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REVIEW

Dental stem cells and their potential role in apexogenesis and apexification

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Abstract

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Injury to an immature permanent tooth may result in cessation of dentine deposition and root maturation leaving an open root apex and thin dentinal walls that are prone to fracture. Endodontic treatment is often complicated and protracted with an uncertain prognosis frequently resulting in premature tooth loss. Postnatal stem cells, which are capable of self-renewal, proliferation and differentiation into multiple specialized cell lineages have been isolated and identified within the dental pulp, apical papilla and periodontal ligament. The ability of these cells to produce pulp-dentine and cementum-periodontal ligament complexes *in vivo* suggest potential applications involving stem cells, growth factors and scaffolds for apexification or apexogenesis. Similar protein expression amongst dental stem cells possibly implicates a common origin; however, the dominant cells to repopulate an open apex will be directed by local environmental cues. A greater understanding of the structure and function of cells within their environment is necessary to regulate and facilitate cellular differentiation along a certain developmental path with subsequent tissue regeneration. This review focuses on development of the apical tissues, dental stem cells and their possible involvement clinically in closing the open root apex. MEDLINE and EMBASE computer databases were searched up to January 2009. Abstracts of all potentially relevant articles were scanned and their contents identified before retrieval of full articles. A manual search of article reference lists as well as a forward search on selected authors of these articles was undertaken. It appears that dental stem cells have the potential for continued cell division and regeneration to replace dental tissues lost through trauma or disease. Clinical applications using these cells for apexogenesis and apexification will be dependent on a greater understanding of the environment at the immature root end and what stimulates dental stem cells to begin dividing and then express a certain phenotype.

Keywords: apexification, apexogenesis, dental stem cells, growth factors, immature permanent teeth, regeneration, root development.

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Introduction

The completion of root development and closure of the root apex of a permanent tooth occurs up to 3 years after tooth eruption. Irreversible injury to the dental pulp of an immature permanent tooth from either infection or dental trauma before complete root development poses a clinical challenge. Dentine formation and tooth maturation ceases, creating difficulties in providing endodontic treatment. Apexification enables a calcified barrier to form at the root apex by placing a biocompatible material against the periapical tissues via the root canal. Calcium hydroxide and mineral trioxide aggregate (MTA) have been the materials of choice for apexification procedures, but neither material is ideal (Shabahang & Torabinejad 2000, Witherspoon *et al.* 2008). MTA is favoured for its sealing

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properties, biocompatibility and ability to induce cementoblast attachment to the barrier, but the sealing of MTA as a root-end filling material when placed via the root canal is not as great as that achieved following retrograde placement (Hachmeister *et al.* 2002, Thomson *et al.* 2003). Internal bonding techniques using composite resin materials within the root canal have demonstrated improved fracture resistance of immature teeth, with MTA apexification reducing the treatment time (Pene *et al.* 2001, Hachmeister *et al.* 2002). However, neither calcium hydroxide nor MTA is able to stimulate regeneration of pulp tissue, and continued root development, so a tooth at risk of fracture with thin dentine walls remains.

Predictable regeneration of hard tissue into the open root apex is desirable, but not yet possible. Postnatal stem cells with the capacity to self-replicate and differentiate into specialized tissue types have recently been identified in the dental tissues. This paper reviews the current knowledge of stem cells from the dental pulp (DPSC), exfoliated deciduous teeth (SHED), apical papilla (SCAP) and periodontal ligament (PDLSC), giving consideration to the role they may have in continued root development, or apical closure after the pulp has been irreversibly damaged. Ovid SP MEDLINE computer database from 1950 to 2009 and EMBASE computer database from 1988 to 2009 were searched using the following MeSH headings: apexification, apexogenesis, dental stem cells, growth factors, regeneration and root development. Title and abstracts of all potentially relevant articles were scanned and their contents identified before retrieval of full articles. A manual search of article reference lists as well as a forward search on selected authors of these articles was undertaken to locate potentially relevant research.

Tissue regeneration attempts to mimic the events of development. A revision of root development and biology is followed by discussion involving dental stem cell populations. Repopulating the open apex with immature cells capable of being directed towards a specific cell fate and regenerating natural tissue may provide an alternative treatment for patients who have sustained serious injury to immature permanent teeth.

Biology of the root apex

The apical region of an immature permanent tooth is comprised of dental pulp, apical papilla and periodontal tissues, which have developed through a series of ectomesenchymal interactions. During the bell stage of tooth development, the dental papilla becomes partially enclosed by the envaginating epithelium, and the condensed ectomesenchyme surrounding the enamel organ and dental papilla forms the dental follicle (Ten Cate 1997).

The dental pulp is soft tissue of ectomesenchymal and mesenchymal origin, which develops from the dental papilla. It is composed of water, ground substance, connective tissue, blood vessels, nerves, lymphatics, fibroblasts, immune cells and odontoblasts (Trowbridge 2003). The odontoblasts secrete dentine and are integral to the pulp–dentine complex. Primary dentine is formed until completed root development, following which dentine formation proceeds as secondary dentinogenesis and continues at a slower rate throughout the lifetime of the individual. As the root and pulp develop, the dental papilla is located apical to the developing pulp and is called the apical papilla. Clinically, this is a gelatinous soft tissue, which is easily detached from the root apex. Histologically, it is distinct from the pulp, is less vascular and cellular, with the two tissues separated by a cell-rich zone (Sonoyama *et al.* 2006, 2008).

The dental follicle surrounding the developing tooth root contains progenitor cells for the developing periodontium: cementum, alveolar bone and PDL. Meanwhile, the inner and outer enamel epithelia fuse to form a structure known as Hertwig's epithelial root sheath (HERS). As HERS migrates apically, the ectomesenchymal tissues are divided into the dental papilla on one side and dental follicle on the other. HERS has a role in root development and shape, but the exact function of the cells is less certain. They may be involved in regulating the differentiation of odontoblasts or cementoblasts with the formation of dentine and cementum (Ten Cate 1978, Sonoyama *et al.* 2007). Once the first layer of mantle dentine has been laid down, the root sheath begins to disintegrate, allowing the attachment of cells from the dental follicle onto the exposed root dentine with the subsequent deposition of cementum (Handa *et al.* 2002). Individual cells from the root sheath migrate away from the root to the region of the future periodontal ligament to form the rests of Malassez. HERS is very sensitive to trauma and once destroyed, there is cessation in normal root development with no further odontoblast differentiation. In an immature permanent tooth, this leaves an open root apex, thin weak root walls and a discontinuous periodontal ligament. The periodontal ligament has a role in supporting the teeth in the jaw as well as contributing to tooth nutrition, homeostasis and repair of damaged tissues.

Stem cells

All tissues originate from a small population of stem cells which play an essential role in embryonic development and tissue regeneration. These immature cells are capable of self-renewal, i.e. the ability to go through numerous cycles of cell division whilst maintaining the undifferentiated state; proliferation, and differentiation into multiple mature cell types. Stem cell potency describes the potential of the cell to divide and express different cell phenotypes. Totipotent stem cells are able to divide and produce all the cells in an individual, including extraembryonic tissues. Pluripotent stem cells have not completely divided and can become many cells. They are able to differentiate into any of the three germ layers: endoderm, mesoderm or ectoderm, where the progeny have multiple distinct phenotypes, whilst multipotent stem cells can differentiate into cells from multiple, but a limited number of lineages (Robey 2000). There are two types of stem cells: embryonic and postnatal. Embryonic stem cells are pluripotent cells capable of differentiating into virtually any cell type as well as maintaining an undifferentiated state. These cells are very plastic by virtue of their capacity to develop into various specialized cell types with a huge potential for tissue regeneration. The immunological and ethical problems of using allogenic embryonic stem cells (Antoniou 2001) may be overcome to some extent, by the use of postnatal stem cells.

Postnatal cells with stem cell-like qualities have been identified in tissues where they make up only 1–4% of cells, which may include progenitor cells (Smith *et al.* 2005). Progenitor cells are left over from development and more committed, having retained the differentiation and proliferation abilities but lack the ability to self-replicate. Postnatal stem cells are multipotent and can be classified on their origin, i.e. haematopoietic or mesenchymal (MSC), and differentiation potential. They are less plastic and more limited in their differentiation potential than embryonic cells with a finite lifespan, but still fulfil the criteria of stem cells. MSC reside in a variety of tissues, but were first discovered in aspirates of bone marrow stroma (BMSSC), which remains the gold standard in terms of identifying stem cell markers (Friedenstein *et al.* 1966, Gronthos *et al.* 1994, 2003). MSC share similar markers with haematopoietic cells and so the identification of markers that are 'specific' to MSC is important for their isolation. The stromal-derived factor-1 (STRO-1) antigen has become recognized as a putative marker in the isolation and identification of MSC. Anti-STRO-1 identifies a cell surface antigen expressed by MSC which is minimally reactive with haematopoietic progenitors (Gronthos *et al.* 1994). Immunohistochemistry and gene profile analysis have identified perivascular cell markers, CD146/MUC18, 3G5, CD-44, VCAM-1; alkaline phosphatase and α-smooth muscle actin in differing proportions on STRO-1 positive cells from dental tissues (Gronthos *et al.* 2000, Shi and Gronthos 2003, Seo *et al.* 2004).

Dental pulp stem cells

Severe injury to a dental pulp from either infection or trauma leads to death of odontoblasts with a limited ability for regeneration. Healing depends on the intensity and duration of the injury, presence of bacteria, and host factors such as the level of innate and systemic immunity. Coronally in the tooth, a new generation of odontoblast-like cells develops from an immature population of cells during the process of reparative dentinogenesis (Mjör *et al.* 1991). It is unknown where in the pulp the cells are recruited from. The cell-rich sub-odontoblast layer of Höhl, perivascular cells or immature mesenchymal cells and fibroblasts have all been suggested (Tziafas *et al.* 2000). A postnatal population of human DPSC has been identified and isolated which show a higher proliferation capacity compared with osteogenic cells, have the ability to differentiate into odontoblast-like cells which express the early odontoblast cell marker, dentine sialophosphoprotein, and can form a dentine–pulp complex when transplanted *in vivo* (Gronthos *et al.* 2000, Shi *et al.* 2005). DPSC are capable of generating new stem cells or multilineage differentiation into odontoblasts, adipocytes and neural-like cells, suggesting a hierarchy of progenitors within the pulp, including a small population of stem cells amongst a larger population of more committed cells (Gronthos *et al.* 2002). This stem cell behaviour occurs following cryopreservation, signifying the potential use of frozen tissues for stem cell isolation (Zhang *et al.* 2006). Pulp cells are able to proliferate and differentiate into odontoblast-like cells with processes, extending into dentinal tubules when in contact with chemo-mechanically treated dentine surfaces in an *in vitro* situation, which is a requirement for the secretion of new dentine (Huang *et al.* 2006).

The exact origin and location of DPSC within the pulp remains unclear; however, these cells display phenotypes consistent with a perivascular niche (Shi & Gronthos 2003). The stem cell population in the pulp is very small; approximately 1% of the total cells (Smith *et al.* 2005) and the effect of aging reduces the cell pool

available to participate in regeneration which reflects the better healing outcomes seen in younger patients. The pulp tissue of third molar teeth has mostly been used to investigate DPSC; however, stem cells have also been identified in supernumery teeth, and permanent tooth germs (Huang *et al.* 2008). When isolated at the stage of crown development, DPSC are more proliferative than later on (Takeda *et al.* 2008). The isolation of stem cells is not restricted to the permanent dentition. SHED have been identified in the remnant pulp of human exfoliated deciduous teeth as a population of highly proliferative, colony-forming cells able to differentiate into more specialized cell lines, and capable of producing bone and dentine when transplanted *in vivo* (Miura *et al.* 2003). SHED are distinct from DPSC with a greater proliferation rate and increased population doublings.

Stem cells from the apical papilla

Stem cells from the apical papilla are a population of multipotent stem cells isolated from the root apical papilla of human teeth (Sonoyama *et al.* 2006, 2008). The soft tissue on the exterior of the apical foramen area expresses markers for STRO-1 and CD24, a surface marker for SCAP, which is lost during odontogenic differentiation. Compared with DPSC, SCAP have greater numbers of STRO-1 positive cells, faster proliferation, a greater number of population doublings and increased capacity for *in vivo* dentine regeneration. Unlike DPSC and other MSC, SCAP are positive for telomerase activity which is present in embryonic stem cells and suggests a very immature source of cells available for hard tissue regeneration which has been demonstrated by the use of SCAP to engineer bioroots in minipigs (Sonoyama *et al.* 2006, 2008, Yang *et al.* 2008).

Further studies are required to more clearly define the apical papilla at a molecular level. SCAP might be the source of primary odontoblasts involved in the development of root dentine, in contrast to DPSC, which are most likely involved in reparative dentine formation.

Periodontal ligament stem cells

Earlier researchers hypothesized that cementoblasts, alveolar bone cells and PDL cells may be derived from a single population of immature cells which were capable of migrating from endosteal spaces into the PDL where they express osteoblast or cementoblast phenotypes (McCulloch 1985, Melcher 1985, McCulloch *et al.* 1987). Recently, isolation and characterization of a stem cell population within the PDL has been confirmed (Seo *et al.* 2004).

Periodontal ligament stem cells are more proliferative than BMSSC, with a longer lifespan, and higher number of population doublings *in vitro*. The potential of PDLSC to develop into other cell lineages and obtain periodontal ligament-like characteristics has been established by the ability of cultured PDLSC to differentiate into cementoblast-like cells, adipocytes and collagen-forming cells *in vitro* and the capacity to generate a cementum/PDL-like structure *in vivo*. PDLSC express similar MSC markers to other dental stem cells, but express a high level of scleraxis, a tendon-specific transcription factor which is weakly expressed in DPSC or BMSSC (Seo *et al.* 2004, Nagatomo *et al.* 2006). In common with DPSC, PDLSC maintain their stem cell characteristics and continue to express STRO-1 after cryopreservation (Seo *et al.* 2005).

Dental pulp stem cells, SHED and PDLSC have similar gene expression profiles for extracellular matrix proteins, growth factors, receptors and adhesion molecules, suggesting the existence of a common origin and molecular pathway regulating the formation of dentine, cementum and bone, but as yet no genes are exclusively expressed by either cell population (Shi & Gronthos 2003, Shi *et al.* 2005).

Growth factors and signals

Growth factors are extracellular secreted proteins that bind to cell receptors and modulate cellular activity. Numerous growth factors play a role in development and repair of dental tissues and their full discussion is beyond the scope of this review. However, several key factors regulate dental stem cell proliferation, differentiation and the secretion of mineralized tissue. Fibroblast growth factor, transforming growth factor beta (TGFβ) superfamily including bone morphogenic proteins (BMPs), platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF) have specific and sometimes overlapping functions in stem cell control, but BMPs appear to be the key regulators (King *et al.* 1997, Iohara *et al.* 2004, Saito *et al.* 2004).

There are six BMPs (BMP-2 to BMP-7) that, together with their receptors, act similarly on DPSC and PDLSC; however, their role in SCAP regulation is largely unknown. Under the influence of BMPs, DPSC differentiate into odontoblast-like cells capable of dentine secretion (Nakashima 1994, Sloan *et al.* 2000, Saito *et al.* 2004) and stimulation of PDLSC results in enhanced periodontal regeneration and cementogene-

sis (King & Hughes 2001). TGFβ-1 and its receptors, and BMP-2, -3, -4, -7 have been identified in pulpal and periodontal cells even, very early in development (Toyono *et al.* 1997, Kémoun *et al.* 2007). PDGF and IGF are both capable of promoting dental stem cell proliferation and may act synergistically to promote the differentiation of immature cells into phenotypes involved in periodontal and pulpal regeneration (Howell *et al.* 1997, Denholm *et al.* 1998).

Following pulpal injury, an inflammatory response ensues which may negatively influence the cellular response to growth factors. Rutherford & Gu (2000) demonstrated that a single application of BMP-7 was insufficient to induce reparative dentinogenesis in teeth with inflamed pulps compared with healthy pulps, which might partially explain the individual variation of healing responses following dental injury.

The type of tissue occupying the immature root apex will be determined by the local environment and what molecular pathways are activated. As in the coronal region of the periodontium, repopulating the area is likely to be dominated by one cell phenotype. Bone, cementum, PDL and apical papilla cells will proliferate and differentiate at different rates in response to growth factors and cytokines, but less clear, is a defined understanding of what pathways are involved in switching cells on. At a molecular level, Notch and Wnt signalling pathways play a critical role in development, and control of stem cell fate with both negatively inhibiting odontoblast differentiation (Lovschall *et al.* 2005, Scheller *et al.* 2008, Zhang *et al.* 2008). These signals may play a role in maintaining stem cells in an undifferentiated state and so contribute to cellular expansion required for tissue regeneration.

Stem cells and apexogenesis or apexification

Regeneration of tissue into the apex of an immature permanent tooth may come from stem cells already residing in vital pulp tissue, the apical papilla, PDL or alveolar bone; alternatively, stem cells and growth factors seeded on scaffolds may be used to regenerate tissue *in vitro* or *in vivo*.

Irreversible pulpal injury results in pulp necrosis and is commonly because of endodontic infection. In younger patients, where the possibility exists to retain some vital pulp tissue and allow continued root development, a conservative approach is desirable. When infection extends throughout the root canal system, endodontic treatment involves the removal of remaining pulp tissue to the level of the developing root apex, i.e. at its loose physical connection with the apical papilla. Clinicians are guided by radiographs, apex locators, tactile sensation and reproducible drying points, but it is impossible to know where the pulp tissue terminates and if all pulpal cells are removed. Immature permanent teeth have a rich cellular and vascular supply and so DPSC and SCAP may survive disinfection, as suggested by case reports showing immature teeth with pulpal necrosis undergoing apexogenesis (Banchs & Trope 2004, Chueh & Huang 2006, Jung *et al.* 2008). Revascularization following pulpal severing has been studied *in vitro* using tooth slices implanted into mice. The application of angiogenic growth factors markedly enhanced vascular sprouting, highlighting the role of the environment on favourable healing (Mullane *et al.* 2008). Histologically, vital tissue has been shown within the root canal space following 'revascularization procedures', but the origin of this tissue remains unproved (Thibodeau *et al.* 2007).

Periodontal studies show cells may proliferate and migrate from adjacent undamaged PDL into the wounded area (King *et al.* 1997, King & Hughes 2001). This suggests stem cells present within PDL, and alveolar bone marrow might be able to be stimulated at a distance and migrate towards the immature root apex. Stem cells have been identified in greater numbers within the PDL of diseased teeth where the inflammatory process actively recruited immature cells (Chen *et al.* 2006). Vojinović & Vojinović (1993) traced periodontal cell migration into the apical pulp during the repair process following pulpectomy in immature dogs' teeth and found inflammation stimulated cellular recruitment. Periapical inflammation of an immature permanent tooth occurs after trauma or infection of the root canal system. The question remaining is what environmental signals and critical level of inflammation are necessary to preferentially stimulate stem cell migration towards the open apex, with the potential to deposit dentine, cementum and/or alveolar bone, but to not permanently injure the cells?

Mooney *et al.* (1996) first described an *in vitro* technique to engineer new pulp-like tissues from cultured human pulpal fibroblasts. Regeneration of pulp or periodontal tissues relies on the provision of appropriate biodegradable scaffolds which are capable of containing or being seeded with growth factors and bioactive signalling molecules, supporting cell organization and growth of a vascular supply. Natural scaffolds like collagen offer good biocompatibility and bioactivity; however, synthetic scaffolds such as polylactic acid, polyglycolic acid, foams and hydrogels have more predictable mechanical properties and offer

greater control of degradation time (Dobie *et al.* 2002, Young *et al.* 2002). As yet no matrix has proved ideal; collagen and polymer scaffolds are able to support *in vitro* survival of DPSC and PDLSC unlike constructs of calcium phosphate (Gebhardt *et al.* 2009).

There are several problems with *in vitro* regenerative procedures. The cell line needs to be grown and expanded before being implanted into the root canal, resulting in protracted clinical treatment times. The implanted cells then need to reliably adhere to the disinfected root canal walls which may dictate a change in the way clinicians currently debride and disinfect root canals. Lastly, the implanted tissue lacks a crucial vascular supply, and it is technically difficult to replant the three-dimensional regenerated pulp without damaging the cells. *In vivo* therapy overcomes some of these obstacles as well as some problems associated with replantation. Cordeiro *et al.* (2008) seeded SHED and endothelial cells onto biodegradable scaffolds within human tooth slices then implanted them into immunocompromised mice. It was observed that cells differentiated into odontoblast-like and endothelial-like cells *in vivo* with the resulting tissue closely resembling dental pulp with a viable blood supply. Gomez Flores *et al.* (2008) developed a novel approach for *in vivo* periodontal regeneration using a multilayer human PDL cell sheet technique which resulted in formation of immature cementum-like tissue and PDL with perpendicular orientation to dentine surfaces. Where an open root apex exists, a similar scaffold design adjacent to a vascular supply may assist apexification by thickening and closing the apical portion of the root with hard tissue.

The viability of dental stem cells in frozen tissue might offer the possibility of banking exfoliated deciduous teeth, supernumerary teeth, third molars or teeth extracted for orthodontic reasons for later use in regenerative therapies. Tissue banking of one's own cells may overcome immunological and ethical considerations involved with the use of allogenic cells. In particular, the banking of SHED or stem cells from immature third molar teeth would be of benefit given the high proliferative nature of these cells and the high incidence of traumatic dental injuries in the early permanent dentition.

The use of gene therapy to regenerate dental tissue by the local delivery of cells that have been genetically manipulated to deliver physiological levels of specific growth factors may be a possibility in future. There has been limited investigation into its use for endodontic and periodontic tissue engineering, with *in vivo* delivery of BMPs giving inconsistent results (Rutherford 2001, Nakashima *et al.* 2002, 2003, 2004, Jin *et al.* 2003) and no published reports involving use of genetically manipulated cells for apexogenesis or apexification procedures. Research is in its early stages in terms of identifying novel genes and finding appropriate vectors for controlled cell-specific safe delivery. Together with ethical constraints for the use of gene technology, the clinical applications for dental tissue regeneration are a long way off.

To fully understand how stem cells can be manipulated by environmental cues to generate new tissue at the root apex, we need to understand how proteins behave in and around cells. Proteomics studies an organism's complete complement of proteins, their structure and function. Research in this area will provide greater clarity not available with gene analysis and bring us closer to the therapeutic use of dental stem cells in clinical practice.

Conclusion

Postnatal stem cells residing in the dental tissues are extremely promising in terms of regenerating tissue; however, there use in a clinical setting to induce apexogenesis or apexification using stem cells, morphogens and scaffolds is presently unpredictable and its applications in endodontic practice are some way off.

The expression of common proteins for DPSC, SHED, SCAP, PDLSC and BMSSC may implicate a common origin and molecular pathway regulating dentine, cementum and bone formation; however, the phenotype repopulating the open root apex will be selected by environmental factors. Further knowledge will more clearly define the behaviour of these cells within their environment, especially the region of the apical foramen and disinfected root canal.

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