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REVIEW

Dental pulp stem cells: what, where, how?

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Introduction. It is now accepted that progenitor/stem cells reside within the post-natal dental pulp. Studies have identified several niches of multipotent mesenchymal progenitor cells, known as dental pulp stem cells, which have a high proliferative potential for self-renewal. These progenitor stem cells are now recognized as being vital to the dentine regeneration process following injury. Understanding the nature of these progenitor/stem cell populations in the pulp is important in determining their potentialities and development of isolation or recruitment strategies for use in regeneration and tissue engineering. Characterization of these cells, and determination of their potentialities in terms of specificity of regenerative response, may help direct new clinical treatment modalities. Such novel treatments may involve controlled direct recruitment of the cells *in situ* and possible 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 may provide an innovative and novel biologically based new generation of clinical materials and/or treatments for dental disease.

Aim. This study aimed to review the body of knowledge relating to stem cells and to consider the possibility of these cell populations, and related technology, in future clinical applications.

Introduction

The dentine–pulp complex has a natural regenerative potential leading to the formation of tertiary dentine. Odontoblasts may survive mild injury, such as attrition or early caries, and secrete a reactionary dentine matrix[1,2]. However, trauma of greater intensity, such as advanced caries or restorative procedures, may lead to the death of the pre-existing odontoblasts[1,2]. In response to stimuli at the dentine–pulp interface, new odontoblasts are recruited and differentiate at the site of injury to synthesize an atubular reparative dentine, also sometimes referred to as osteodentine. This reparative dentine provides a 'bridge' of mineralized tissue immediately below the extensively damaged tissue, in order to preserve pulp vitality.

A growing number of studies have now indicated that the post-natal pulp contains several niches of potential progenitor/stem cells, which may have importance in mediating reparative dentine formation. Indeed, progenitor/stem cell niches are continually being identified in all connective tissues of the body, where they play a fundamental role in wound repair processes. This subset of undifferentiated cells can represent as little as 1% of the total cell population. However, they produce multiple highly differentiated progeny in response to specific extracellular signals[3].

Central to the niche is the 'true' adult or 'mother' stem cell which displays an infrequent, yet almost unlimited self-renewal[3]. At mitosis, these cells give rise to a renewed mother stem cell and a daughter transit amplifying progenitor cell. These daughter progenitor cells possess a more limited capacity for self-renewal, but are highly proliferative. They also appear to control multi-potentiality, and are capable of following along several cell lineages to ultimately produce terminally differentiated cells such as osteoblasts, odontoblasts, adipocytes, chondrocytes, and neural cells (Fig. 1). Within these progenitor/stem cell niches, cell–cell and cell–matrix communication is important in maintaining the status of the stem cell, whereas

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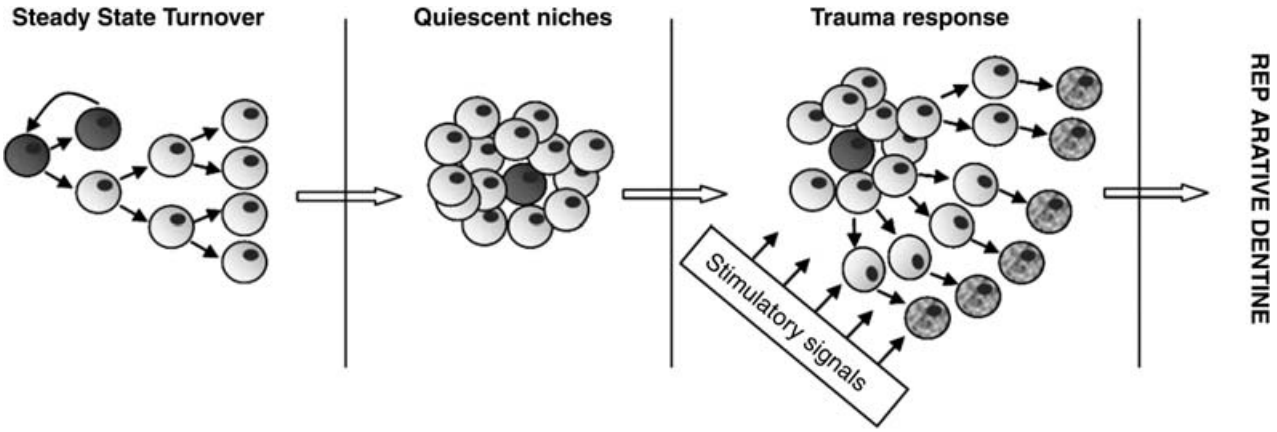

Fig. 1. The stem cell niche consists of both the 'true' adult stem cell surrounded by the transit amplifying progenitor cells. The 'true' adult stem cell has the capacity for infrequent, but almost unlimited self-renewal, whereas the daughter transit amplifying cells are highly proliferative and are capable of differentiating along several cell lineages to produce the desired differentiated cell upon stimulation.

their responsiveness to external stimuli provides a dynamic system for tissue repair[4].

Stem cell niches in dental pulp

Within healthy tissues, the progenitor/stem cell niches usually maintain a quiescent state[3,5], due to the influence of the environment in which they are found. Injury or trauma, leading to death of the post-mitotic odontoblasts within the dentine matrix, stimulates a cascade of complex and as yet unclarified events, whereby signals are released into the matrix. These signals then cause the progenitor/stem cell population to produce a high proliferative activity and the generation of the terminally differentiated odontoblast cell. The identification of progenitor/stem cell niches is thus best observed *in situ* following their activation in response to injury. Studies performed during the 1980s first indicated that the replacement odontoblasts, which synthesized reparative dentine, are derived from undifferentiated mesenchymal cells in the pulp proper[6–9]. Labelling of cells with tritiated thymidine, following pulp capping, showed an initial proliferation of cells in a deep pulpal area below the site of injury, followed by an apparent migration of these cells to the wound site where they undergo further proliferation and differentiation to functional odontoblast-like cells[6]. The source of these replacement odontoblast-like cells (progenitor/stem cell niches) was, and perhaps still, is a subject of some debate. The early studies of Fitzgerald and co-workers[6] proposed that these progenitor cells were derived from a population of fibroblast-like cells. The investigators, however, also noted a potential contribution from daughter cells derived from the perivascular cell population. More recent studies monitoring *ex vivo* BrdU uptake by proliferative cells in response to injury have suggested that the progenitor/stem cell niches reside predominately in the perivascular regions of the pulpal cavity, from where they migrate to the site of injury[10]. However, determination of stem/progenitor cell niches, as determined by elevation in Notch expression, following pulpal injury *in vivo* has proposed niches additional to the perivascular niche[11].

Notch is purported to be an important signalling molecule which controls stem cell fate. A recent study in rats explored Notch expression following pulp capping[11]. It was found that 1–3 days following pulp capping, Notch 1 expression was increased in the odontoblast–sub-odontoblast layers; Notch 2 expression increased in the pulpal stroma, whereas Notch 1 and 3 increased in cells associated with the perivascular structures[11]. This finding suggests that progenitor/stem cell niches reside in different locations throughout the pulpal tissue (Fig. 2). But the responsiveness of each of the progenitor/stem cell niches to injury would appear to vary according to location.

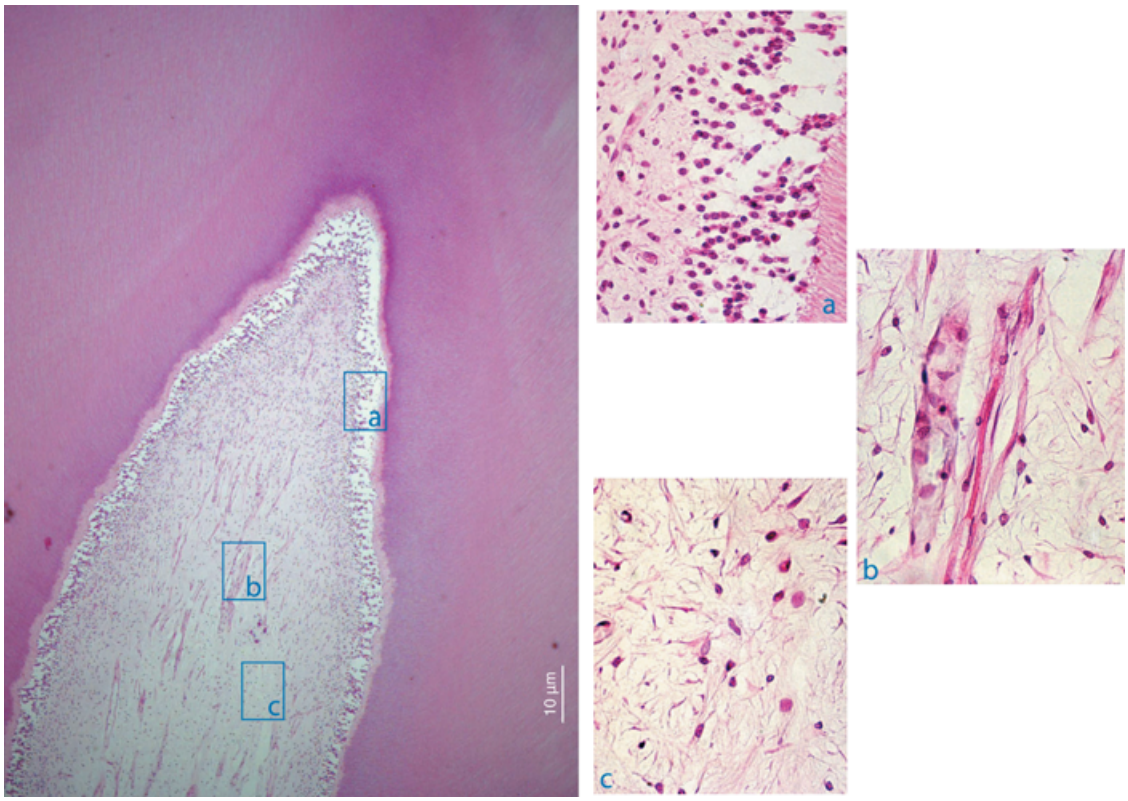

Fig. 2. Several stem/progenitor cell niches may exist in the mature dental pulp. These include (a) undifferentiated mesenchymal cells (so-called sub-odontoblasts) residing in the cell-rich layer close to the existing post-mitotic odontoblasts; (b) a perivascular cell population associated with the pulpal vasculature; and (c) a Notch-2 positive cell population within the central pulpal stroma.

Isolation and characterization of stem cell populations in dental pulp

The identification and isolation of an odontogenic progenitor population in adult dental pulp were first reported by Gronthos and co-workers in 2000[12]. This group described the identification of dental pulp stem cells (DPSCs) by virtue of their clonogenic abilities, rapid proliferative rates, and capacity to form mineralized tissues both *in vitro* and *in vivo*. Subsequent studies isolated single cell colony-derived populations of DPSCs which demonstrated multi-potentiality. This meant they were able to form adipocytes and neural precursors *in vitro*, in addition to dentin-like tissue following transplantation into immunocompromised mice[13]. These studies also demonstrated differences in the odontogenic potential of the single colony-derived populations, with only 67% of cells found capable of forming abundant ectopic dentine *in vivo*[13]. This may suggest the existence of differing progenitor/stem cell niches within dental pulp, which, although highly proliferative, display a hierarchy for cellular differentiation and multi-potentiality.

The cellular characteristics of these DPSCs have been compared with those of bone marrow stem cells. Both dental pulp and bone marrow stem cell populations express similar putative stem cell surface markers, including CD44, CD106, CD146, 3G5, and Stro-1[14]. They both expressed matrix proteins associated with mineral tissue formation, such as alkaline phosphatase, osteocalcin, and osteopontin. Similar expression patterns were also noted for stem cells isolated from periodontal ligament[14]. However, in contrast to bone marrow stem cells, DPSCs have been shown to maintain a 30% higher proliferation rate and a higher growth potential[14]. This higher rate of proliferation has been linked to the increased pulp cell expression of specific cell cycling mediators, namely cyclin-dependant kinase 6 and insulin-like growth factor[15]. Transplantation of DPSCs

into immunocompromised mice resulted in the formation of a dentine-like tissue, whereas bone marrow stem cells produced a tissue resembling that of lamellar bone[12]. This suggests that inherently different regulatory mechanisms exist within the two stem cell populations, evidenced by the elevated synthesis of dentine-specific proteins during the formation of the dentine tissue at the cell transplant sites[16].

Various attempts have subsequently been made to isolate and characterize progenitor/stem cell populations from adult dental pulp, with the intention of achieving a more defined clonal population of cells. A mesenchymal stem progenitor population, expressing the cell surface receptor Stro-1, has been isolated from adult dental pulp[17–20]. The isolation strategy was similar to those previously used for the isolation of bone marrow stem cells[21]. However, the presence of clonogenic cells, with high proliferative capacity, is reported to be much less common in dental pulp compared to bone marrow[17]. These Stro-1 positive cells were found to differentiate down neurogenic, adipogenic, myogenic, and chondrogenic lineages[18], and make a mineralized matrix when cultured in 'odontogenic'-inducing conditions[19]. When cells isolated for Stro-1 were compared with those which were negative for this mesenchymal stem cell marker, only Stro-1 positive cells were capable of differentiating into odontoblast-like cells, indicating the importance of these cells in dentine repair processes[20].

In alternative strategies, subsets of cell populations with progenitor/stem cell characteristics have been selected utilizing the haematopoietic/endothelial marker CD34 and putative stem cell proto-oncogene marker c-kit[22,23]. By virtue of their infrequent, albeit unlimited self-renewal, attempts have been made to preferentially isolate the 'true' or 'mother' adult stem cell from porcine dental pulp, by selecting cells which fail to incorporate the DNA binding dye Hoechst 33342 during cell division[24]. A long proliferative lifespan has been confirmed for these cells, as has their ability to form reparative dentine following autologous transplantation. Primitive cells express high levels of ß1 integrins, which may assist in the constrained localization of stem cell populations through interaction with matrix proteins such as fibronectin[25,26]. This observation has recently been utilized to obtain a highly proliferative, mesenchymal progenitor population with multi-potentiality from rat dental pulp[27]. Cells expressing the neural crest cell markers of the Eph family have also been localized in mature dental pulp, suggesting these embryonic cells persist in the post-natal tissue[28]. Through the selection of cells expressing the neural crest-associated low-affinity nerve growth factor receptor (LANGFR), a small population (less than 1%) has been isolated from mature dental pulp[29]. Subsequent characterization of this cell population, by the authors of this review, has shown the expression of several putative stem cell markers and an ability to differentiate down adipocytic, chondrocytic, and osteogenic lineages[27].

Hierarchical ordering of stem cell niches

In view of the known hierarchical order of stem cells in dental pulp, the utilization of different isolation strategies could potentially lead to the isolation of different progenitor/stem cell populations, which reside in different niches throughout the pulpal tissue (Fig. 2). At present, work in this field is in its infancy, and the phenotypic analysis in terms of putative stem cell markers expressed by the various progenitor/stem cell populations is incomplete. Preliminary data have recently been presented which directly compare the expression of several putative stem cell markers of two progenitor cell populations isolated utilizing different stem cell characteristics. These have included LANGFR, which is found on the cell surface of neural crest cells, and ß1 integrin, which is highly expressed by primitive cells[27]. The results provisionally suggest that more than one progenitor/stem cell population exists within mature dental pulp, which differ in their embryonic status, one of neural crest character and one of mesenchymal origin[27].

If more than one progenitor stem cell population exists in mature dental pulp, then this may provide an explanation for the observed differences in odontogenic responses following transplantation of single-cell clonal populations[13]. It may also explain differences in proliferative responses[4,10] and patterns in Notch signalling[11] in response to trauma. The possibility that

more than one progenitor stem cell population may exist in mature dental pulp is also supported by the finding that stem cell populations in other tissues, such as primary tooth pulp and periodontal ligament, have differing genotypic and protein expression patterns[14].

Comparison of human dental-derived progenitor cells

It is now well established that bone marrow stromal cells (BMSCs) have the ability to form adherent colonies when plated at reasonably low densities in supplemented growth medium[30]. Studies have demonstrated that each colony is originally derived from the clonal expansion of a single progenitor cell[31]. In addition to these, and stem cell populations derived from adult dental pulp, other so-called mesenchymal stem cell populations have been recently identified from human exfoliated deciduous teeth (SHED cells), and adult periodontal ligament (PDLSCs) by their ability to generate clonogenic adherent colonies when grown and expanded under the same conditions as the BMSCs[12,14,32]. Comparison of dental pulp, SHEDs, PDLSCs, and BMSCs has demonstrated that DPSC, SHED, and PDLSCs maintain a higher growth potential compared with BMSCs. However, there are reported differences in their protein and gene expression profile. Interestingly, the SHED cells are distinct from DPSCs by virtue of their higher proliferation rate, increased cell population doublings, and osteoinductive capacity *in vivo*[14,33]. In addition to this, although SHED cells are able to differentiate into putative odontoblasts, and are immunoreactive to dentine sialophosphoprotein, they failed to reconstitute a complete dentine–pulp complex which was observed with DPSCs. Such data suggest that SHED cells are distinctly different from DPSCs in regard to odontogenic differentiation and osteogenic induction. It is therefore possible that these SHED cells may represent a population of multipotent stem cells that are more immature than the DPSCs obtained from adult teeth. Although these SHED cells may contain stem/progenitor cells, they may not be a single-cell type, and it is now thought that they may well be a heterogeneous population of cells from the pulp[12,13,34].

Stem/progenitor cells in dentine regeneration and novel therapeutics

The identification and characterization of stem/progenitor cells within the dental and craniofacial tissues are now directing applied research into the use of such cells in clinical treatments. Such novel treatment modalities, however, will not be easy to develop due to the complex natural regenerative processes within the injured pulp. It is well understood that uncontrolled inflammation within the pulpal environment negates any natural capacity for repair[35]. This inflammatory response and its progression are an important consideration in harnessing the reparative potential of the tooth and its progenitor cells. Severe injury may lead to odontoblast cell death at specific foci directly beneath the area of injury, and the reparative dentinogenic response will have implications for the survival of the sub-odontoblasts within the underlying cell-rich zone[36], possibly one of the stem cell niches outlined earlier in this review. The ability of such cells to migrate to areas of injury from other niches in unaffected areas of the pulp, in order to replace damaged/lost cells, remains an area of ongoing enquiry[10]. The potent mix of bacterial infection and subsequent inflammation will undoubtedly moderate the regenerative process as mentioned earlier. However, it is still unclear how these inflammatory processes effect stem/progenitor cells or the molecular signalling processes responsible for their differentiation.

The utilization of post-natal stem cells in clinical applications may be best served by developing materials that stimulate migration of stem/progenitor cells to the site of injury. These would then differentiate into a new generation of odontoblast-like cells when the vitality of the existing post-mitotic odontoblasts is compromised by carious injury or trauma. There is good evidence that such migration occurs in response to pulp capping procedures, with subsequent reparative dentinogenesis and dentine bridge formation[37–39]. The recruitment of a progenitor cell population to differentiate into a new generation of odontoblast-like cells, leading to reparative dentinogenesis, is a natural reparative response of the dentine–pulp

complex. The finding that dentine bridge formation is stimulated by calcium hydroxide indicates that this process may be exploited clinically[38,40]. It is tempting to consider the exciting possibilities of clinically directing this aspect of natural regeneration, both by maximizing recruitment of progenitor cells to areas of injury and disease and also through influencing the nature of the cell populations recruited. If the specific chemotactic signals for these cell populations can be determined, this could be harnessed for directed recruitment of these cells, to provide greater specificity and control to the tissue response. It is now clear that growth factors, sequestered within the dentine matrix, influence and direct the processes of reactionary and reparative dentinogenesis[41–43]. With increasing understanding of pulpal regeneration in recent years, we have been able to suggest more robust hypotheses regarding the molecular and cellular processes responsible for dental regeneration and subsequent novel clinical therapies[44–47].

Although recruitment of progenitor cells to sites of injury occurs naturally, it may be considered random (given the potential environment of the compromised pulp) and uncontrolled. Although it may be more relevant to try to harness and exploit this natural process, it has been suggested that directed recruitment of progenitor cells might be achieved through local application of enriched populations of cells, either by harvesting cells from non-autologous teeth or autologous exfoliated primary teeth[33]. BMP-2-treated cultured pulp cells[24] and *Gdf11*-electrotransfected pulp cells[48] have been successfully transplanted to surgically amputated pulps, suggesting a possible therapeutic approach to dental regeneration. Interestingly, the initial regenerated tissue exhibits an osteo-dentine-like appearance, similar to that of reparative dentine (atubular) rather than tubular dentine. It is worth considering that this may be of benefit in dentine bridge formation where an atubular dentine would provide a more effective barrier to bacterial progression during any further carious challenge. However, this may not be entirely feasible due to the difficulties of obtaining adequate sources and volume of autologous cells to reduce any immune response to the cell transplant. In order to improve yield, cells sourced from non-dental sites have been explored and have been found to be capable of differentiation into odontoblasts in an experimental model system[49]. If such approaches to dental regeneration are to be explored further, tissue banks to source autologous cells will be required. This raises inevitable questions regarding clinical feasibility and cost. Are such approaches justified for vital pulp therapy or should we focus our attention on understanding the role played by these progenitor cell populations during natural tissue regeneration and exploit these clinically *in situ*?

In addition to reparative dentinogenesis, the success of vital pulp therapy also depends on an adequate vascular supply. Local angiogenesis is essential during healing at all wound sites, and pulpal injury and healing are no different. In addition to providing nutrition during the healing process, a good blood supply may also benefit the perivascular progenitor cell niche by increasing perivascular progenitor cell recruitment during regeneration. As discussed earlier, populations of DPSCs express the perivascular cell marker CD146, among other markers[12,13], which are co-localized to perivascular sites in the pulp[17]. It is now known that angiogenic growth factors are present among the cocktail of growth factors found within the mature dentine matrix[50]. It would appear that their release after injury may be key to the local up-regulation of angiogenesis at sites of trauma and, thereby indirectly, influence the pool progenitor cells for regeneration.

Engineering a whole tooth

Although the concept of engineering a whole tooth offers exciting potential and has been shown to be potentially feasible in controlled *in vivo* animal models[51–53], significant clinical challenges remain. Issues surrounding the control of tooth shape, size, availability of dental epithelium, growth, and eruption of an engineering bio-tooth have yet to be resolved[54]. Investigations are currently in progress to develop methods to engineer a whole tooth and dental pulps. These range from tissue recombination studies to more complex scaffold-based

engineering strategies, assembly of different bioengineered components, novel cell pellet engineering, chimeric tooth engineering, and gene-manipulated tooth regeneration[51–53,55–61]. Although a discussion regarding these methodologies is beyond the scope of this review, it is worth noting that recent advances in stem cell biology and tissue engineering have highlighted the possibilities for tooth reconstruction. Future investigations will no doubt focus on the enrichment of dental progenitor cells and bioprocessing of such cells for clinical use. However, the technical obstacles associated with this invariably question whether stem cell-based whole tooth engineering can be successfully translated into clinical strategies.

Dental pulp stem cells and ageing

Histomorphometric analyses of pulpal cell populations indicate that age-related reductions in pulpal cell numbers occur. This includes sub-odontoblastic cells which may be a potential progenitor cell niche[62,63]. This laboratory finding would support clinical observations that pulpal wound healing and regeneration may be compromised with increasing age. Preliminary studies have identified a potential progenitor cell population in dental pulp, which comprises less than 1% of the total cells[27,64]. It is thus likely that the size of the pool of stem/progenitor cells is relatively small and that a relatively low number of cells are able to participate in specific dentinogenic regenerative responses. Taken together, these factors raise significant issues regarding the development of novel therapeutics to target a progenitor cell population. Are such progenitor cell niches exhaustive? Are they replenished via the vasculature in sufficient numbers? Do the specific populations reduce in number at different rates, hence influencing the specificity of regenerative response? Would novel biomimetic materials be as effective in older patients or is there a window of opportunity for biologically mediated vital pulp therapy linked to age? In essence, the smaller the population of those cells able to participate, the less likely it is that novel clinical treatments will be effective in harnessing their potential to stimulate regeneration. It is speculated that biomimetic materials or 'biofillings' may be effective in stimulating natural tissue repair and influencing the effectiveness and lifespan of restorations, and maintaining the vitality of the compromised pulp. The stage of dental development, however, may have important relevance when predicting the likely success of such novel biological treatment modalities.

Conclusions

Regeneration of the dental tissues provides an attractive alternative to more traditional restorative approaches because the diseased tissue is replaced by natural tissue, which forms an integral part of the tooth. Novel methods based on progenitor cell recruitment and subsequent stimulation offer considerable potential to significantly impact on dental disease treatment and the promotion of vital pulp therapy. The development of such approaches, however, requires precise regulation of the regenerative events if they are to be effective. If uncontrolled, obliteration of the pulp chamber will arise with inevitable loss of tooth vitality. An understanding of this issue is critical to our future development of any dental regenerative therapies based on exploitation of the progenitor cells in the dental pulp. It is tempting to speculate that such novel clinical treatments will come to fruition, but we are some way from taking this knowledge from laboratory to clinic. We clearly have an opportunity to move restorative dentistry into a new era, harnessing the biological activity of the dental tissues to facilitate wound healing and tissue regeneration. Indeed, current moves towards isolation, collection, and cryopreservation of dental pulp progenitor cells for banking and clinical are now feasible and commercially possible[65]. However, we must approach the challenges with careful consideration. We are still some distance from fully understanding the potentiality and behaviour of dental pulp progenitor cells, and subsequent clinical treatment modalities. Nonetheless, the opportunities for their exploitation in dental tissue regeneration are becoming clearer and will lead to significant benefits in the management of the effects of dental disease.

What this paper adds
- This paper provides an overview of recent developments in the field of DPSC research.
- New knowledge relating to the mechanisms underlying reparative dentinogenesis is explored and the potential for therapeutic manipulation is considered.

Why this paper is important to paediatric dentists
- We live in an era of rapid biomedical advances, and it is important that paediatric dentists keep informed of technologies that may have relevance to their clinical practice.
- Stem cell research may provide possibilities for tissue regeneration in the compromised dentition, and clinicians should be aware of this line of enquiry.
- Identification of progenitor/stem cell lines in exfoliated primary teeth may be of particular interest to the paediatric dentist.

References

1 Arana-Chavez VE, Massa LF. Odontoblasts: the cells forming and maintaining dentine. *Int J Biochem Cell Biol* 2004; **36**: 1367–1373.
2 Smith AJ, Cassidy N, Perry H, Begue-Kirn C, Ruch J-V, Lesot H. Reactionary dentinogenesis. *Int J Dev Biol* 1995; **39**: 273–280.
3 Stocum DL. Stem cells in regenerative biology and medicine. *Wound Repair Regen* 2001; **9**: 429–442.
4 Scadden DT. The stem-cell niche as an entity of action. *Nature* 2006; **441**: 1075–1079.
5 Nishikawa S-I, Osawa M. Generating quiescent stem cells. *Pigment Cell Res* 2007; **20**: 263–270.
6 Fitzgerald M, Chiego JD, Heys DR. Autoradiographic analysis of odontoblast replacement following pulp exposure in primate teeth. *Arch Oral Biol* 1990; **35**: 707–715.
7 Tziafas D, Kolkuris I. Inductive influences of demineralized dentin and bone matrix on pulp cells: an approach of secondary dentinogenesis. *J Dent Res* 1990; **69**: 75–81.
8 Yamamura T. Differentiation of pulpal cells and inductive influences of various matrices with reference to pulpal wound healing. *J Dent Res* 1985; **64**: 530–540.
9 Yamamura T, Shimono M, Koike H, *et al*. Differentiation and induction of undifferentiated mesenchymal cells in tooth and periodontal tissue during wound healing and regeneration. *Bull Tokyo Dent Coll* 1980; **21**: 181–221.
10 Tecles O, Laurent P, Zygouritsas S, *et al*. Activation of human dental pulp progenitor/stem cells in response to odontoblast injury. *Arch Oral Biol* 2005; **50**: 103–108.
11 Lovschall H, Tummers M, Thesleff I, Fuchtbauer EM, Poulsen K. Activation of the Notch signalling pathway in response to pulp capping of rat molars. *Eur J Oral Sci* 2005; **113**: 312–317.
12 Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci USA* 2000; **97**: 13625–13630.
13 Gronthos S, Brahim J, Li W, *et al*. Stem cell properties of human dental pulp stem cells. *J Dent Res* 2002; **81**: 531–535.
14 Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. *Orthod Craniofac Res* 2005; **8**: 191–199.
15 Shi S, Robey PG, Gronthos S. Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. *Bone* 2001; **29**: 532–539.
16 Batouli S, Miura M, Brahim J, *et al*. Comparison of stem-cell-mediated osteogenesis and dentinogenesis. *J Dent Res* 2003; **82**: 976–981.
17 Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res* 2003; **18**: 696–704.
18 Yang X, Zhang W, van den Dolder J, *et al*. Multilineage potential of STRO-1+ rat dental pulp cells *in vitro*. *J Tissue Eng Regen Med* 2007; **1**: 128–135.
19 Yang X, Walboomers XF, van den Beucken JJ, Bian Z, Fan M, Jansen JA. Hard tissue formation of STRO-1-selected rat dental pulp stem cells *in vivo*. *Tissue Eng Part A* 2008; Epub ahead of print.
20 Yang X, van den Dolder J, Walboomers XF, *et al*. The odontogenic potential of STRO-1 sorted rat dental pulp stem cells *in vitro*. *J Tissue Eng Regen Med* 2007; **1**: 66–73.
21 Gronthos S, Graves SE, Ohta S, Simmons PJ. The STRO-1+ fraction of adult human bone marrow contains the osteogenic precursors. *Blood* 1994; **84**: 4164–4173.
22 Laino G, d'Aquino R, Graziano A, *et al*. A new population of human adult dental pulp stem cells: a useful source of living autologous fibrous bone tissue (LAB). *J Bone Miner Res* 2005; **20**: 1394–1402.
23 Laino G, Carinci F, Graziano A, *et al*. *In vitro* bone production using stem cells derived from human dental pulp. *J Craniofac Surg* 2006; **17**: 511–515.
24 Iohara K, Zheng L, Ito M, Tomokiyo A, Matsushita K, Nakashima M. Side population cells isolated from porcine dental pulp tissue with self-renewal and multipotency for dentinogenesis, chondrogenesis, adipogenesis, and neurogenesis. *Stem Cells* 2006; **24**: 2493–2503.
25 Jones PH, Watt FM. Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. *Cell* 1993; **73**: 713–724.
26 Jensen UB, Lowell S, Watt FM. The spatial relationship between stem cells and their progeny in the basal layer of human epidermis: a new view based on whole-mount labelling and lineage analysis. *Development* 1999; **126**: 2409–2418.
27 Waddington RJ, Youde SJ, Chi PL, Sloan AJ. Isolation of distinct progenitor stem cell populations from dental pulp. *Cells, Tissues, Organs* 2008; EPub ahead of print.

28 Stokowski A, Shi S, Sun T, Bartold PM, Koblar SA, Gronthos S. EphB/Ephrin-B interaction mediates adult stem cell attachment, spreading and migration: implications for dental tissue repair. *Stem Cells* 2007; **25**: 156–164.

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

30 Castro-Malaspina H, Gay RE, Resnick G, Kapoor N, Meyers P, Chiarieri D. Characterisation of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. *Blood* 1980; **56**: 289–301.

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

32 Seo BM, Miura M, Gronthos S, *et al*. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004; **364**: 149–155.

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

34 Liu H, Gronthos S, Shi S. Dental pulp stem cells. *Methods Enzymol* 2006; **419**: 99–113.

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

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

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

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

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

40 Graham L, Cooper PR, Cassidy N, Nor JE, Sloan AJ, Smith AJ. The effect of calcium hydroxide on solubilisation of bio-active dentine matrix components. *Biomaterials* 2006; **27**: 2865–2873.

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

42 Zhao S, Sloan AJ, Murray PE, Lumley PJ, Smith AJ. Ultrastructural localisation of TGF-beta exposure in dentine by chemical treatment. *Histochem J* 2000; **32**: 489–494.

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

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

45 Trope M. Regenerative potential of dental pulp. *J Endod 2008* 2008; **34**: S13–S17.

46 Nör J. Tooth regeneration in operative dentistry. *Oper Dent* 2006; **31**: 633–642.

47 Sloan AJ, Smith AJ. Stem cells and the dental pulp: potential roles in dentine regeneration and repair. *Oral Dis* 2007; **13**: 151–157.

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

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

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

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

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

53 Duailibi SE, Duailibi MT, Zhang W, Asrican R, Vacanti JP, Yelick PC. Bioengineering dental tissues grown in the rat jaw. *J Dent Res* 2008; **87**: 745–750.

54 Yu J, Shi J, Jin Y. Current approaches and challenges in making a bio-tooth. *Tissue Eng* 2008; **14**: 307–319.

55 Young CS, Terada S, Vacant JP, Honda M, Bartlett JD, Yelick PC. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. *J Dent Res* 2002; **81**: 695–700.

56 Yu JH, Deng ZH, Shi JN, *et al*. Differentiation of dental pulp stem cells into regular shaped dentin–pulp complex induced by tooth germ cell conditioned medium. *Tissue Eng* 2006; **12**: 3097–3105.

57 Young CS, Abukawa H, Asrican R, *et al*. Tissue engineering hybrid tooth and bone. *Tissue Eng* 2005; **11**: 1599–1610.

58 Yu JH, Shi JN, Deng ZH, *et al*. Cell pellets from dental papillae can reexhibit dental morphogenesis and dentinogenesis. *Biochem Biophys Res Commun* 2006; **346**: 116–124.

59 Edwards PC, Mason JM. Gene enhanced tissue engineering for dental hard tissue regeneration: (1) overview and practical considerations. *Head Face Med* 2006; **2**: 12.

60 Edwards PC, Mason JM. Gene enhanced tissue engineering for dental hard tissue regeneration: (2) dentin–pulp and periodontal regeneration. *Head Face Med* 2006; **2**: 12.

61 Cordeiro MM, Dong Z, Kaneko T, *et al*. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. *J Endod* 2008; **34**: 962–969.

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

63 Murray PE, Matthews JB, Sloan AJ, Smith AJ. Analysis of incisor cell populations in Wistar rats of different ages. *Arch Oral Biol* 2002b; **47**: 709–715.
64 Smith AJ, Patel M, Graham L, Sloan AJ, Cooper PR. Dentine regeneration: the role of stem cells and molecular signalling. *Oral Biosci Med* 2005; **2**: 127–132.
65 Perry BC, Zhou D, Wu X, *et al*. Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. *Tissue Eng* 2008; **14**: 149–156.