International Endodontic Journal

doi:10.1111/j.1365-2591.2011.01915.x

Review

Repair and regeneration in endodontics

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Abstract

Lin LM, Rosenberg PA. Repair and regeneration in endodontics. *International Endodontic Journal*, **44**, 889–906, 2011.

The ideal objective of treatment of established diseases, including irreversible pulpitis and apical periodontitis, is to achieve wound healing. Wound healing can result in repair or regeneration. The ultimate goal of wound healing is to restore the original architecture and biological function of the injured tissue or organ. Although humans are equipped with powerful innate and adaptive immune defence mechanisms, many intrinsic and extrinsic factors can affect wound healing. Complete regeneration following injury in humans can occur only in the pre-natal foetus within 24 weeks of gestation. Post-natal wounds including irreversible pulpitis or apical periodontitis always heal by repair or by a combination of repair and regeneration. Somatic cells, such as fibroblasts, macrophages, cementoblasts and osteoblasts, in the pulp and periapical tissues have limited potential for regeneration following injury and lack of telomerase. Wound healing of irreversible pulpitis and apical periodontitis requires recruitment and differentiation of progenitor/stem cells into tissue-committed somatic cells. Stem cell differentiation is regulated by intrinsic factors and extrinsic micro-environmental cues. Functionality of stem cells appears to show an age-related decline because of the change in intrinsic properties and diminished signals within the extrinsic local and systemic environment that modulate the function of stem cells or their progeny. Infection induces an immuno-inflammatory response and tissue destruction, which hinders the potential of tissue regeneration. Therefore, prevention, early detection and treatment of inflammation/infection of pulpal and periapical disease can enhance regeneration and minimize the repair of pulpal and periapical tissues after endodontic therapy.

Keywords: nonvital pulp therapy, post-natal wound healing, pre-natal wound healing, progenitor/stem cells, vital pulp therapy.

Received 22 March 2011; accepted 6 June 2011

Introduction

Although pulpal and periapical disease can be caused by traumatic injury, physical insult, chemical irritation or periodontal disease, microbial infection is the primary aetiology (Kakehashi *et al.* 1965, Nair 1997, 2004). The ideal objective of the treatment of established diseases, including irreversible pulpitis and apical periodontitis, is to achieve wound healing (Slauson & Cooper 2002, Majno & Joris 2004, Kumar *et al.* 2009). The ultimate goal of wound healing is to reconstitute the original architecture and biological function of the injured tissue (Martin 1997). Wound healing can be achieved by the host's innate and adaptive immune defence mechanisms (Park & Barbul 2004, Abbas *et al.* 2007) as in an uninfected simple surgical incision through the skin or by combination of the host's defence mechanisms and therapeutic modalities, such as pulp capping in vital pulp therapy of immature permanent teeth and root canal treatment in teeth with apical periodontitis (Ørstavik & Pitt Ford 2007, Ingle *et al.* 2008, Hargreaves & Cohen 2010).

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The purpose of this article was to review repair and regeneration in endodontics based on cellular and molecular biology. It will include vital pulp therapy (indirect and direct pulp capping, pulpotomy and apexogenesis) and nonvital pulp therapy (pulpectomy/pulp debridement, apexification and revascularization) to provide a better understanding of wound healing of the dentine–pulp complex and pulpal–periapical tissue complex after different modalities of endodontic therapy. Increased knowledge in this area should lead to the development of biologically based treatment strategies, which can enhance regeneration and minimize the repair of the damaged dentine–pulp complex and pulpal–periapical tissue complex during wound healing. Most histological studies of wound healing, following vital and nonvital pulp therapy as well as the treatment of apical periodontitis, have been conducted in animal experiments (Cox & Bergenholtz 1986, Harrison & Jurosky 1992, Oguntebi *et al.* 1995, Leonardo *et al.* 1997, Dominguez *et al.* 2003, Sabeti *et al.* 2006), because human studies are not readily available. The results of animal studies are then extrapolated to human clinical outcome assessments. Although animals are different from humans in many biological aspects, animal studies are a necessary prerequisite before clinical trials, according to the International Organization of Standardization concerning the biological evaluation of medical devices (ISO).

Regardless of the biomaterials and techniques used in endodontic therapy, control of infection plays the most important role in the regeneration and repair of the pulp and periapical wound healing. As elegantly stated by Smith *et al.* (2002), 'An appreciation of the underlying biological processes taking place in the dentine-pulp complex during injury and repair, and how treatment events can modify the dentine-pulp complex, offer considerable potential for exploiting the regenerative potential of these tissues'.

Indications, treatment procedures and outcomes of vital and nonvital pulp therapy will not be included in the scope of this review. They are described, in detail, in several excellent endodontic textbooks (Ørstavik & Pitt Ford 2007, Ingle *et al.* 2008, Hargreaves & Cohen 2010).

Repair and regeneration

Wound healing is the programmed tissue response to injury of a living organism that involves complex cellular and molecular biological processes (Clark 1996, Majno & Joris 2004, Kumar *et al.* 2009). The wound healing process is considered to recapitulate embryonic tissue development (Gerstenfeld *et al.* 2003, Martin & Parkhurst 2004) and can result in repair or regeneration. Repair is the replacement of the damaged tissue by different tissue, such as fibrosis or scarring, and usually causes the loss of biological function of the injured tissue. In contrast, regeneration is the replacement of the damaged tissue by the same cells with the restoration of the biological function of the injured tissue (Slauson & Cooper 2002, Majno & Joris 2004, Kumar *et al.* 2009). Therefore, wound healing does not necessarily imply tissue regeneration. Repair and regeneration is regulated by cell–cell and cell–extracellular matrix cross-talk and by the expression of growth factors/cytokines and other bioactive molecules at different temporal and spatial stages during wound healing (Clark 1996, Werner & Gross 2003, Werner *et al.* 2007, Gurtner *et al.* 2008, Barrientos *et al.* 2008). Growth factors/cytokines are multifunctional and serve as signalling molecules between cells (Clark 1996, Werner & Gross 2003). They are capable of stimulating cell growth, proliferation, differentiation, activation and metabolic activity and also act as chemotactic factors (Werner & Gross 2003, Gurtner *et al.* 2008, Barrientos *et al.* 2008).

Tissue wound healing can be affected by intrinsic and extrinsic factors such as (i) the level of biological evolution (Tanaka 2003), (ii) the immune response (innate and adaptive) (Regan & Barbul 1991, Chang *et al.* 2000), (iii) regenerative potential of resident cells, for example, in the heart and skin (Zhang & Fu 2008, Mercola *et al.* 2011), (iv) function of specific genes at particular stages of wound healing and in particular cell types (Stelnicki *et al.* 1997, 1998), (v) ability of the injured tissue to promote progenitor/stem cell differentiation, for instance, in the central nerve system and liver (Alvarez-Buylla & Lois 1995), (vi) expression of growth factors (Whitby & Ferguson 1991, Sullivan *et al.* 1993), (vii) extracellular matrix and associated noncollagenous protein molecules (Bullard *et al.* 2003, Colwell *et al.* 2003), (viii) infection or foreign bodies (Nair 2004), (ix) the rate of turnover of the tissues (Rando 2006) and (x) angiogenesis (Tonnesen *et al.* 2000).

Depending on the regenerative potential of tissues and organs, wound healing of injured tissues in adults may restore its integrity but the cost is replacement of normal tissue with fibrosis or scar tissue and potential loss of biological function (Bullard *et al.* 2003, Kumar *et al.* 2009). The foetus during the early stages of gestation (within 24 weeks) has the ability to heal

injured tissues by complete regeneration without a scar (Adzick & Longaker 1992, Bullard *et al.* 2003, Colwell *et al.* 2003, Dang *et al.* 2003). This is attributable to several factors, such as immuno-inflammatory response, the expression of transforming growth factor-beta and fibroblast behaviour, regulating wound healing (Bullard *et al.* 2003, Colwell *et al.* 2003). Any kind of injury including trauma that induces an inflammatory reaction is always accompanied by at least some scar tissue formation in post-natal wound healing (Adzick & Longaker 1992, Bullard *et al.* 2003, Colwell *et al.* 2003). A post-natal wound cannot heal by complete regeneration without a scar (Longaker *et al.* 1994, Bullard *et al.* 2003, Colwell *et al.* 2003). Therefore, wound healing of irreversible pulpitis, pulpal necrosis and apical periodontitis following endodontic treatment does not necessarily imply complete regeneration of the pulp or periapical tissues because they are post-natal wounds.

Some low vertebrates, such as the salamander and zebrafish, have a remarkable capacity to regenerate entire limbs, the lens of eyes and portions of the heart (Poss *et al.* 2002, Tanaka 2003, Morrison *et al.* 2006, Tanaka & Weidinger 2008). Despite that capacity, the salamander and zebrafish, like humans, have a limited lifespan. Tissue regeneration in humans is limited, and that constitutes a major challenge to the repair of a damaged organ and tissue function (Tanaka 2003, Pajcini *et al.* 2010). Humans cannot regenerate large portions of lost tissue after injury. An exception is liver regeneration after injury (Michalopoulos & DeFrances 1997). An important question is: What is the specific mechanism that salamanders have and humans do not have, which regulates the regenerative capacity of injured tissues during wound healing (Tanaka 2003)?

Regenerative potential of the pulp and periapical tissues

The dental pulp consists of cells such as odontoblasts, fibroblasts, macrophages, endothelial cells, dendritic cells, lymphocytes, Schwann cells and progenitor/stem cells (Nanci 2007). Fibroblasts, macrophages, lymphocytes and Schwann cells have a limited lifespan and a limited capability for cell division called the Hayflick limit (Hayflick & Moorhead 1961). The Hayflick limit is the number of times that a normal cell population can divide before it stops dividing (Hayflick & Moorhead 1961). It is related to the length of telomeres on the DNA of the cell. Telomeres are DNA sequence repeats, TTAGGG, located at the ends of the linear chromosome (Blackburn 1991, Greider 1996). Telomeres are essential for chromosome stability and allow the complete replication of the ends of the chromosomes (Blackburn 1991, Greider 1996). Each time a cell divides; the length of telomeres is shortened owing to the loss of pieces of telomeres (Blackburn 1991, Greider 1996). When the length of telomeres becomes shortened to a critical point, the cell is prevented from dividing. This is called replicative senescence (Blackburn 1991, Greider 1996). Odontoblasts are post-mitotic cells incapable of cell division (Nanci 2007, Tziafas & Kodonas 2010). Differentiation of primary odontoblasts during embryonic tooth development requires cross-talk between the epithelial cells of the inner enamel epithelium and neural crest-derived ectomesenchymal cells in the dental papilla (D'Souza 2002, Nanci 2007, Tziafas & Kodonas 2010). During crown dentinogenesis, the ectomesenchymal cells in the dental papilla, which are aligned adjacent to the inner enamel epithelium, receive inductive signalling molecules sequestered in the basement membrane from the epithelial cells and differentiate into primary odontoblasts (Ruch 1998, Smith & Lesot 2001, Goldberg & Smith 2004, Tziafas & Kodonas 2010). They subsequently produce crown dentine (Smith & Lesot 2001). In teeth with complete crown formation, the inner enamel epithelium disintegrates. Similar to primary odontoblast differentiation in crown dentinogenesis, the primary odontoblast differentiation in root dentinogenesis also requires cross-talk between the inner epithelial cells of Hertwig's epithelial root sheath (HERS) and ectomesenchymal cells of the apical papilla (Sonoyama *et al.* 2008). When primary odontoblasts are destroyed by caries, trauma, mechanical insult or chemical cytotoxicity, progenitor/stem cells in the dental pulp are capable of differentiating into odontoblast-like cells upon stimulation by appropriate inductive signalling molecules (Gronthos *et al.* 2000, 2002).

The periapical tissues consist of cementum, periodontal ligament and alveolar bone. Fibroblasts, epithelial cells, cementoblasts, osteoblasts, macrophages, endothelial cells, Schwann cells and undifferentiated mesenchymal cells (progenitor/stem cells) are resident cells of the periodontal ligament (Nanci 2007). Except for progenitor/stem cells, other resident cells in the periodontal ligament have a limited lifespan and a limited capability for cell division. Differentiation of primary cementoblasts requires cross-talk between HERS cells and neural crest-derived ectomesenchymal cells in the dental follicle. The ectomesenchymal cells receive inductive signalling molecules from the

epithelial cells of HERS and differentiate into cementoblasts (D'Souza 2002, Nanci 2007, Tziafas & Kodonas 2010). Therefore, HERS cells play crucial roles in root development and root dentine and cementum formation (Zeichner-David *et al.* 2003, Sonoyama *et al.* 2007). In mature teeth, the HERS cells break down into nests of epithelial cell rests of Malassez in the periodontal ligament (Nanci 2007, Sonoyama *et al.* 2007). When primary cementoblasts are destroyed by trauma or periodontal disease, progenitor/stem cells in the periodontal ligament are capable of differentiating into cementoblast-like cells, adipocytes and collagen-forming cells upon stimulation by appropriate inductive signalling molecules (Seo *et al.* 2004). Bone marrow-derived mesenchymal stem cells in the alveolar bone are also capable of differentiating into osteoblasts, chondrocytes and adipocytes upon stimulation by appropriate inductive signalling molecules (Prockop 1997, Pittenger *et al.* 1999).

Stem cells and wound healing

Wound healing is closely related to recruitment and differentiation of progenitor/stem cells into somatic cells of the injured tissue (Clark 1996, Wu *et al.* 2007a,b, Stappenbeck & Miyoshi 2009). Growth factors/cytokines, bioactive molecules, extracellular matrix and cell adhesion molecules play an important role in recruitment, proliferation and differentiation of progenitor/stem cells at the site of tissue injury, micro-environmental cues (Watt & Hogan 2000, Scadden 2006, Kolf *et al.* 2009). Nevertheless, the signals that control progenitor/stem cells from proliferation and differentiation during wound healing are not clear. It is likely that micro-environmental cues at the injury site may also signal progenitor/stem cells to stop proliferation and differentiation because of down-regulation of factors, which promote stem cell proliferation and differentiation (Ogawa 1993).

Stem cells are capable of self-renewal and differentiation into multiple cell lineages (Morrison *et al.* 1997). The replicative lifespan of stem cells is limited by the length of telomeric DNA and may be maintained by the expression of a low level of telomerase activity (Hiyama & Hiyama 2007). Telomerase is a ribonucleoprotein reverse transcriptase (Blackburn 1991, Greider 1996). It can replace lost ends of telomeric DNA when a cell divides and maintains the replicative lifespan of a cell (Greider 1996). Stem cells express telomerase activity but somatic cells do not (Hiyama & Hiyama 2007). Not all stem cells self-renew into adulthood, and not all adult stem cells reflect self-renewal of foetal cells (Morrison *et al.* 1997).

Stem cells normally remain quiescent in a steady state until stimulated by the signals triggered by tissue damage or remodelling (Morrison *et al.* 1997, Abbott *et al.* 2004). The interaction between stem cells and their niche is critical for maintaining the stem cell properties, which include self-renewal capacity and the ability of differentiation into multiple cell lineages (Moore & Lemischka 2006, Arai & Suda 2007). The niche consists of stromal cells, signalling molecules, extracellular matrix and cell adhesion molecules (Moore & Lemischka 2006, Arai & Suda 2007). Stem cells are positioned at a perivascular niche (Shi & Gronthos 2003) and ready to travel through the circulation to the target site of tissue injury (Abbott *et al.* 2004). Stem cell differentiation is regulated by intrinsic properties and extrinsic micro-environmental cues, such as stromal cells, extracellular matrix (e.g. collagen, fibrin, fibronectin, laminin, osteopontin and proteoglycans), growth factors/cytokines and bioactive molecules as well as cell surface adhesion molecules (e.g. integrins and cadherins) (Morrison *et al.* 1997, Watt & Hogan 2000, Scadden 2006, Kolf *et al.* 2009). The entire process of the molecular mechanism that governs stem cell differentiation is not fully understood. Alteration in intrinsic epigenetic mechanisms, such as changes in DNA cytosine methylation, histone modifications or small noncoding RNA-controlled pre- and post-transcriptional regulation of gene expression, appears to regulate stem cell differentiation and fate (Wu & Sun 2006, Lunyak & Rosenfeld 2008). During cell differentiation, some genes are turned on, whilst other genes are turned off. Once the stem cells leave their niche, they are committed to differentiation (Scadden 2006). Homing of stem cells to the target site of tissue injury is directed by soluble factors released by damaged tissue and cells, such as stromal-cell-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes (Askari *et al.* 2003, Son *et al.* 2006).

Tissue-resident stem cells clearly play an important role in tissue regeneration. However, their relatively low frequency and limited regenerative potential in a given tissue may not be sufficient enough to account for the massive regeneration in severe tissue injury (Pajcini *et al.* 2010) as in a large inflammatory periapical lesion. Recently, it was proposed that the transient inactivation of Arf and Rb genes (tumour suppressor genes) in mammalian post-mitotic somatic cells could cause dedifferentiation of somatic cells to become regenerative cells (Pajcini *et al.* 2010). Thus,

transient induction of dedifferentiation of post-mitotic cells could serve as an adjunct to classical tissue-specific stem cells (Pajcini *et al.* 2010).

Stem cell functionality, such as differentiation potential, shows an age-related decline owing to change in the intrinsic regulatory factors (e.g. DNA damage and epigenetic dysregulation) of stem cells and diminished extrinsic signals (e.g. growth factors/cytokines and bioactive molecules) within the local and systemic environmental cues (Ho *et al.* 2005, Rossi *et al.* 2005, 2007, Carlson & Conboy 2007, Silva & Conboy 2008). These factors modulate the function of ageing stem cells or their progeny. The intrinsic factors and micro-environmental cues of ageing stem cells may affect their repair and regenerative potential. For example, older hematopoietic stem cells (HSCs) were less able to regenerate the blood system than young hematopoietic stem cells (Chambers *et al.* 2007). HSCs from young animals, when transplanted into either young or old hosts, produced successful regenerative responses, whilst aged HSCs failed to regenerate properly regardless of the age of the host (Conboy *et al.* 2005). Therefore, with advancing age, adult stem cells lose the ability to differentiate into functional tissue-specific cells (Conboy *et al.* 2005). This may be similar to the scenario that occurs during the successful outcome of vital pulp therapy, which depends not only on the blood supply but also on the age of the patients and stage of root development (Mjor 2002, Høsted-Bindslev & Løvschall 2003).

Progenitor/stem cells in the dental pulp, periodontal ligament and alveolar bone marrow play an important role in wound healing by replenishing damaged post-mitotic cells and cells with limited division potential in the pulp and periapical tissues.

Indirect pulp capping

Indirect pulp capping is a procedure in which a material is placed on a thin partition of remaining carious dentine that, if removed, might expose the pulp in immature permanent teeth (AAE 2003). Once dentine is damaged by caries or trauma, the integrity of the dentin–pulp complex is compromised because damaged dentine cannot be regenerated by primary odontoblasts. Indirect pulp capping is one of the most controversial issues in vital pulp therapy. A critical traditional review of treatment of deep carious lesions by complete excavation or partial removal indicates that removing all vestiges of infected dentine from carious lesions approaching the pulp is not required for successful caries management (Thompson *et al.* 2008). However, systematic reviews of caries and pulp treatment from the Cochrane Database indicate a lack of randomized clinical trials to support incomplete removal of caries (Ricketts *et al.* 2006, Miyashita *et al.* 2007).

It has been demonstrated that when the thickness of remaining dentine including reactionary dentine between the front of bacterial penetration and the pulp was 1.1 mm or more, the pulpal inflammatory response to bacterial infection of the dentinal tubules was negligible. However, when the bacterial penetration in the dentinal tubules reached to within 0.5 mm from the pulp, there was a significant increase in the extent of pulpal inflammation. The pulp became severely inflamed when bacteria had invaded the reactionary dentine (Reeves & Stanley 1966). Indirect pulp capping is intended to protect primary odontoblasts and the pulp from further injury. It promotes reactionary dentine formation at the pulp–dentine junction (Smith *et al.* 1995, Smith 2002, Tziafas 2004). Depending on the severity of carious involvement of the dentine–pulp complex, some primary odontoblasts may be destroyed even without pulp exposure and reparative dentine is formed in conjunction with reactionary dentine (Smith 2002, Tziafas 2004). Reactionary dentine has more matrix and fewer regular tubules than primary and secondary dentine (Smith *et al.* 1995, Tziafas 2004). Reactionary dentine is formed limited to only those areas where the dentinal tubules are in communication with the cavity floor (Smith *et al.* 1995, Tziafas 2004).

Reactionary dentinogenesis is usually caused by milder injury of the dentine–pulp complex. An example would be an attack by slowly progressing caries, cavity preparation and the biomaterials used as indirect pulp capping agents (Smith *et al.* 1995, 2001). Reactionary dentinogenesis is secreted by functional upregulation of surviving primary odontoblasts, stimulated by signalling molecules such as transforming growth factor-beta (TGF-β) family proteins or bioactive molecules released from the dentine matrix during injury to the dentine–pulp complex (Smith *et al.* 1995, 2001, Tziafas 2004). In addition, bone morphogenetic proteins (BMPs) (Bessho *et al.* 1991, Casagrande *et al.* 2010), angiogenetic growth factors (Roberts-Clark & Smith 2000) and insulin-like growth factors (IGFs) (Finkelman *et al.* 1990) released from the dentine matrix may also participate in reactionary dentinogenesis. Functional upregulation of primary odontoblasts during reactionary dentinogenesis is a result of an increased activity in

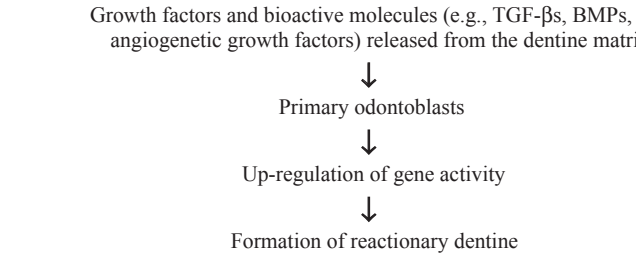


Figure 1 Indirect pulp capping.

dentine collagen protein synthesis and therefore a result of increased activity in gene transcription (Smith *et al.* 1995) (Fig. 1). Growth factors can act as extracellular signalling molecules and bind to odontoblast surface receptors. Binding of ligand to surface receptor initiates the transmission of a signal across the plasma membrane and induces conformational change in the intracellular domain of the receptor. The activated receptor can acquire enzymatic activity and trigger one or more intracellular signal-transduction pathways, which cause the phosphorylation of transcription factors either in the cytosol or in the nucleus (Lodish *et al.* 2008). Following these complex biological processes, binding of ligand to its cognate receptor leads to the regulation of gene activity or gene expression in the nucleus by active transcription factors during gene transcription (Lodish *et al.* 2008).

It is believed that many growth factors for the induction and differentiation of odontoblasts are embedded in the dentine matrix during primary dentinogenesis and may be released following demineralization of the dentine matrix caused by injury to the dentine–pulp complex (Smith & Lesot 2001, Goldberg & Smith 2004). Caries, cavity preparation and biomaterials used as etching and capping agents can cause the release of growth factors and bioactive molecules from the dentine matrix (Smith *et al.* 1995, 2001, Tziafas 2004). Calcium hydroxide and glass–ionomer cement have been successfully used as indirect pulp capping agents (Marchi *et al.* 2006). They can cause the release of growth factors from the dentine matrix (Smith *et al.* 1995, Tziafas 2004). The extent of reactionary dentine formation appears to be related to the residual dentine thickness (Smith 2002).

Although reactionary dentine is formed by primary odontoblasts and similar to primary and secondary dentine, it is formed at the pulp–dentine border corresponding to the dentinal tubules involved by caries and not where the original dentin was destroyed. Strictly speaking, reactionary dentine formation following indirect pulp capping is neither a regenerative nor a reparative process. Functional upregulation of biosynthesis and secretion of dentine matrix by surviving primary odontoblasts after insult is best described as reactionary dentinogenesis (Smith *et al.* 1995, 2001, Tziafas 2004).

Direct pulp capping

Direct pulp capping is a dental material placed directly on a mechanical or traumatic vital pulp exposure (AAE 2003). Treatment of an exposed vital pulp is accomplished by sealing the pulpal wound with a material such as calcium hydroxide or mineral trioxide aggregate (MTA) to facilitate the formation of reparative dentine and the maintenance of a vital pulp (Cvek 1978, Pitt Ford *et al.* 1996, Faraco & Holland 2001, AAE 2003, Witherspoon *et al.* 2006, Bogen *et al.* 2008). There are insufficient high-quality, randomized controlled clinical trials to assess the outcome of using calcium hydroxide or MTA as direct pulp capping agents (Roberts *et al.* 2008), although recent observational studies claim a high success rates of direct pulp capping using MTA (Farsi *et al.* 2006, Bogen *et al.* 2008).

At the site of pulp exposure, primary odontoblasts together with other pulpal cells are destroyed and inflammation is initiated. If infection/inflammation in the coronal pulp is under control, resident cells other than odontoblasts will regenerate. Histologically, reparative dentine has more matrix and fewer tubules than primary and secondary dentine. It often contains cell

inclusions and the tubules are irregular (Smith *et al.* 2001, Tziafas 2004, Nanci 2007). Reparative dentinogenesis requires recruitment and differentiation of progenitor/stem cells in the underlying uninfected vital pulp into odontoblast-like cells, which produce reparative dentine or dentine-like mineralized scar tissue or osteodentine (Smith 2002, Tziafas 2004, Lesot *et al.* 1993, Smith *et al.* 1994). Post-natal stem cells have been demonstrated in human dental pulps and are capable of differentiating into odontoblast-like cells upon receiving appropriate inductive signals (Gronthos *et al.* 2000, 2002). Growth factors, such as TGF-1, TGF-3, BMP-2 and IGF-1, and bioactive molecules released from the dentine matrix and extracellular matrix molecules can signal progenitor/stem cells in the dental pulp to differentiate into odontoblast-like cells (Lesot *et al.* 1994, Begue-Kirn *et al.* 1994). It is possible that the same growth factors and bioactive molecules might also stimulate odontoblast-like cells to produce reparative dentine (Fig. 2). Pulp capping materials such as calcium hydroxide and MTA induce reparative dentin formation most likely through an indirect mechanism by causing the release of growth factors from the dentine matrix (Lesot *et al.* 1993, 1994, Smith *et al.* 1994, Begue-Kirn *et al.* 1994). However, little is known of gene regulation and activation of transcription factors that are involved in the recruitment and differentiation of odontoblast-like cells in the pulp to produce dentine matrix (Goldberg & Smith 2004).

Reparative dentine is not formed by primary odontoblasts but by odontoblast-like cells differentiated from pulp progenitor/stem cells. Reparative dentine is not similar to primary dentine. Therefore, reparative dentine formation following direct pulp capping is a reparative process of the dentine–pulp complex.

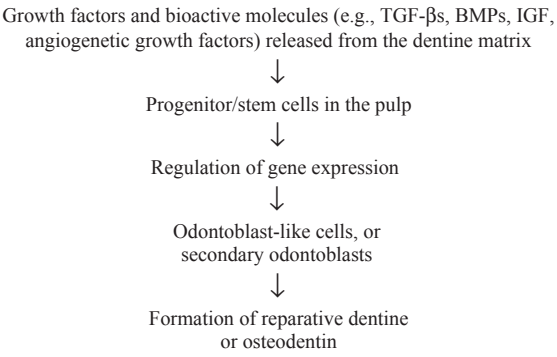


Figure 2 Direct pulp capping and pulpotomy.

Pulpotomy

Pulpotomy is the surgical removal of the coronal portion of a vital pulp as a means of preserving the vitality of the remaining radicular portion (AAE 2003). The remaining uninfected radicular vital pulp is usually sealed with calcium hydroxide to encourage dentine bridge formation (Cvek & Lundberg 1983, Fuks *et al.* 1987, Fong & Davis 2002). Although a review of the literature indicates that MTA is promising as a pulpotomy material (El Meligy & Avery 2006, Barrieshi-Nisair & Qudeimat 2006, Witherspoon 2008), there are insufficient randomized, double-blind, clinical studies that can be used for critical evaluation (Roberts *et al.* 2008).

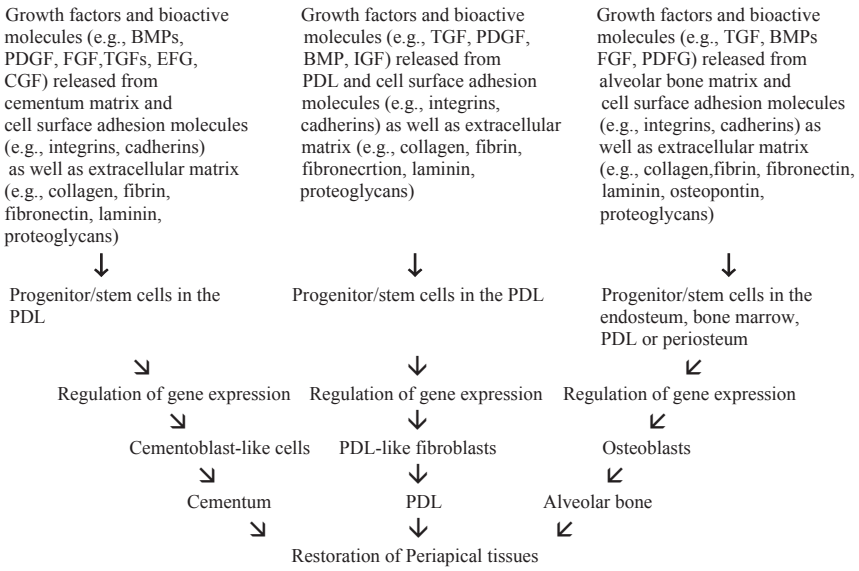
A dentine bridge can also be formed in surgically exposed pulps in germ-free but not in conventional laboratory rats without pulp capping (Kakehashi *et al.* 1965). Therefore, similar to direct pulp capping, calcium hydroxide and MTA induce dentine bridge formation most likely by an indirect mechanism through the release of growth factors from the dentine matrix (Fig. 2). Histologically, the nature of a dentine bridge is similar to that of reparative dentine (Smith 2002, Smith *et al.* 1995). The dentine bridge is formed across the remaining radicular vital pulp tissue. A dentine bridge is not impermeable or completely protective of the underlying vital pulp, and tunnel defects have been demonstrated in 89% of them (Cox *et al.* 1997).

After pulpotomy, the primary odontoblasts and other pulp cells in the coronal pulp are destroyed completely. The tissue injury and inflammation are more severe following a pulpotomy than after a direct pulp capping. Therefore, a pulpotomy requires more recruitment and differentiation of progenitor/stem cells in the remaining uninfected vital pulp into odontoblast-like cells. The cellular and molecular mechanism of dentine bridge formation following pulpotomy is similar to that of reparative dentinogenesis after direct pulp capping (Smith 2002, Smith *et al.* 1995).

Dentine bridge formation following pulpotomy is a reparative process of the dentine–pulp complex.

Pulpectomy/pulp debridement

Pulpectomy is the complete surgical removal of the vital dental pulp (AAE 2003). Pulpectomy, control of root canal infection and bacteria-tight seal of the root canal system are the necessary steps in root canal treatment (Ørstavik & Pitt Ford 2007, Ingle *et al.* 2008,



PDFG, platelet derived growth factor, FGF, fibroblast growth factor, EGF, epidermal growth factor, CGF, cementum-derived growth factor, PDL, periodontal ligament.

Figure 3 Periapical wound healing.

Hargreaves & Cohen 2010). In teeth with irreversible pulpitis/pulp necrosis without apical periodontitis after pulpectomy and chemomechanical debridement, the root canals are filled with root canal sealer and gutta-percha to prevent root canal reinfection. In teeth with apical periodontitis/abscess, root canal treatment can encourage periapical wound healing.

Periapical wound healing is a complex biological process. The restoration of damaged periapical tissues requires the recruitment of progenitor/stem cells from the periodontal ligament, endosteum, bone marrow and possibly periosteum to differentiate into PDL fibroblasts, cementoblast-like cells and osteoblasts (Lin *et al.* 2010). Subsequently, these tissue-committed cells will produce PDL ligament, cementum and alveolar bone (Fig. 3).

After root canal treatment, a highly orchestrated biological process enables wounded periapical tissues to be restored almost back to their original architecture radiographically. Although cell–cell and cell–matrix cross-talk and signalling between complex bioactive molecules play an important role in tissue wound healing (Clark 1996, Werner & Gross 2003, Werner *et al.* 2007), the master biological mechanism that regulates the temporal and spatial relationship between cementum, PDL and alveolar bone during periapical wound healing is not clear. Why are PDL, cementum and alveolar bone restored in such an orderly fashion without irregularity during periapical wound healing? Do newly differentiated cementoblast, PDL-like cells and osteoblasts have a position- or pattern-specific memory in the periapical area?

The wound healing process following periapical surgery is similar to that following nonsurgical root canal treatment (Lin *et al.* 2009). Both surgical and nonsurgical endodontic therapies are primarily regenerative processes with some fibrosis of the periapical tissues (Ricucci *et al.* 2009), even though radiographs may show complete restoration of the periapical structures. Periapical wound healing will never achieve complete regeneration because it is a post-natal wound, which always results in some scar formation (Bullard *et al.* 2003).

Apexogenesis

Apexogenesis is a vital pulp therapy procedure performed to encourage continued physiological development and formation of the root end (AAE 2003). Apexogenesis is similar to pulpotomy and indicated in immature permanent teeth with vital pulp (Ørstavik & Pitt Ford 2007, Ingle *et al.* 2008, Hargreaves & Cohen 2010). The biomaterial usually used for an apexogenesis procedure is calcium hydroxide (Saad 1988). A

dentine bridge is commonly formed in the remaining uninfected radicular vital pulp. The cellular and molecular mechanism of dentine bridge formation in apexogenesis is similar to that in pulpotomy.

Dentine bridge formation following apexogenesis is a reparative process of the dentine–pulp complex. However, the continued root development is a normal physiological process.

Apexification

Apexification is a method to induce a calcified barrier in a root with an open apex or the continued apical development of an incompletely formed root in teeth with necrotic pulps (AAE 2003). Calcium hydroxide has been the most commonly used biomaterial, although recently MTA is gaining more acceptances for apexification procedures (Rafter 2005). In apexification procedures, the infected necrotic pulp is removed up to the apex by means of mechanical debridement and antiseptic chemical irrigation (Rafter 2005). Accordingly, it is unlikely that reparative dentine can be formed because of absence of the pulp tissue. The calcified barrier formed at the blunt open apex has been described as cementum-like tissue or osteodentine (Steiner & Van Hassel 1971, Torneck *et al.* 1973, Baldassari-Cruz *et al.* 1998, Andreasen & Andreasen 1994). MTA has been shown to promote cementogenesis (Torabinejad *et al.* 1997, Shabahang *et al.* 1999, Thomson *et al.* 2003) and osteogenesis (Perinpanayagam & Al-Rabeah 2009). However, it is not known how calcium hydroxide or MTA signals progenitor/stem cells in the periodontal ligament or alveolar bone marrow to differentiate into cementoblast-like cells or osteoblasts and produce cementoid or osteoid tissue barrier at the blunt open apex. If calcium hydroxide and MTA can cause the release of growth factors from the dentine matrix as described in pulp capping and pulpotomy (Lesot *et al.* 1993, 1994, Smith *et al.* 1994, Begue-Kirn *et al.* 1994, Smith 2002), it is conceivable that they might also be able to induce the release of growth factors such as BMPs, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), TGF-, IGF-1, epidermal growth factor (EGF), cementum-derived growth factor (CGF) from cementum matrix (MacNeil & Sommerman 1993, Grzesilk & Narayanan 2002) and IGFs, TGF-β, BMPs, FGF, PDFG from alveolar bone matrix (Linkhart *et al.* 1996, Solheim 1998) and signal progenitor/stem cells in the periodontal ligament and alveolar bone marrow to differentiate into

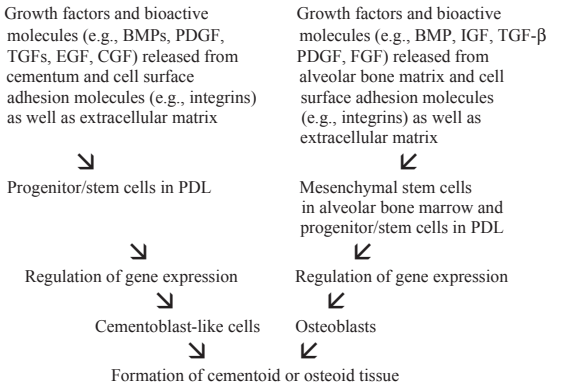


Figure 4 Apexification.

cementoblast-like cells and osteoblasts (Fig. 4). Recently, it was shown that MTA induced BMP-2 expression and calcification in human periodontal ligament cells through calcium-sensing receptor gene expression (Maeda *et al.* 2010).

Heithersay (1970) and Dominguez *et al.* (2005) in their case series studies reported a high success rate of complete root development in immature permanent teeth with infected necrotic pulp and apical periodontitis following apexification procedures using calcium hydroxide. They speculated that HERS cells might have survived in apical periodontitis in immature permanent teeth with infected necrotic pulps after apexification procedures. If HERS cells survive in inflammatory periapical lesions after apexification procedures, they will be capable of inducing progenitor/stem cells in the periodontal ligament to differentiate into cementoblast-like cells and produce cementum-like tissue to promote root development (Sonoyama *et al.* 2007). Yang *et al.* (1990) reported continued physiological root development of an immature permanent tooth with infected necrotic pulps and apical periodontitis after apexification procedures treated with calcium hydroxide. The newly formed root apical to the hard tissue barrier had normal root structure with pulp tissue in the canal space surrounded by dentine and cementum. It appears in this case that the apical papilla and HERS have survived despite apical periodontitis. HERS cells are capable of signalling stem cells in the apical papilla to differentiate into odontoblasts and produce root dentin (Sonoyama *et al.* 2007). Following apexification procedures, HERS cells appear to be more resistant than apical papilla cells to apical periodontitis/abscess in immature permanent teeth (Heithersay 1970, Dominguez *et al.* 2005).

Apical hard tissue barrier formation following apexification is a reparative process of the dentine–pulp complex.

Revascularization

Revascularization is a new treatment procedure that may replace apexification procedures (Iwaya *et al.* 2001, Banchs & Trope 2004, Chueh & Huang 2006, Jung *et al.* 2008, Ding *et al.* 2009, Chueh *et al.* 2009). It is designed to promote the revascularization of an immature permanent tooth with an infected necrotic pulp and apical periodontitis/abscess. The procedure requires minimal or no mechanical instrumentation but copious antiseptic irrigation of the canals in conjunction with disinfection using a triple antibiotic mixture of ciprofloxacin, metronidazole and minocycline or calcium hydroxide. Revascularization procedures have been shown radiographically to induce increased thickening of the canal walls by the deposition of hard tissue and continued root development in immature permanent teeth with necrotic pulp and apical periodontitis/abscess (Iwaya *et al.* 2001, Banchs & Trope 2004, Chueh & Huang 2006, Jung *et al.* 2008, Ding *et al.* 2009). With one exception (Chueh & Huang 2006), intracanal bleeding was induced in all reported cases involving revascularization procedures (Iwaya *et al.* 2001, Banchs & Trope 2004, Jung *et al.* 2008, Ding *et al.* 2009).

The precise nature of the hard tissue formed inside the canal space and continued root development of immature permanent teeth with apical periodontitis/abscess following revascularization procedures in humans is not known, because no histological studies are available. It has been assumed that periodontal ligament tissue might grow into the canal space and deposit cementum on the canal walls after revascularization procedures (Chueh & Huang 2006). Vojinovic & Vojinovic (1993), using autoradiographic examination, showed that PDL cells could migrate into the apical root canal after pulpectomy in immature dog's teeth. Stem cells in the periodontal ligament are capable of differentiating into cementoblast- and osteoblast-like cells upon stimulation by appropriate inductive signal (Seo *et al.* 2004, Bartold *et al.* 2006). It was also speculated that there might be direct in-growth of cementum and bone from the periapical tissues into the canal space (Cotti *et al.* 2008). In studying pulpal changes in replanted and autotransplanted immature dog's teeth, Skoglund & Tronstad (1981) showed that osteoid tissue formed inside the root canal space was continuous with the bone of the alveolar socket through open apical foramina. The same finding was also demonstrated in dog's immature teeth with apical periodontitis after revascularization procedures (Bezerra da Silva *et al.* 2010, Yamauci *et al.* 2011). Similar observations of hard tissue formation in the canal space were reported in rhesus monkeys when their immature teeth were artificially infected for 1 week. The pulps were then removed and the canals were chemomechanically debrided and filled with collagen/calcium phosphate gel as a scaffold (Nevins *et al.* 1976).

The tissues present inside the canal space of immature teeth with apical periodontitis after revascularization procedures in dog studies were described as cementum- or bone-like tissue and periodontal ligament-like fibrous connective tissue (Thibodeau *et al.* 2007, Bezerra da Silva *et al.* 2010, Wang *et al.* 2010, Yamauci *et al.* 2011). No pulp-like tissue was present regardless of the intracanal medication used (Thibodeau *et al.* 2007, Bezerra da Silva *et al.* 2010, Wang *et al.* 2010, Yamauci *et al.* 2011). Most recently, under controlled conditions without induced apical periodontitis in ferret's immature teeth, no new dentin formation on the root canal walls and no pulp tissue regeneration were observed in the canal spaces after the removal of vital pulp and treatment with revascularization procedures (Buhrley *et al.* 2011). The canal spaces were filled with hard tissue similar to cementoid and/or osteoid tissue (Buhrley *et al.* 2011).

It is generally believed that in immature permanent teeth with apical periodontitis, the pulps are completely necrotic. However, apical periodontitis is an extension of apical pulpitis (Kovacevic *et al.* 2008). Therefore, apical periodontitis may develop in the presence of an inflamed vital pulp in the apical portion of the canal in mature teeth (Lin *et al.* 1984). Histologically, Cvek *et al.* (1990) showed the presence of vital pulp tissue in the apical portion of the canal space in reimplanted immature incisors, despite the presence of intraradicular and periapical abscesses. The possibility exists of some pulp tissue regeneration after revascularization procedures in human immature permanent teeth with apical periodontitis. Accordingly, it has been speculated that pulp tissue regeneration might occur after revascularization procedures (Chueh & Huang 2006, Cotti *et al.* 2008). Histological evidence is required to validate that possibility. At this time, in animal studies, there is no convincing histological evidence, indicating that true pulp regeneration takes place inside the canal space of immature teeth with apical periodontitis/abscess after revascularization procedures (Thibodeau

et al. 2007, Bezerra da Silva *et al.* 2010, Yamauci *et al.* 2011). Similar to periodontium (Bartold *et al.* 2006), pulp tissue has a limited capacity for regeneration once damaged. Complete pulp tissue regeneration is unlikely to occur after revascularization procedures in immature permanent teeth with infected necrotic pulps and apical periodontitis/abscess, as irreversible pulpitis or pulp necrosis is a post-natal wound (Colwell *et al.* 2003, Bullard *et al.* 2003).

HERS cells play an essential role in root development (Nanci 2007, Zeichner-David *et al.* 2003, Sonoyama *et al.* 2007). Similar to apexification, if HERS cells survive in apical periodontitis/abscess after revascularization procedures, they can signal progenitor/stem cells in the periodontal ligament to differentiate into cementoblast-like cells and produce cementum-like tissue to promote root development (Zeichner-David *et al.* 2003, Sonoyama *et al.* 2007). If the apical papilla also survives in apical periodontitis/abscess after revascularization procedures, HERS cells will signal apical papilla cells to differentiate into root primary odontoblasts and promote root dentine formation (Sonoyama *et al.* 2008). Thus, the incompletely formed root will continue its normal physiological development after revascularization procedures.

Continued root development has been reported in human immature permanent teeth with apical periodontitis/abscess after revascularization procedures ((Iwaya *et al.* 2001, Banchs & Trope 2004, Chueh & Huang 2006, Jung *et al.* 2008, Ding *et al.* 2009, Chueh *et al.* 2009). It was assumed that in revascularization cases, the apical papilla was surviving in human immature permanent teeth with apical periodontitis (Ding *et al.* 2009, Chueh *et al.* 2009). In contrast, no significant root development has also been described in human immature permanent teeth with apical periodontitis/abscess following revascularization procedures (Shah *et al.* 2008, Chueh *et al.* 2009, Petrino *et al.* 2010). Root development is regulated by HERS cells (Nanci 2007, Zeichner-David *et al.* 2003) and not by the apical papilla. HERS cells have to survive in order to guide continued root development in immature permanent teeth with apical periodontitis/abscess following revascularization procedures. Heithersay (1970) and Dominguez *et al.* (2005) also reported similar observations of continued root development in apexification cases with calcium hydroxide treatment. They assumed that HERS survived in apical periodontitis. No pulp-like tissue was present in the apical portion of the canal space of immature teeth with apical periodontitis/abscess after revascularization procedures in animal studies (Thibodeau *et al.* 2007, Bezerra da Silva *et al.* 2010, Wang *et al.* 2010, Yamauci *et al.* 2011). This indicates that the apical papilla did not survive. In addition, no evidence of dentine formation was observed inside the canal space or on the canal walls in those animal studies.

The apical papilla cells contribute only to root dentine and not to root cementum formation upon stimulation by appropriate inductive signal from HERS cells (Sonoyama *et al.* 2008). Continued root development was evidenced by apical deposition of cementum without dentin after revascularization procedures in animal studies (Bezerra da Silva *et al.* 2010, Wang *et al.* 2010, Yamauci *et al.* 2011), probably because HERS cells survived in apical periodontitis. When the entire or half of HERS was removed from immature teeth of monkeys before autotransplantation, total or partial arrest of root formation occurred. In contrast, when HERS was not injured before autotransplantation, normal physiological root development took place (Andreasen *et al.* 1988). Increased root length can also occur by excessive deposition of cementum around the apex (hypercementosis) caused by ageing, abnormal occlusal trauma, pulpal or periapical inflammation in adult mature teeth without the presence of HERS cells (Neville *et al.* 2009). It has been demonstrated that the extracellular matrix of cementum and growth factors released from cementum matrix are capable of signalling PDL stem cells to differentiate into cementoblasts and produce cementum (MacNeil & Sommerman 1993, Grzesilk & Narayanan 2002).

Regeneration implies regrowth of a lost or damaged part of original tissue or organ caused by trauma or disease (Kumar *et al.* 2009). An example of this is the regeneration of the liver after it is partially resected (Michalopoulos & DeFrances 1997). The incompletely developed roots of immature permanent teeth with infected necrotic pulp and apical periodontitis are not attributable to the destruction of previously formed roots but are attributable to the inhibition or damage of HERS cells. Therefore, continued root development of human immature permanent teeth with infected necrotic pulp and apical periodontitis/abscess after revascularization procedures does not appear to be a regenerative process. It could be considered a physiological process if both apical papilla and HERS survive in apical periodontitis/abscess after periapical wound healing. Continued root development of human immature permanent teeth with infected necrotic pulp and apical periodontitis was also observed in apexification procedures (Frank 1966, Heithersay 1970, Dominguez

et al. 2005) and has never been considered a regenerative process. A functional root cannot be formed without cementum formation. Acellular cementum provides a primary anchorage of PDL fibres to the alveolar socket, and cellular cementum serves as a minor anchorage (Nanci 2007).

Primary cementogenesis is similar to primary dentinogenesis in that both require cross-talk between epithelial cells and neural crest–derived ectomesenchymal cells (Nanci 2007). It has been proposed that HERS cells are capable of undergoing epithelial–mesenchymal transition to give rise to cementum-forming cells (Zeichner-David *et al.* 2003, Zeichner-David 2006). In addition, it has also been suggested that acellular cementum and cellular cementum are formed by cementoblasts from different cell lineages (Zeichner-David *et al.* 2003, Zeichner-David 2006). Irrespective of regenerative therapy, the newly formed cementum is composed of reparative, cellular, extrinsic and intrinsic fibre types (Sculean *et al.* 2005).

Several questions remain to be answered concerning revascularization procedures. What are the molecular signals, which stimulate migration and proliferation of progenitor/stem cells in the periapical tissues into the canal space? Cell migration and proliferation require a scaffold, adhesion molecules, chemotactic and growth factors (Griffith & Naughton 2002, Delong *et al.* 2005). What is/are the cellular and molecular mechanism(s), which instruct progenitor/stem cells to differentiate into hard tissue formation cells inside the canal space? Stem cell differentiation is tightly regulated by intrinsic epigenetic mechanisms and extrinsic micro-environmental cues (Watt & Hogan 2000, Wu & Sun 2006, Kolf *et al.* 2009, Lunyak & Rosenfeld 2008). Increased thickening of the canal walls should increase the strength of the teeth. Nevertheless, an important question exists. What is the nature of the junction between intracanal hard tissue (cementum-like/bone-like) and the root canal walls? During tooth development, the dentine–cementum complex is formed by interlocking of dentin collagen fibres produced by primary odontoblast and cementum collagen fibres produced by cementoblasts. The interwoven collagen fibres then become mineralized by the deposition of hydroxyapatite crystals (Nanci 2007). In pathological external root resorption involving cementum and dentine, the newly formed cementoblasts differentiated from PDL progenitor/stem cells produce collagen fibres, which intermingle with exposed dentin collage fibres at the resorbed root area. Subsequently, the interlocking collagen fibres become mineralized by the deposition of hydroxyapatite crystals (Bosshardt & Schroeder 1994). A similar finding was observed in the revascularization of immature teeth with apical periodontitis in dogs by Yamauci *et al.* (2011). When the canal dentine was treated with 17% ethylenediaminetetraacetic acid (EDTA) to expose dentine matrix, dentin-associated mineralized tissue appeared to be tightly attached to the canal dentine walls, In addition, EDTA may also cause the release of bioactive growth factors from dentine matrix (Roberts-Clark & Smith 2000). The exact biological process of how hard tissue forms on the canal walls in immature permanent teeth with apical periodontitis/abscess following revascularization remains unknown. What then would be the long-term outcome of immature permanent teeth with ectopic cementoid and osteoid tissues formed in the pulp canal space because they are not normal components of the pulp tissue?

A recent study has demonstrated that mesenchymal stem cells in the root canal space following revascularization procedures are derived from local periapical tissues and they may provide the biological foundation for the continued root development and regenerative response in necrotic immature permanent teeth with open apex (Lovelace *et al.* 2011). For pulp tissue regeneration, the mesenchymal stem cells from the periapical tissues must be capable of differentiating into odontoblast-like cells (Shi *et al.* 2005). Mesenchymal stem cells from different tissues are endowed with tissue-specific potency (Wagers & Weissman 2004, Bianco *et al.* 2008). In addition, if HERS is destroyed by a long-standing apical periodontitis/abscess, periapical mesenchymal stem cells will have to be able to transdifferentiate into HERS to promote continued root development. Transdifferentiation of mesenchymal stem cells into epithelial cells does not appear to occur (Wagers & Weissman 2004, Bianco *et al.* 2008). In tissue regeneration or tissue engineering, it is necessary that stem cells delivered to the target site can be precisely instructed to differentiate into tissue-committed cells (Nakashima & Akamine 2005).

It is reasonable to ask whether revascularization procedures for immature permanent teeth with apical periodontitis/abscess have the same potential for repair in older and younger patients. In immature permanent teeth with a long-standing apical periodontitis/abscess in adult patients, the apical papillae and HERS cells may be severely damaged, thus hindering the potential of continued root development. Furthermore, the functionality of stem cells is age-related decline owing to change in the intrinsic factors of stem cells and

diminished signals within the extrinsic local and systemic environment that modulate the function of stem cells or their progeny (Ho *et al.* 2005, Rossi *et al.* 2005, 2007, Carlson & Conboy 2007, Chambers *et al.* 2007). Recently, it has been demonstrated that ageing dental mesenchymal stem cells lose their replication and odontogenic differentiation potentials, along with loss of Bmi-1 expression, which is a polycomb-group protein required for the transcriptional repression of its target genes through chromatin remodelling (Mehrazarin *et al.* 2011). This may also affect the regenerative potential of immature permanent teeth with apical periodontitis/abscess following revascularization in adult patients.

If the tissues present inside the canal space in human immature permanent teeth with apical periodontitis/abscess after revascularization procedures are similar to that observed in animals, then revascularization is a reparative process with the loss of pulp biological function. Cementoid and osteoid tissues are not normally present as part of the pulp tissue. These tissues inside the canal space may function like periodontal tissues (Huang 2009a). Histological studies are necessary to verify whether repair or regeneration of the pulp tissue occurs inside the root canal space following revascularization procedures in humans.

An important question to be answered is: What is the effect of inflammation/infection, especially chronic inflammation (e.g. chronic apical periodontitis), on stem cell function during wound healing? In a classic germ-free rat study, wound healing of exposed pulps proceeded satisfactorily but in conventional laboratory rats, exposed pulps became necrotic and subsequently resulted in apical periodontitis/abscess owing to infection/inflammation (Kakehashi *et al.* 1965).

Regeneration of the dentine–pulp complex after complete pulp necrosis or irreversible pulpitis with apical periodontitis is likely to occur only by tissue engineering (Nakashima & Akamine 2005, Huang 2009a,b) or gene therapy (Nakashima *et al.* 2006).

Conclusion

Ideal wound healing would achieve maximum regeneration and minimal repair, so that the biological function of the injured tissue would not be jeopardized. Infection is the primary aetiology of pulp and periapical inflammatory disease and has a significant impact on pulp and periapical tissue wound healing. Therefore, control of infection is the key to the success of all endodontic treatment procedures. Infection induces an immuno-inflammatory response, which is not only protective but also destructive to the host (Marton & Kiss 2000, Garlet 2010). The severity of infection is related to the extent of tissue inflammation and destruction (Kumar *et al.* 2009), which hinders the capability of tissue regeneration by progenitor/stem cells. Accordingly, prevention, early detection and treatment of infectious/inflammatory pulpal and periapical disease can enhance regeneration and minimize the repair of pulpal and periapical tissue wound healing following endodontic therapy.

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