsukamoto *et al.* 1992, About *et al.* 2000, Couble *et al.* 2000). However, it was not until it was demonstrated the formation of ectopic dentine/pulp-like complex *in vivo* by isolated pulp cells that the isolation of odontoblast progenitor cells or pulp stem cells was truly confirmed (Gronthos *et al.* 2000). These cells were termed postnatal DPSCs.

Pulp tissue engineering

Before the isolation of DPSCs, pulp regeneration was tested using modern tissue engineering concepts by growing pulp cells onto synthetic polymer scaffolds of polyglycolic acid (PGA) and *in vitro* and *in vivo* analyses performed (Mooney *et al.* 1996, Bohl *et al.* 1998, Buurma *et al.* 1999). These approaches are basically a proof-of-principle to test whether cultured pulp cells can grow well and produce matrix on PGA, and whether the engineered pulp can be vascularized using *in vivo* study models. This approach reflected the emphasis on providing a three-dimensional structure for cells to attach to which simulates the *in vivo* environment. Using a tooth slice model, generation of well-vascularized pulp-like tissue has been reported (Cordeiro *et al.* 2008, Prescott *et al.* 2008).

Issues in cell-based pulp tissue engineering

The following questions must be considered when attempting to engineer and regenerate pulp tissue: (i) vascularization: can the angiogenesis from the limited apical blood supply extend to the coronal end if the entire pulp is to be regenerated? (ii) New odontoblast formation: can the new odontoblasts form against the existing dentinal wall that has been chemically disinfected during the root canal procedures? (iii) New dentine formation: can the newly differentiated odontoblasts produce new dentine and how much would they produce? (iv) Cell source: autologous cells are still the best cell source to avoid potential immune rejection. However, where can one find the cells needed for pulp regeneration in the clinical setting? These points will now be discussed in turn.

Vascularization

While vascularization is a universal issue for an engineered tissue, it is of special concern for pulp tissue engineering because of the lack of a collateral source of blood supply. It was considered that the use of angiogenic inducing factors such as vascular endothelial growth factor (VEGF) could enhance and accelerate pulp angiogenesis. Alternatively, the insertion of engineered pulp tissue may have to be separated into multiple steps to allow progressive vascularization (Huang *et al.* 2008). The choice of scaffold and the source of angiogenic factors have become integrated issues. Artificial synthetic scaffolds such as co-polymer of D,L-lactide and glycolide can be fabricated with impregnated growth factors such as VEGF and/or platelet-derived growth factor (Sheridan *et al.* 2000, Richardson *et al.* 2001, Peters *et al.* 2002, Kanematsu *et al.* 2004, Stiver *et al.* 2004, Sun *et al.* 2005). The size of apical opening would affect the ingrowth of blood vessels into the engineered pulp tissue. It is assumed that the larger the opening, the more likely that angiogenesis can occur. Immature teeth with open apices are therefore the best candidates for pulp tissue regeneration.

It is a misconception to adapt the concept of engineering/regenerating bone for pulp tissue. Certain scaffolds that have osteo-inductive or conductive properties and are suitable for bone regeneration, such as hydroxyapatite and tricalcium phosphate have been proposed as scaffolds for pulp regeneration. The misconception is based on the fact that dentine production has many aspects similar to bone formation. However, it is important to recognize the key differences. An obvious one is the anatomic characteristics. Bone mass contains compact or trabecular bone and marrow, whereas dentine and pulp in a tooth have a rigid anatomic location. When regenerating pulp and dentine, the dentine should be located peripherally to the pulp, not within it. Therefore, the scaffold that carries the cells to regenerate pulp and dentine should not induce dentine formation randomly within the regenerated pulp.

New odontoblast formation

To address the question whether new odontoblasts can form on the existing dentine walls, *in vitro* experiments have shown that by seeding DPSCs onto the existing dentine, some cells transformed into odontoblast-like cells with a cellular process extending into dentinal tubules (Huang *et al.* 2006). A tooth slice model has been utililzed and seeded SHED onto synthetic scaffolds

of poly-L-lactic acid cast in the pulp chamber of the thin tooth slice. They observed odontoblast-like cells arising from the stem cells and localized against the existing dentine surface in their *in vivo* study model (Nör 2006, Cordeiro *et al.* 2008). From these observations, it appears that stem cells seeded in the scaffold will be attracted to the dentinal wall, differentiate into odontoblast-like cells and extend their cellular processes into the dentinal tubules. The mechanism behind this phenomenon has been speculated to be the released growth factors such as TGF-β by the dentine, which attracts and induces the differentiation of odontoblasts (Huang *et al.* 2006). Chemical disinfection of the root canal space may damage these embedded growth factors. Further investigation is needed to seek for ways to avoid this potential damage, and positively promote odontoblast-like colonization.

New dentine formation

The next question is whether these newly formed odontoblast-like cells will make new dentine. In an *in vivo* study model, DPSCs were seeded onto dentine and the construct implanted into the subcutaneous space of immunocompromised mice. Deposition of reparative dentine-like structures by odontoblast-like cells was observed (Batouli *et al.* 2003). This finding suggests the possibility of forming additional new dentine on existing dentine if new odontoblasts can emerge. Huang G.T.-J., Shea L.D., Shi S. & Tuan R.S. (upubl. data) also demonstrated that new dentine-like or osteodentine structure can deposit onto the existing dentine throughout the entire canal wall in an *in vivo* pulp engineering/regeneration study model.

Cell source

With respect to the cell source, there are several potential sources to obtain autologous cells for pulp/dentine tissue regeneration: DPSC, SCAP and SHED. Immature third molars are one of the best sources for DPSCs and SCAP. The latter have been shown to be more potent dental stem cells than DPSCs in terms of their level of immaturity and potentiality. They give rise to odontoblast-like cells and make ectopic dentine in *in vivo* study models (Sonoyama *et al.* 2006) . SHED also produce ectopic dentine *in vivo* (Miura *et al.* 2003). The problem is the availability of this source. Banking personal teeth for future use appears to be a direction that must be explored and established to ensure this availability. Allogenic cells are an alternative and convenient source. The finding of the immunosuppressive capacity of mesenchymal stem cells to avoid immuno-rejection provides a great possibility that allogenic stem cells may be a good source (Pierdomenico *et al.* 2005, Chen *et al.* 2006). However, *in vivo* studies to verify the long-term survival of transplanted allogenic dental stem cells are lacking.

Prospects

The above analysis points out the potential future fate of apexification procedures. Such procedures may no longer be the preferred first option to treat immature permanent teeth with nonvital pulps. Induced generation and regeneration of vital tissues in the pulp space can thicken the root structure leading to a stronger tooth with a potentially reduced fracture risk. The progress of pulp/dentine regeneration so far has been promising and is likely to work in the not so distant future.

There is some concern caused by the uncertainty as to how pulp regeneration would affect the future of endodontic practice (Murray *et al.* 2007b) . One may anticipate that to feasibly deliver stem cell-based endodontic therapy for pulp/dentine regeneration in endodontic practice, an uncomplicated clinical protocol would need to be established. If not, technology transfer to the commercial sector would be difficult (Rutherford 2007).

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