Oral Diseases (2007) 13, 151–157. doi:10.1111/j.1601-0825.2006.01346.x

http://www.blackwellmunksgaard.com

INVITED REVIEW

Stem cells and the dental pulp: potential roles in dentine regeneration and repair

AJ Sloan[1], AJ Smith[2]

[1]*Oral Surgery, Medicine & Pathology, School of Dentistry, Cardiff University, Cardiff, UK;* [2]*Oral Biology, School of Dentistry, The University of Birmingham, Birmingham, UK*

The dentine–pulp complex displays exquisite regenerative potential in response to injury. The postnatal dental pulp contains a variety of potential progenitor/stem cells, which may participate in dental regeneration. A population of multipotent mesenchymal progenitor cells known as dental pulp stem cells with high proliferative potential for self-renewal has been described and may be important to the regenerative capacity of the tissue. The nature of the progenitor/stem cell populations in the pulp is of importance in understanding their potentialities and development of isolation or recruitment strategies, and allowing exploitation of their use in regeneration and tissue engineering. Various strategies will be required to ensure not only effective isolation of these cells, but also controlled signalling of their differentiation and regulation of secretory behaviour. Characterization of these cells and determination of their potentialities in terms of specificity of regenerative response will form the foundation for development of new clinical treatment modalities, whether involving directed recruitment of the cells and seeding of stem cells at sites of injury for regeneration or use of the stem cells with appropriate scaffolds for tissue engineering solutions. Such approaches will provide an innovative and novel biologically based new generation of clinical treatments for dental disease.
Oral Diseases (2007) **13**, 151–157

Keywords: stem cells; pulp; dentine; regeneration; cell signalling

Introduction

During tooth formation, epithelial/mesenchymal interactions within the developing tooth germ initiate differentiation of a population of ectomesenchymal cells in the dental papilla into odontoblasts. Odontoblast cells are post-mitotic and are responsible for the secretion of primary dentine. Following primary dentinogenesis, these odontoblasts remain functional and secrete physiological secondary dentine at a continuing, albeit reduced rate. These cells retain the ability to respond to mild environmental stimuli and focally upregulate their secretory activity during reactionary dentinogenesis leading to dentinal regeneration (Smith *et al*, 1995). However, a more intensive stimulus may lead to death of the existing odontoblast population and in such cases dentin regeneration is mediated by the differentiation of a new generation of odontoblast-like cells from a precursor population during the process of reparative dentinogenesis (Smith *et al*, 1990).

The derivation of the stem/progenitor cells during reparative dentinogenesis remains elusive, although a variety of origins may go some way towards explaining the diversity of responses observed during regeneration of the dentine–pulp complex. Potential derivations suggested for these stem/progenitor cells include the cell-rich layer of Höhl adjacent to the odontoblasts (Cotton, 1968), perivascular cells, undifferentiated mesenchymal cells and fibroblasts (Ruch, 1998). More recently, the presence of a unique population of postnatal dental pulp stem cells has been reported (Gronthos *et al*, 2000, 2002). These studies suggest a hierarchy of progenitors in the adult dental pulp, including a small population of self-renewing, highly proliferative multipotent stem cells resident within a larger compartment of more committed progenitors (Gronthos *et al*, 2002).

Despite our extensive knowledge regarding the pathology of dental disease, restoration of diseased dental tissue to date remains fairly empirical. However, our increasing understanding of the exquisite regenerative potential of the dentine–pulp complex highlights the importance of characterizing fully the cellular and molecular processes underpinning dentine regeneration. Sophisticated tissue engineering approaches have emerged as prospective alternatives to conventional treatments, including the use of cytokines and artificial scaffolds, or administration of growth factors such as

Correspondence: Dr Alastair J Sloan, Oral Surgery, Medicine and Pathology, School of Dentistry, Cardiff University, Heath Park, Cardiff, CF14 4XY. Tel: +44 29 2074 6779, Fax: +44 29 2074 2442, E-mail: sloanaj@cardiff.ac.uk
Received 20 July 2006; revised 4 September 2006; accepted 21 September 2006

bone morphogenetic proteins (BMPs) and transforming growth factor-βs (TGF-βs) (Rutherford *et al*, 1995; Mooney *et al*, 1996; Smith and Lesot, 2001). The identification of putative dental stem cell populations capable of regenerating organized tooth-like structures has increased interest in the potential use of postnatal stem cell-based therapies for dental tissue regeneration following trauma or disease (Gronthos *et al*, 2000; Miura *et al*, 2004; Ohazama *et al*, 2004; Duailibi *et al*, 2004; Shi *et al*, 2005).

Derivation of dental cells: the developmental blueprint

Tooth development involves a series of critical, sequential reciprocal interactions between the oral epithelium and cranial neural crest-derived mesenchymal cells (Jernvall and Thesleff, 2000). Studies have demonstrated the influence of the first arch epithelium in the induction of tooth development (Mina and Kollar, 1987) and such interactions, involving a highly co-ordinated expression of growth factors, homeobox genes and transcription factors, progressively lead to the development of the tooth primordia into a complex mineralized structure of specific size, shape and location within the dental arch (Thesleff and Sharpe, 1997; Cobourne and Sharpe, 2003). Studies using a genetic marker to follow neural crest cell migration and differentiation in the mouse have clearly demonstrated that in the developing tooth germ, cranial neural crest derived ectomesenchyme contributes to the condensed dental ectomesenchyme during the bud stage and subsequently to the formation of the dental papilla and surrounding dental follicle. Such studies have also demonstrated that odontoblasts, dentine matrix and much of the pulpal tissue are of cranial neural crest origin (Chai *et al*, 2000).

The primary odontoblasts are induced to differentiate from dental papilla cells through signals derived from the inner dental epithelium and mediated by the basement membrane (Ruch *et al*, 1995). The last division of these papilla cells coming into contact with the basement membrane gives rise to two daughter cells, one of which responds to the molecular signals mediated through the basement membrane to become fully differentiated odontoblasts, the other remaining undifferentiated in the cell rich layer of Höhl as the sub-odontoblast population (Ruch *et al*, 1995; Smith and Lesot, 2001). Many interdependent steps lead to odontoblast differentiation and ultimately the secretion of dentine matrix. These include commitment, restriction of fate and expression of specific competence (ability to respond appropriately to epigenetic signals). Only the cells in contact with the basement membrane are able to differentiate into odontoblasts and the need for a specific number of cell cycles, cytoskeletal reorganization and cell matrix interactions have been postulated to be essential for this specific competence (Lesot *et al*, 1988; Ruch *et al*, 1995; Ruch, 1998). The regulation of odontoblast differentiation may be achieved at the level of transcription factors (Bègue-Kirn *et al*, 1994; Ruch *et al*, 1995) but ultimately, differentiation is dependent on matrix-mediated epigenetic interactions and several molecules (BMPs, TGF-βs, fibroblast growth factors, insulin-like growth factors) have been implicated in signalling odontoblast differentiation (Ruch *et al*, 1995).

With development, the dental pulp becomes further populated with cells of non-neural crest origin, and such cells are most likely derived from the first branchial arch mesenchyme (Chai *et al*, 2000). Thus, the mature pulp comprises a heterogenous population of cells.

A comparison of the events during tooth development and dental tissue repair highlights the many similarities in the two processes (Smith and Lesot, 2001). Presentation of inner dental epithelium-derived growth factors, particularly those of the TGF-β family by the basement membrane leads to odontoblast differentiation during the bell stage of development. Expression of TGF-β isoforms by odontoblasts (Sloan *et al*, 2000) leads to their sequestration in the dentine matrix (Finkelman *et al*, 1990; Cassidy *et al*, 1997) where they are bound within the matrix (Smith *et al*, 1998) unless released during tissue injury. The release of such bioactive molecules during carious demineralization or other tissue injury may lead to stimulation of the secretion of tertiary dentine matrices and differentiation of odontoblast-like cells. Here, the dentine matrix may mimic the basement membrane in the presentation of the inductive signal. However, as during development, where tempro-spatial regulation of odontoblast differentiation is provided in part by the presentation of the inductive signal where only certain cells may respond, in repair it is probable that the cells that respond to the inductive signal are not simply residing in immediately adjacent areas of the pulp, but may be recruited to the site of signal presentation. It is likely that various extracellular matrix components locally released during the injury process may provide a chemotactic stimulus for cell migration. However, there is a paucity of information on the specificities of these molecules in terms of their chemotactic attraction of any particular cell population. Such knowledge may prove invaluable in the development of regenerative therapeutic strategies aimed at targeting specific stem/progenitor cell populations. The ability of such progenitors to respond to such signals may be dictated by their embryonic lineage; however, the need for migration on their part and their subsequent chemotaxis indicate subtle differences in the reparative response, and highlight the importance of understanding such progenitor cell niches.

Mature pulp tissue: niches of stem/progenitor cell populations

Pluripotent stem cells are isolated from the embryonic inner cell mass. In the adult organism, however, although many of the cells are committed, most tissues generally contain a small subpopulation of cells (adult stem cells) with the innate ability to maintain a stem-cell pool by self-replication and generate more committed progenitors through differentiation along multiple lineages (Weissman, 2000). Such cells usually remain quiescent within the adult tissue; however, they may

respond to tissue injury and play an integral role in the tissue repair processes. In dental tissues, it has been suggested that the newly generated odontoblast-like cells were the pulp cells and undifferentiated mesenchymal cells, which had de-differentiated from pulp cells, and pericytes (Yamamura, 1985). Many studies have isolated pulp cells from adult tissues of various species and demonstrated their high proliferative rate and ability to differentiate into cells forming mineralized nodules in supporting media (Nakashima, 1991; Nakashima *et al*, 1994; Kettunen *et al*, 1998; Buchaille *et al*, 2000; Yokose *et al*, 2000; About *et al*, 2000). However, the dentinogenic specificity of such mineralized deposits is not always clear. Recently, Gronthos *et al* (2000, 2002) have attempted to characterize a unique population of postnatal human dental pulp stem cells (DPSCs). These cells showed capacity for self-renewal and differentiation into odontoblast-like cells, which formed the dentine matrix with some tubular features *in vivo*. The same group has also identified a potential mesenchymal stem cell population derived from exfoliated deciduous human teeth (SHED), capable of extensive proliferation and multipotential differentiation (Miura *et al*, 2003). The possible role for DPSCs in regeneration is demonstrated by their *in vitro* differentiation into odontoblast-like cells and deposition of mineralized deposits after treatment with dentine matrix extracts in association with a mineralization supplement of B-glycerophosphate and ascorbic acid (Liu *et al*, 2005). Identifying clear markers for such a cell population is important if we are to attempt to isolate these cells for downstream tissue engineering approaches.

Further attempts to identify a stem cell niche in the dental pulp suggested that the putative stem cell marker, STRO-1 was expressed by dental-derived stem cells using immunomagnetic activated cell selection (Shi and Gronthos, 2003; Shi *et al*, 2005). It has also been reported that DPSCs express the perivascular cell marker CD146, and a proportion of these cells also positively co-expresses α smooth muscle actin and the pericyte-associated antigen 3G5 (Gronthos *et al*, 2003; Miura *et al*, 2004). These findings concur with co-localization studies of these markers to perivascular cells *in situ*, and it is possible that a population of DPSCs may reside in this perivascular niche within the adult pulp derived from outside the tooth.

It is interesting to note that expansion cultures of rodent mature dental pulp cells gives rise to cells of myofibroblast appearance and strong expression of smooth muscle α-actin (Smith *et al*, 2005). This may be simply be due to a stronger competitive growth by myofibroblast progenitors in the cell population being cultured; however, it has been speculated that myofibroblasts are a form of 'default' differentiation as the neural crest phenotype has been suggested to be unstable, with Schwann cells able to trans-differentiate into this cell type (Real *et al*, 2005; Smith *et al*, 2005). This also raises interesting questions regarding the 'site specificity' of any primary explant of dental pulp which may contain more or less vascular tissue and therefore differing numbers of vascular-derived progenitors or myofibroblast progenitors. Should we really be isolating and culturing a mixed population of primary cells, or is it better to select cells early on the basis of surface antigens prior to culturing on?

Attempts to isolate stem cells from first branchial arch tissue, which are capable of differentiating into odontoblasts has facilitated the search for appropriate markers for these cells (Deng *et al*, 2004). The use of the cell surface marker low-affinity nerve growth factor receptor to identify possible postnatal stem/progenitor cells from mature rodent dental pulp using flow cytometry has yielded a small population of cells whose potentiality is now being examined (Smith *et al*, 2005).

It has been shown that DPSCs and SHEDs express dentin sialophosphoprotein (DSPP) in xenogenic transplants and that this expression is not present in bone formed by bone marrow stromal cells in similar transplants, suggesting that the clonogenic dental pulp-derived cells represent an undifferentiated pre-odontogenic phenotype *in vitro* (Shi *et al*, 2005). Conversely, a DPSC fraction has been shown to exhibit a degree of pluripotency (Nakashima, 2005) and bone marrow cells have also been demonstrated to have the ability to differentiate into odontoblasts cells within a developing tooth tissue engineering model (Hu *et al*, 2006). Despite a gene expression profile of human DPSCs having been compared with bone marrow stromal cells, only relatively few differentially expressed genes (including collagen XVIII α1, IGF-2cyclin-dependent kinase 6) were highly expressed in human DPSCs and there are still no specific markers for DPSCs (Shi and Gronthos, 2003). Thus, whilst it appears that there are potentially several niches of stem/progenitor cell within the dental pulp, more information is required to further understand whether all clonogenic cells are derived from a single highly proliferative pluripotent stem cell population or from committed progenitors belonging to distinct lineages.

Diversity of regenerative responses in the dentine–pulp complex

While any regenerative response in the dentine–pulp complex has been commonly referred to as tertiary dentinogenesis, examination of the appearances of the tissue responses clearly indicates that these represent a diverse spectrum ranging from a tissue structure with regular tubularity resembling physiological primary dentine to an atubular appearance displaying few morphological features characteristic of the appearance of dentine. Such diversity in tissue structure raises questions as to the dentinogenic specificity of some of these responses and suggests that the term 'tertiary dentinogenesis' is perhaps rather loosely used as a term of description.

As discussed previously, physiological tooth development involves a series of carefully orchestrated epithelial-mesenchymal interactions and this implies a high degree of regulation as initially uncommitted cells are induced to differentiate and give rise to the elegant three-dimensional structure of the tooth organ. In contrast, many of these regulatory controls may be

absent during natural tissue regeneration in the tooth and be responsible, at least in part for the diversity of the dentinogenic nature of the regenerative response after injury.

A vital epithelial cellular compartment is missing in the mature, fully erupted tooth and any recapitulation of embryonic developmental events during regeneration requires an alternative epigenetic cell-signalling source. As described earlier in this review, growth factors and other bio-active molecules sequestered in the dentine matrix may be able to substitute for the enamel organ of the tooth germ in the signalling of regeneration in the mature dentine–pulp complex if these molecules are released during injury to the tissue. However, release of such molecules from the dentine matrix is likely to proceed in an uncontrolled manner with consequent potential effects on the nature of the cellular signalling processes. Indeed, the lack of control on release of these molecules may be such that the resultant signalling may even compromise cell survival causing apoptosis during more intense injury and account for the absence of regenerative responses in rapidly progressing caries (Bjorndal, 2001). While isolated dentine matrix preparations can stimulate regeneration (Smith *et al*, 1995), higher concentrations of these preparations (Smith *et al*, 2005) and similar concentrations of TGF-β1 (He *et al*, 2005) compromise cell survival and cause apoptosis in the immortalized MDPC-23 odontoblast-like cell line. Thus, different concentrations of the same molecules can show a spectrum of effects ranging from stimulation of regeneration to cell death. Such diversity of effects on cell responses indicates the opportunities for different signalling events, possibly including variation in dentinogenic specificity.

While the molecules responsible for cellular signalling may be one of the determinants of the diversity of the dentinogenic specificity of any regenerative response, it is also probable that a major determinant will be the variety of cell populations found in the mature dental pulp which can potentially respond to the signalling molecules released after injury in the tooth.

Earlier in this review, we have discussed several different cell populations, which have been implicated in regeneration in the dentine–pulp complex including cells from the layer of Höhl (Cotton, 1968) and perivascular cells, undifferentiated mesenchymal cells and fibroblasts (Fitzgerald *et al*, 1990). Despite significant research in this area over several decades, no single cell population from the pulp has been identified as the sole progenitor for odontoblast-like cell differentiation during regeneration. It is thus easy to speculate that a number of different cell populations in the pulp are able to respond during regeneration and that this may account for some of the diversity in specificity of the dentinogenic response. Those cell populations with a similar developmental lineage to primary odontoblasts may provide a more specific dentinogenic response, whilst cells of differing origin may be more limited in their capacity to express a true odontoblastic phenotype. The definition of this phenotype is important to the functional properties of the dentine–pulp complex. A simple molecular definition of the odontoblast phenotype alone is probably insufficient and morphological features of the cells (i.e. what determines their secretory behaviour) and their extracellular matrices (i.e. the tubular matrix of dentine) are also important to distinguish how dentine differs morphologically and functionally from other mineralized tissues, such as the bone and cementum.

Potential involvement of stem/progenitor cells in dentine regeneration

It is apparent that there may be considerable heterogeneity in cell types participating in tertiary dentinogenesis, which could contribute to a variety of dentinogenic specificities in the resultant regenerative responses. The events in the post-injury pulpal environment, which lead to the involvement of any one particular cell type are likely to be complex and not easy to predict. Clearly, important factors controlling the pulpal environment will be the intensity and extent of the injurious challenge leading to the responses and level of inflammation prevailing in the pulp. Injury of sufficient intensity to cause local odontoblast death and subsequent reparative dentinogenesis also may well lead to death of cells in the cell-rich zone of Höhl, one of the putative sites of progenitor cells for repair and regeneration (Cotton, 1968), immediately beneath the affected odontoblasts. However, this may only represent a local effect and such cells may well be able to migrate from other unaffected areas in the pulp. The presence of inflammation, which will be exacerbated by bacterial infection, is well recognized as a moderator of regeneration and will probably inhibit regenerative processes as long as it is maintained (Rutherford and Gu, 2000). It is unclear whether this reflects a direct effect on the stem/progenitor cells for regeneration or the molecular signalling processes responsible for their differentiation. Nevertheless, the presence of inflammation will compromise stem/progenitor cell recruitment and differentiation during regeneration.

As mentioned previously in this review, migration of stem/progenitor cells to the site of injury for differentiation into a new generation of odontoblast-like cells will be an important event for cell recruitment during regeneration when the vitality of the primary odontoblasts is compromised. Evidence that such migration occurs is provided from the wealth of studies reporting that reparative dentinogenesis and dentine bridge formation occur during pulp capping procedures (Schröder, 1985; Murray *et al*, 2002a,b, 2002c; Tziafas, 2004). Exciting possibilities exist to exploit this aspect of regeneration, both in respect of maximizing recruitment of progenitor cells and also, perhaps through influencing the nature of the cell populations recruited. If the necessary chemotactic signals for specific cell populations can be determined, this could be harnessed for directed recruitment of those cells to provide greater specificity to the tissue response.

Local angiogenesis is a common feature during healing at all wound sites, including those in the pulp, and apart from its obvious importance in providing

nutrition during the healing process, it may also increase the opportunities for perivascular stem/progenitor cell recruitment during regeneration. The DPSCs described by Gronthos *et al* (2000, 2002) express the perivascular cell marker CD146, amongst other markers, which have been co-localized to perivascular sites in the pulp (Shi and Gronthos, 2003). Sequestration of angiogenic growth factors in dentine matrix (Roberts-Clark and Smith, 2000) and their release after injury are likely to be key to the local upregulation of angiogenesis at injury sites during healing and may indirectly contribute to the available pool of stem/progenitor cells for regeneration.

In fact, the relative size of any stem/progenitor cell population is likely to be a significant factor in determining successful outcomes for tissue regeneration. During DPSC isolation from pulp, approximately 400 CFU-F colonies were generated per 10^5 cells plated (Gronthos *et al*, 2000, 2002). Preliminary studies using affinity cell sorting have identified a potential stem/progenitor cell population in dental pulp, which comprises $<1\%$ of the total cells (Smith *et al*, 2005). It is thus likely that the size of the pool of stem/progenitor cells is relatively small unless de- or trans-differentiation of some larger pulpal cell populations, such as fibroblasts, can occur. The latter possibility is without evidence at this stage and thus, it should be assumed that relatively few cells are able to participate in a specific dentinogenic regenerative response. The smaller the population of those cells able to participate, the less likely it is that regeneration may occur. Histomorphometric analyses of pulpal cell populations with ageing indicate that some reduction in pulpal cell numbers occurs, including the sub-odontoblastic cells which may be prime candidates for participation in regeneration (Murray *et al*, 2002a,b). These observations concur with anecdotal reports that pulpal wound healing and regeneration may be compromised with increasing age.

Conclusions

Our understanding of the biology of the pulp has improved significantly in recent years and this has allowed us to present more robust hypotheses regarding the molecular and cellular processes responsible for dental regeneration. However, it is still not possible to state with any certainty which cell populations and which specific molecular signalling pathways predominate during dental regeneration, although the variety of cell populations potentially involved and many of the signalling events are becoming clearer. This is, in part, due to the fact that no one episode of regeneration will be the same. The intensity and duration of the tissue injury, the involvement of inflammatory processes and their possible exacerbation by bacteria will all impact on the tissue environment in which regeneration takes place. This environment will be further influenced by host factors, such as ageing, which will modify the cell populations present and systemic and innate immunity. A number of these factors may also influence the availability and/or the ability of cell-signalling molecules, which may be sequestered within the dentine matrix and released during injury. Therefore, it is not surprising that a diversity of tissue responses can be observed during dental regeneration as the latter represents a pathophysiological response.

It certainly raises the question as to whether the nature of the regenerative responses has biological or clinical significance. From a functional viewpoint, there may well be significance. The primary dentine matrix secreted during tooth formation has a characteristic tubular structure through which the odontoblast maintains communication with its extracellular matrix environment and which is also implicated in the transmission of sensation through the tissue. Thus, loss of this tubular structure in instances of atubular dentine regeneration would be expected to impact on tissue function. However, this may be of lesser importance clinically in some situations than providing an impermeable hard-tissue barrier to the dental pulp to prevent further bacterial ingress and consequent tissue reactions. It could be envisaged that directed regeneration might be usefully exploited for 'designer' therapies whereby stimulation of tubular dentine matrix regeneration in crown tissue restorations might be targeted, whilst in endodontic applications, where there is a need for an impermeable seal for protection of the apical/periapical tissues, atubular dentine regeneration might be stimulated. It is probable that strategies to investigate therapies directed at stimulating tubular *vs* atubular matrix formation during regeneration may well need to target both the progenitor/stem cell phenotype and the signalling processes for cellular differentiation as the dentinogenic specificity of the response may determine whether the matrix secreted during regeneration is tubular or not.

Such approaches to clinical therapies, however, require close control or regulation of the regenerative events taking place if they are to be effective. If regeneration is allowed to proceed in an uncontrolled manner, then obliteration of the pulp chamber and loss of tooth vitality will be inevitable. This raises a fundamental question about the regulation of odontoblast secretory activity during dentinogenesis, which is very pertinent to the regulation of dentine regeneration; namely, do odontoblasts have to be stimulated to secrete the dentine matrix components or is their inherent state one of active secretion and has this secretory behaviour to be turned off when not required? During secondary dentinogenesis, there is a marked downregulation of odontoblast secretory activity and it is unclear whether this is due to lack of stimulus or inhibition of secretion as the cells enter into this phase of their life cycle. Understanding of this point is critical to our future development of dental regenerative therapies and exploiting the progenitor/stem cells in the dental pulp.

If pulpal progenitor/stem cells are to be optimally harnessed for dental regeneration, then strategies are required to ensure their effective recruitment at sites of injury. Whilst clearly such recruitment does occur during natural regeneration, it is a somewhat haphazard process lacking in control. Directed recruitment of these cells might be achieved through local application of enriched populations of cells, either by harvesting cells

from non-autologous teeth or autologous shed deciduous primary teeth (Miura *et al*, 2003). Transplantation of BMP-2-treated cultured pulp cells (Iohara *et al*, 2004) and *Gdf11*-electrotransfected pulp cells (Nakashima *et al*, 2004) to surgically amputated pulps shows promise for *ex vivo* therapeutic approaches to dental regeneration, although initial tissue regenerated was osteo-dentine-like in appearance and adequate sources of autologous cells are required if immune responses to the implanted cells are to be avoided. Cells sourced from non-dental sites for implantation may also prove feasible. A c-kit$^+$-enriched bone marrow cell population has recently been reported to be capable of selecting stem/progenitor cells capable of differentiation into odontoblasts in an experimental model of tooth development (Hu *et al*, 2006). If such approaches to dental regeneration are developed to a clinical stage, tissue banks for sourcing autologous cells will become increasingly important. However, although it is tempting to speculate that such clinical therapeutics will prove successful, we are some way from taking this knowledge from bench to clinic.

We are at an exciting point of a new era of restorative dentistry harnessing the biological activity of the dental tissues to facilitate wound healing and tissue regeneration. There is still much to learn of the nature, potentiality and behaviour of dental stem/progenitor cells, but the opportunities for their exploitation in dental tissue regeneration are immense and will lead to significant benefits for the management of the effects of dental disease.

References

About I, Bottero MJ, de Denato P, Camps J, Franquin JC, Mitsiadis TA (2000). Human dentin production *in vitro*. *Exp Cell Res* **258:** 33–41.

Bègue-Kirn C, Smith AJ, Loriot M, Kupferle C, Ruch JV, Lesot H (1994). Comparative analysis of TGF betas, BMPs, IGF1, msxs, fibronectin, osteonectin and bone sialoprotein gene expression during normal and *in vitro*-induced odontoblast differentiation. *Int J Dev Biol* **38:** 405–420.

Bjorndal L (2001). Presence or absence of tertiary dentinogenesis in relation to caries progression. *Adv Dent Res* **15:** 80–83.

Buchaille R, Couble ML, Magloire H, Bleicher F (2000). A substractive PCR-based cDNA library from human odontoblast cells: identification of novel genes expressed in tooth forming cells. *Matrix Biol* **19:** 421–430.

Cassidy N, Fahey M, Prime SS, Smith AJ (1997). Comparative analysis of Transforming Growth Factor-Beta (TGF-β) isoforms 1–3 in human and rabbit dentine matrices. *Arch Oral Biol* **42:** 219–223.

Chai Y, Jiang X, Ito Y *et al*. (2000). Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* **127:** 1671–1679.

Cobourne MT, Sharpe PT (2003). Tooth and jaw: molecular mechanisms or patterning in the first branchial arch. *Arch Oral Biol* **48:** 1–14.

Cotton WR (1968). Pulp response to cavity preparation as studied by the method of thymidine 3H autoradiography. In: Finn SB, ed. *Biology of the dental pulp organ*. Univ Alabama Press: Tuscaloosa, AL, **69**.

Deng MJ, Jin Y, Shi JN *et al*. (2004). Multi-lineage differentiation of ecto-mesenchymal cells isolated from the first branchial arch. *Tiss Eng* **10:** 1597–1606.

Duailibi MT, Duailibi SE, Young CS, Bartlett JD, Vacanti JP, Yelick PC (2004). Bioengineered teeth from cultured rat tooth bud cells. *J Dent Res* **83:** 523–528.

Finkelman RD, Mohan S, Jennings JC, Taylor AK, Jepsen S, Baylink DJ (1990). Quantitation of growth factors IGF-I, SSGF/IGF-II and TGF-beta in human dentin. *J Bone Miner Res* **5:** 717–723.

Fitzgerald M, Chiego JD, Heys DR (1990). Autoradiographic analysis of odontoblast replacement following pulp exposure in primate teeth. *Arch Oral Biol* **35:** 707–715.

Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000). Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci USA* **97:** 13625–13630.

Gronthos S, Brahim J, Li W *et al*. (2002). Stem cell properties of human dental pulp stem cells. *J Dent Res* **81:** 531–535.

Gronthos S, Zannettino AC, Hay SJ *et al*. (2003). Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. *J Cell Sci* **116:** 1827–1835.

He WX, Niu ZY, Zhao S, Smith AJ (2005). Smad protein mediated transforming growth factor beta1 induction of apoptosis in the MDPC-23 odontoblast-like cell line. *Arch Oral Biol* **50:** 929–936.

Hu B, Unda F, Bopp-Kuchler S *et al*. (2006). Bone marrow cells give rise to ameloblast-like cells. *J Dent Res* **85**, 416–421.

Iohara K, Nakashima M, Ito M, Ishikawa M, Nakashima A, Akamine A (2004). Dentin regeneration by dental pulp stem cell therapy with recombinant human bone morphogenetic protein 1. *J Dent Res* **83:** 590–595.

Jernvall J, Thesleff I (2000). Reiterative signaling and patterning during mammalian tooth morphogenesis. *Mech Dev* **92:** 19–29.

Kettunen P, Karavanova I, Thesleff I (1998). Responsiveness of developing dental tissues to fibroblast growth factors: expression of splicing alternatives of FGFR1, -2, -3, and of FGFR4; and stimulation of cell proliferation by FGF-2, -4, -8, and -9. *Dev Genet* **22:** 374–385.

Lesot H, Karcher-Djuricic V, Kubler MD, Ruch JV (1988). Membrane-cytoskeleton interactions: inhibition of odontoblast differentiation by a monoclonal antibody directed against a membrane protein. *Differentiation* **37:** 62–72.

Liu J, Jin T, Ritchie HH, Smith AJ, Clarkson BH (2005). *In vitro* differentiation and mineralization of human dental pulp cells induced by dentin extract. *In Vitro Cellular Dev Biol* **41:** 232–238.

Mina M, Kollar EJ (1987). The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Arch Oral Biol* **32:** 123–127.

Miura M, Gronthos S, Zhao M *et al*. (2003). SHED: Stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci USA* **100:** 5807–5812.

Miura M, Chen XD, Allen MR *et al*. (2004). Crucial role of caspase-3 in osteogenic differentiation of bone marrow stromal stem cells. *J Clin Invest* **114:** 1704–1713.

Mooney DJ, Powell C, Piana J, Rutherford B (1996). Engineering dental pulp-like tissue *in vitro*. *Biotechnol Prog* **12:** 865–868.

Murray PE, Stanley HR, Matthews JB, Sloan AJ, Smith AJ (2002a). Ageing human odontometric analysis. *Oral Surg Oral Med Oral Pathol Oral Radiol* **93:** 474–482.

Murray PE, Matthews JB, Sloan AJ, Smith AJ (2002b). Analysis of incisor cell populations in Wistar rats of different ages. *Arch Oral Biol* **47:** 709–715.

Murray PE, Kitasako Y, Tagami J, Windsor LJ, Smith AJ (2002c). Hierarchy of variables correlated to odontoblast-like cell numbers following pulp capping. *J Dent* **30:** 297–304.

Nakashima M (1991). Establishment of primary cultures of pulp cells from bovine permanent incisors. *Arch Oral Biol* **36:** 655–663.

Nakashima M (2005). Bone morphogenetic proteins in dentine regeneration for potential use in endodontic therapy. *Cytokine Growth Factor Revs* **16:** 369–376.

Nakashima M, Nagasawa H, Yamada Y, Reddi AH (1994). Regulatory role of Transforming Growth Factor-beta, bone morphogenetic protein-2 and protein-4 on gene expression of extracellular matrix proteins and differentiation of pulp cells. *Dev. Biol* **162:** 18–28.

Nakashima M, Iohara K, Ishikawa M *et al*. (2004). Stimulation of reparative dentin formation by ex vivo gene therapy using dental pulp stem cells electrotransfected with Growth/differentiation factor11(Gdf11). *Hum Gene Ther* **15:** 1045–1053.

Ohazama A, Modino SA, Miletich I, Sharpe PT (2004). Stem cell based tissue engineering of murine teeth. *J. Dent. Res.* **83:** 518–522.

Real C, Galvieux-Pardanaud C, Vaigot P, Le-Douarin N, Dupin E (2005). The instability of the neural crest phenotypes: Schwann cells can differentiate into myofibroblasts. *Int J Dev Biol* **49:** 151–159.

Roberts-Clark D, Smith AJ (2000). Angiogenic growth factors in human dentine matrix. *Arch Oral Biol* **45:** 1013–1016.

Ruch JV (1998). Odontoblast commitment and differentiation. *Biochem Cell Biol* **76:** 923–938.

Ruch JV, Lesot H, Begue-Kirn C (1995). Odontoblast differentiation. *Int J Dev Biol* **39:** 51–68.

Rutherford RB, Gu K (2000). Treatment of inflamed ferret dental pulps with recombinant bone morphogenetic protein-7. *Eur J Oral Sci* **108:** 202–206.

Rutherford RB, Spangberg L, Tucker M, Charette M (1995). Transdentinal stimulation of reparative dentine formation by osteogenic protein-1 in monkeys. *Arch Oral Biol* **40:** 681–683.

Schröder U (1985). Effects of calcium hydroxide-containing pulp-capping agents on pulp cell migration, proliferation, and differentiation. *J Dent Res* **64:** 541–548.

Shi S, Gronthos S (2003). Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res* **18:** 696–704.

Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S (2005). The efficacy of mesenchymal stem cells to regenerate and repair dental structures Orthod. *Craniofacial Res* **8:** 191–199.

Sloan AJ, Perry H, Matthews JB, Smith AJ (2000). Transforming growth factor-β expression in mature human molar teeth. *Histochem J* **32:** 247–252.

Smith AJ, Lesot H (2001). Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? *Crit Rev Oral Biol Med* **12:** 425–437.

Smith AJ, Tobias RS, Plant CG, Browne RM, Lesot H, Ruch JV (1990). *In vivo* morphogenetic activity of dentine matrix proteins. *J Biol Buccale* **18:** 123–129.

Smith AJ, Cassidy N, Perry H, Begue-Kirn C, Ruch JV, Lesot H (1995). Reactionary dentinogenesis. *Int J Dev Biol* **39:** 273–280.

Smith AJ, Matthews JB, Hall RC (1998). Transforming Growth Factor-β1 (TGF-β1) in dentine matrix: ligand activation and receptor expression. *Eur J Oral Sci* **106:** 179.

Smith AJ, Patel M, Graham L, Sloan AJ, Cooper PR (2005). Dentine regeneration: the role of stem cells and molecular signaling. *Oral Biosci Med* **2:** 127–132.

Thesleff I, Sharpe PT (1997). Signalling networks regulating dental development. *Mech Dev* **67:** 111–123.

Tziafas D (2004). The future role of a molecular approach to pulp-dentinal regeneration. *Caries Res* **38:** 314–320.

Weissman IL (2000). Stem cells: units of development, units of regeneration, and units in evolution. *Cell* **100:** 157–168.

Yamamura T (1985). Differentiation of pulpal cells and inductive influences of various matrices with reference to pulpal wound healing. *J Dent Res* **64:** 530–540.

Yokose S, Kadokura H, Tajima Y, Fujieda K, Kateyoma I, Masuoka T, Katayama T (2000). Establishment and characterisation of a culture system for enzymatically released rat dental pulp cells. *Calcif. Tiss. Int.* **66:** 139–144.