REVIEW

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Stem cells and periodontal regeneration

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ABSTRACT

Periodontitis is an inflammatory disease which manifests clinically as loss of supporting periodontal tissues including periodontal ligament and alveolar bone. For decades periodontists have sought ways to repair the damage which occurs during periodontitis. This has included the use of a range of surgical procedures, the use of a variety of grafting materials and growth factors, and the use of barrier membranes. To date periodontal regeneration is considered to be biologically possible but clinically unpredictable. Recently, reports have begun to emerge demonstrating that populations of adult stem cells reside in the periodontal ligament of humans and other animals. This opens the way for new cell-based therapies for periodontal regeneration. For this to become a reality a thorough understanding of adult human stem cells is needed. This review provides an overview of adult human stem cells and their potential use in periodontal regeneration.

Key words: Mesenchymal stem cells, periodontal ligament stem cells, periodontal regeneration.

Abbreviations and acronyms: BMP = bone morphogenetic proteins; BMP-7 = bone morphogenetic protein-7; BMSSCs = bone marrow stromal stem cells; EGF = epidermal growth factor; ePTFE = expanded polytetrafluoroethylene; ES = embryonic stem; FGF = fibroblast growth factor; IGF = insulin-like growth factor; HA/TCP = hydroxyapatite/tricalcium phosphate ceramic; MHC = major histocompatibility; MSCs = mesenchymal stem cells; PDGF = platelet-derived growth factor; PDLSCs = periodontal ligament stem cells.

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INTRODUCTION

Periodontitis is a disease of the periodontium characterized by irreversible loss of connective tissue attachment and supporting alveolar bone.[1] These changes often lead to an aesthetically and functionally compromised dentition. For many decades, periodontists have been interested in regenerating tissues destroyed by periodontitis. Periodontal regeneration can be defined as the complete restoration of the lost tissues to their original architecture and function by recapitulating the crucial wound healing events associated with their development.[2] Conventional open flap debridement falls short of regenerating tissues destroyed by the disease,[3,4] and current regenerative procedures offer a limited potential towards attaining complete periodontal restoration.[5–9] Recently, the isolation of adult stem cells from human periodontal ligament has presented new opportunities for tissue engineering.[10,11] Clearly, in order for such therapies to be successful, a thorough understanding of stem cells and their role in regenerating periodontal tissues is required.

The aim of this review is to discuss the current state of our understanding of adult human stem cells in dental tissues and their potential application in regenerative periodontal therapy. Current regenerative procedures, in particular guided tissue regeneration, are critically assessed. Furthermore, potential clinical implications of dental stem cells as well as the challenges for further research are also highlighted.

Definition and types of stem cells

The term ''stem cell'' first appeared in the literature during the 19th century. Like many other terms in biology, the concept of a stem cell has expanded greatly with identification of novel sites and functions. A ''stem cell'' refers to a clonogenic, undifferentiated cell that is capable of self-renewal and multi-lineage differentiation.[12] In other words, a stem cell is capable of propagating and generating additional stem cells, while some of its progeny can differentiate and commit to maturation along multiple lineages giving rise to a range of specialized cell types. Depending on intrinsic signals modulated by extrinsic factors in the stem cell niche, these cells may either undergo prolonged self-renewal or differentiation.[13] A pluripotent stem cell can give rise to cell types from all three

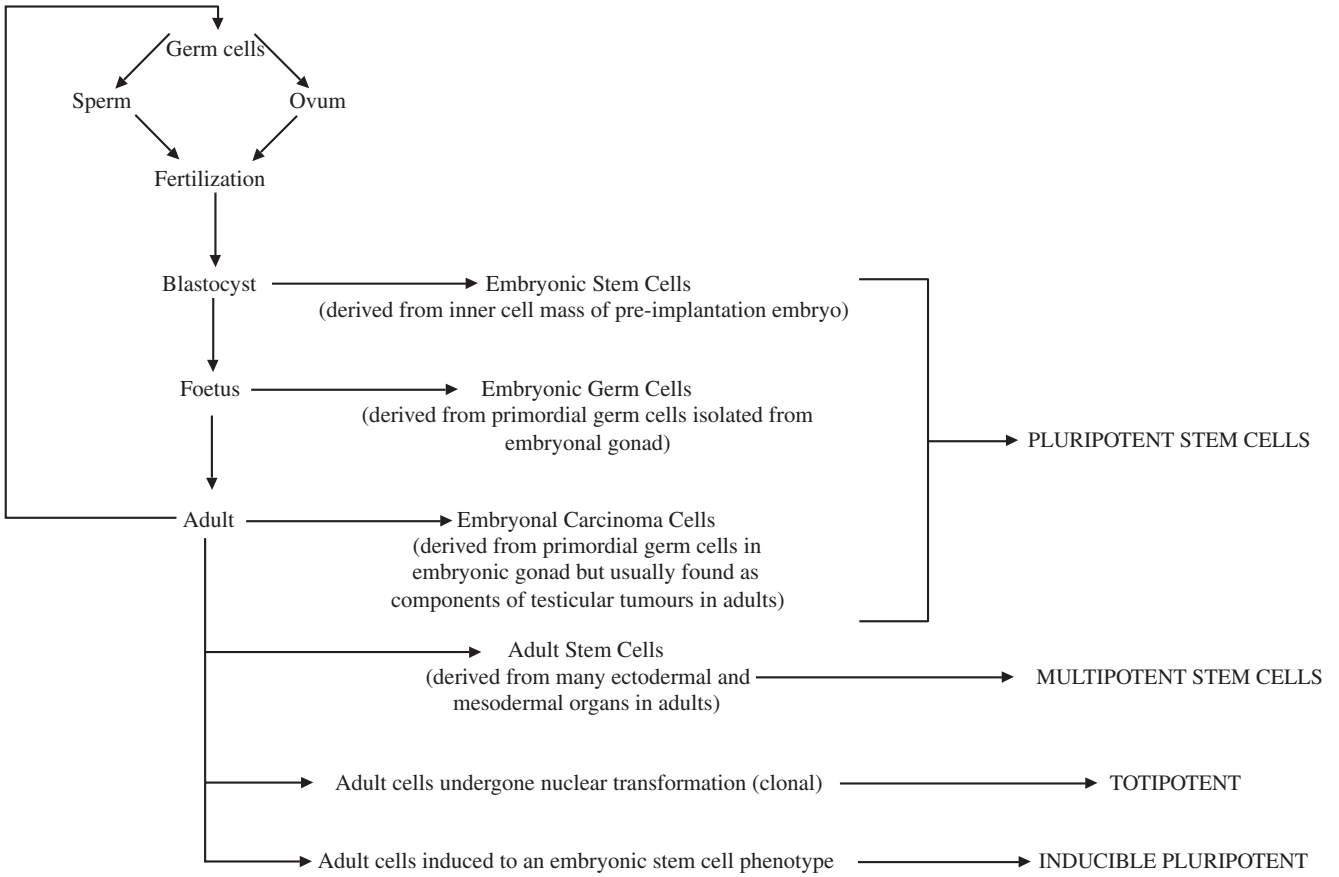

Fig 1. Sources and derivation of stem cell populations. Depending on the site, stage of development or cell culture induction environment human stem cells can be classified as being of pluripotent, multipotent, totipotent or inducible pluripotent potential.

germ layers of the body (i.e., ectoderm, mesoderm and endoderm) whereas a multipotent stem cell can produce cell types from more than one (but not all) lineages. Descriptive and experimental studies support the notion that stem cells exist in both embryonic and adult tissues.[14] To date, six types of stem cells have been isolated in humans[15–17] and these are depicted in Figure 1.

Embryonic stem cells

In 1998, Thomson and co-workers derived the first human embryonic stem (ES) cell line from the inner cell mass of 4- to 7-day-old blastocyst-stage embryos donated by couples undergoing fertility treatment.[16] A list of essential characteristics that define human ES cells is presented in Table 1. The capacity of human ES cells to form teratomas containing derivatives of all three germ layers highlights their potential to differentiate into a range of cell types. To date, human ES cells have not been tested for their ability to participate in human embryonic development *in vivo* or to contribute to germ lines because of ethical concerns. The use of embryonic stem cells for clinical therapies is a relatively

Table 1. Defining properties of embryonic stem cells

1. Derived from the inner cell mass/epiblast of the blastocyst of pre-implantation or peri-implantation embryo.
2. Capable of undergoing unlimited proliferation in an undifferentiated state.
3. Exhibit and maintain a stable, diploid normal complement of chromosomes.
4. Can give rise to differentiated cell types that are derivatives of all three embryonic germ layers (ectoderm, mesoderm and endoderm) even after prolonged culture.
5. Capable of integrating into all foetal tissues during development.†
6. Capable of colonizing the germ line and giving rise to egg or sperm cells.†
7. Clonogenic, i.e. a single ES cell can give rise to a colony of genetically identical cells or clones, which have the same properties as the original cell.
8. Expresses the transcription factor Oct-4, which then activates or inhibits a host of target genes and maintains ES cells in a proliferative, non-differentiating state.
9. Can be induced to continue proliferating or to differentiate.
10. Lacks the G1 checkpoint in the cell cycle. ES cells spend most of their time in the S phase of the cell cycle, during which they synthesize DNA. Unlike differentiated somatic cells, ES cells do not require any external stimulus to initiate DNA replication.
11. Do not show X inactivation. In every somatic cell of a female mammal, one of the two X chromosomes becomes permanently inactivated but this does not occur in undifferentiated ES cells.

†Not shown in human ES cells. All of the criteria have been met by mouse ES cells.

new endeavour and currently this development has been hampered by ethical concerns.

Adult stem cells and mesenchymal stem cells

Adult stem cells, also known as somatic stem cells, are undifferentiated cells found in specialized tissues and organs of adults.[12] Compared to the pluripotent and almost immortal nature of embryonic stem cells, adult stem cells appear more mature with a finite lifespan and only multipotent differentiation capacity. It appears that all specialized tissues with renewal capacity throughout life probably contain adult stem cells in very small numbers that probably help replenish cell loss during normal senescence or tissue injury.[18–20] Haematopoietic stem cells from bone marrow were the first type of adult stem cells to be identified.[21] Over the years these cells have been extensively studied and are currently used therapeutically. Another population of adult non-haematopoietic stem cells also resides in the bone marrow microenvironment.[22–26] These are termed bone marrow stromal stem cells (BMSSCs) or mesenchymal stem cells (MSCs) and their biological properties are less well understood.

In recent years, human MSCs have been identified in many tissues throughout the adult body. However, the primary source of MSCs is the bone marrow where they exist at a low frequency (one per 34 000 nucleated cells), which declines with age.[25,27] MSC-like cell populations have also been identified in other tissues, including adipose tissue, muscle, peripheral blood, foetal pancreas and liver.[28–33] Because of their widespread distribution, it has been proposed that MSCs arise from a perivascular stem cell niche[26,33,34] where it has been suggested that MSC exhibit a phenotype characteristic of pericytes.[34,35]

Mesenchymal stem cells have been characterized both morphologically and immuno-phenotypically using various surface markers. While the morphology of MSCs typically falls into one of two types (large and flat or elongated and fibroblastic), this is not a defining or distinguishing feature of these cells. Of more relevance to their identification is the expression of a number of phenotypic characteristics of osteoblasts, endothelial, perivascular cells, neural or muscle cells and a range of surface markers (including CD49a/CD29, CD44, STRO-1, CD90, CD105, CD106, CD146, CD140b, CD166, CD271). This broad expression of cell surface molecules suggests a common link between different cellular types since most of these markers are expressed by all MSC.[25,26,36–38] However, the heterogeneous nature of these cells is highlighted in clonal studies demonstrating functional differences between MSCs, based on their proliferative potentials and developmental capacities *in vitro* and *in vivo*.[25,26,39–43] A particularly distinguishing feature of human MSCs is their ability to form colonies (i.e., they are clonogenic). In addition, under special inductive culture conditions, these cells can differentiate along numerous lineages including those for osteoblasts, adipocytes, myelosupportive stroma, chondrocytes and neuronal cells.[26,42–44] The ability of MSCs to give rise to multiple specialized cell types along with their extensive distribution in many adult tissues (including those of dental origin) have made them an attractive target for use in periodontal regeneration.

Goal of periodontal therapy

Following disease control, periodontal regeneration represents the ultimate goal of periodontal therapy and entails the re-formation of all components of the periodontium: gingival connective tissue, periodontal ligament, cementum and alveolar bone.[45] Periodontal regeneration aims to restore these lost tissues to their original form and function by recapitulating the crucial wound healing events associated with periodontal development.[45,46] Hence an understanding of the processes involved in the development of the periodontium is necessary in order to appreciate the cellular and molecular events that might occur during periodontal regeneration.

Development and formation of periodontium

Periodontal development commences at the end of crown stage, when cells of the inner and outer enamel epithelium proliferate from the cervical loop of the enamel organ to form Hertwig's epithelial root sheath (Fig 2). The root sheath separates cells of the dental papilla from the dental follicle, and initiates the differentiation of odontoblasts from the dental papilla to form root dentine. After root dentine is deposited, cells of the root sheath secrete a fine matrix of proteins onto the dentine surface, known as the hyaline layer of Hopewell Smith.[47,48] It is thought that subsequent fragmentation of the root sheath allows cells of the dental follicle to attach to this protein matrix and subsequently differentiate into cementoblasts, although the exact events are unclear.[49,50] Apical development of the root continues with continuing cementum deposition. Coronal to the developing root front, collagen fibres become embedded in the newly-formed cementum, known as Sharpey's fibres. This process initiates the formation of the periodontal ligament through the activities of the periodontal ligament fibroblasts which are also derived from the dental follicle.[51,52] At this stage of root development, bundle bone (i.e., the portion of the alveolar process that lines the tooth socket) is also formed and this is derived from osteoblasts, also originating in the dental follicle.[53] Insertion of Sharpey's fibres into this newly-forming

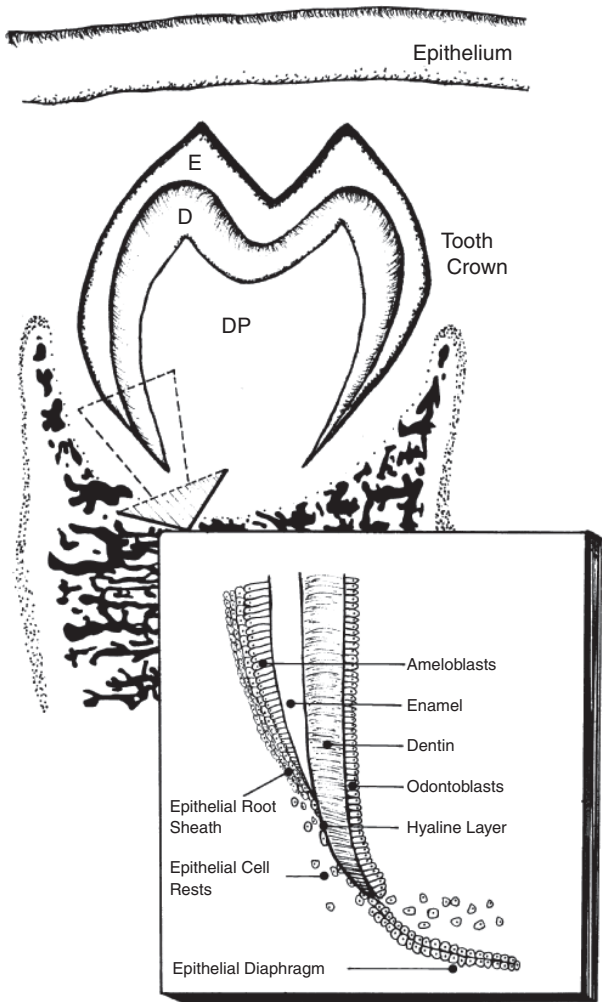

Fig 2. Schematic representation of root development. At the end of crown stage, root formation and periodontal development commence. The inset illustrates deposition of root dentine by odontoblasts and subsequent fragmentation of epithelial root sheath. Enamel (E), dentine (D), and dental papilla (DP) (reproduced, with permission from reference 45).

bone completes the development of the attachment apparatus of the periodontium. The formation of cementum, periodontal ligament and alveolar bone in a spatially and temporally coordinated manner, along with the transformation of reduced enamel epithelium to sulcular and junctional epithelium during tooth eruption, give rise to a complete periodontal attachment apparatus.[54] To facilitate regeneration of lost connective tissue attachment, further research is needed to fully elucidate the developmental processes of the periodontium as well as the wound healing events following periodontal therapy.

Periodontal wound healing and regeneration

Wound healing is the process by which an injured tissue repairs itself. This consists of three interdependent, sequential phases that overlap with each other: inflammation, granulation tissue formation and remodelling of the newly-formed tissue (Fig 3).[55] The duration of each phase varies depending on a range of local and systemic factors, particularly wound morphology and the condition of adjacent tissues.[55,56]

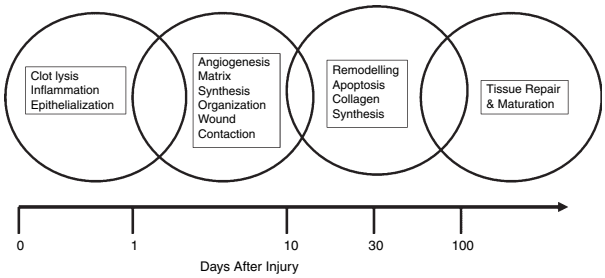

Fig 3. A time course of wound repair events that overlap in time and vary in duration depending on local and systemic factors (adapted from reference 45).

Immediately following injury, a complex cascade of molecular and cellular events occurs to initiate healing. An inflammatory response is mounted and a blood clot fills the site to provide tensile strength to the wound, and to serve as a reservoir of growth factors and a provisional matrix for cell migration. Infiltrates of neutrophils and monocytes enter the wound site to phagocytose dead or damaged tissue and foreign matter. Epithelial cells originating from wound margins also migrate to close the epithelial breach. The fibrin clot is subsequently organized into granulation tissue as new capillaries form and fibroblasts differentiate into myofibroblasts to contract the wound. In the final phase, the granulation tissue is remodelled into either repaired (scar) tissue or regenerated tissue. Healing by repair results in a tissue that does not completely restore the architecture or function of the original tissue, whereas healing by regeneration produces a new tissue that is identical in both structure and function to the original tissue.[57]

Healing following conventional periodontal therapy is more complicated than simple soft tissue healing because of the involvement of mineralized tissues (i.e., cementum and bone) in addition to soft tissue components. Most mechanical and surgical periodontal procedures (e.g., scaling, debridement and flap procedures of various types) favour the healing of anatomical defects produced by periodontitis. This largely involves repair of the gingival connective tissues and the coronal portion of the periodontal ligament with virtually no repair of the cementum or alveolar bone. These events, by definition, do not constitute regeneration of the periodontium.[45] Indeed, healing after flap surgery is mediated by a range of reparative responses (Table 2), none of which can be considered to be tissue regeneration.

For regeneration to occur, healing events should progress in an ordered and programmed sequence both

Table 2. Possible healing responses following conventional periodontal therapy

Repair	Control of inflammation Long junctional epithelium Connective tissue attachment to the root surface (reattachment or new attachment) New bone separated from the root surface by long functional epithelium New bone with root resorption or ankylosis or both
Regeneration	New functional attachment apparatus with formation of cementum periodontal ligament and alveolar bone

(Adapted from reference 45.)

temporally and spatially, replicating the key events in periodontal development.[46,58] The course of healing is dependent on the availability of appropriate cells, inductive factors and extracellular matrix secreted by these cells.[59] Although the exact events are unclear, appropriate progenitor cells must migrate and attach to the denuded root surface, and then proliferate and mature into all of the tissue components which constitute a functional attachment apparatus. The correct proliferation, migration and maturation of these cells is dependent partly on the presence of inductive factors and the contact with extracellular matrix, controlling gene expression and release of specific inductive factors.[59,60] In the absence of appropriate cellular, molecular or matrix components, healing may be compromised and occur by repair rather than regeneration.

Progenitor and stem cells are of particular interest in periodontal wound healing and regeneration as they are most likely the parental cells of synthetic cells (e.g., osteoblasts, cementoblasts and fibroblasts) responsible for the restoration of lost periodontal tissues (Fig 4).[61–63] Whether progenitor cells arise from blood vessels in the periodontal ligament or surrounding bone stroma has been debated.[64] However, one recent study has clearly demonstrated the presence of periodontal

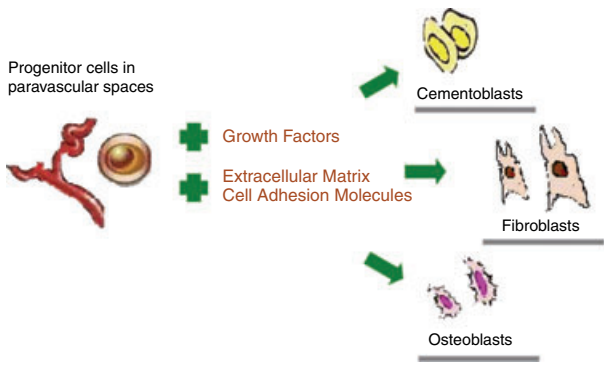

Fig 4. The role of stem cells in periodontal regeneration. Cells in the paravascular areas of mature periodontal ligament have the potential to differentiate into mature osteoblasts, periodontal ligament fibroblasts and cementoblasts.

Table 3. Phases of periodontal regeneration

Surgical techniques	1950–1970
Root surface conditioning	1970–1980
Guided tissue regeneration	1980–1990
Growth factors	1990–2000
Tissue engineering	2000–????
Stem cells	????

ligament stem cells in perivascular niches of periodontal ligament.[65] Cells cultured from bone have the capacity to form cementum-like material *in vitro*[66] and these cells appear to be transferred to the periodontal ligament via numerous vascular channels in the alveolar bone.[67] Recent evidence shows that some clonal cell lines isolated from the periodontal ligament have characteristics of stem cells.[68–71] A better understanding of these cells in the periodontal tissues will facilitate use of these cells for regenerative periodontal therapy.

Periodontal regeneration

For decades attempts have been made to develop clinical procedures which might lead to predictable periodontal regeneration (Table 3). Most of these have been based on surgical solutions to the problem. However, until recent times many of the approaches were not based on sound biological principles and thus were eventually found to be of limited value.

Root surface conditioning

In an early approach, root surfaces were "conditioned" in early attempts either by demineralization of root surfaces, or by coating root surfaces with chemical agents such as fibronectin, or both. The demineralization procedure was believed to reverse periodontitis-induced root surface hypermineralization and to expose collagen fibres with which newly-formed fibres could interdigitate. Exposed collagen fibres were also expected to discourage the attachment of unwanted epithelial cells. However, this procedure did not yield predictable regeneration, and often caused ankylosis and root resorption as side effects instead.[72] The advantage of using fibronectin root surface coating was also unclear because serum contains high fibronectin levels and providing additional protein is unlikely to have any beneficial effect.[73]

Bone filling materials

Another approach to periodontal "regeneration" involved the introduction of a "filler" material into periodontal defects in the hope of inducing bone regeneration. Various types of bone grafts have been investigated to determine their ability to stimulate new bone formation. Of these, the following have been

studied in detail: (1) alloplastic materials which are generally synthetic filler materials; (2) autografts which are grafted tissue from one site to another in the same individual; (3) allografts of tissue between individuals of the same species but with different genetic composition; and (4) xenografts which consist of grafted materials between different species. Although utilization of such grafting materials for periodontal defects may result in some gain in clinical attachment levels and radiographic evidence of bone fill, careful histologic assessment usually reveals that these materials have little osteoinductive capacity (let alone cementogenic capacity) and generally become encased in a dense fibrous connective tissue.[74]

Guided tissue regeneration

In recent years guided tissue regeneration has come to be considered the "gold standard" upon which to compare regenerative technologies. This procedure involves draping a barrier membrane over the periodontal defect from the root surface and onto adjacent alveolar bone prior to replacement of the mucoperiosteal flap. The barrier membrane prevents unwanted epithelium and gingival connective tissue from entering the healing site while promoting re-population of the defect site by cells from the periodontal ligament (Fig 5). The concept of using barrier membranes in periodontal regeneration is based on findings of a series of experiments carried out to address the regenerative capacity of different periodontal tissues.[75–77] From these studies it was reported that only cells of the periodontal ligament possessed regenerative capacity, and that exclusion of gingival tissues from the wound site allowed periodontal ligament cells to re-populate the site, making regeneration biologically possible.[78]

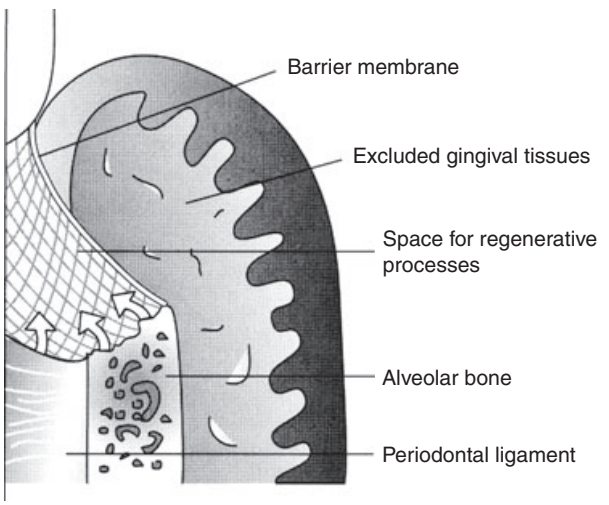

Fig 5. Schematic representation of guided tissue regeneration. The membrane physically excludes gingival tissues from the wound site while providing space to allow cells of the periodontal ligament to migrate into the site and promote regeneration (reproduced, with permission from reference 45).

Table 4. Essential design criteria for guided tissue regeneration membranes

1. Tissue integration	An open microstructure to encourage tissue integration and limit epithelial migration, while creating a stable site for wound healing
2. Cell occlusivity	Separate all cell types so that the desired cells can repopulate the defect area
3. Clinical manageability	Easy to cut and shape to fit particular periodontal defects
4. Space provision	Resist collapse from the pressure of overlying tissue so that they can maintain adequate space during the healing period
5. Biocompatibility	Non-toxic, non-antigenic and induce minimal inflammatory response from the host
6. Membrane stability*	Remain *in situ* to allow progenitor cells adequate time to repopulate the defect site without interference from gingival connective tissue or epithelium
7. Membrane resorption*	Be degraded, replaced, or incorporated into the healing flap after cell selection is complete

*Apply only to resorbable membranes. (Adapted from references 79, 80 and 81.)

Barrier membranes require at least seven essential criteria for their design to be effective (Table 4).[79–81] Resorbable membranes are made of collagen, polylactic acid and polyglycolic acid[82] as well as autograft and allograft materials.[83] Non-resorbable membranes are commonly made of expanded polytetrafluoroethylene (ePTFE).[84] Resorbable membranes have the advantage of bio-disintegration which negates the need for a second surgical procedure to retrieve the membrane. In contrast, non-resorbable membranes are used in a two-stage procedure involving removal of the membrane 6–8 weeks after initial placement.

Histological analysis of guided tissue regeneration-mediated healing shows that new connective tissue attachment to the root surface forms with minor contributions from new cementum and bone which, by definition, is not true regeneration.[5] Guided tissue regeneration procedures represent an attempt to achieve concordance between biological principles and clinical practice. However, their limited clinical and histological success, particularly in advanced periodontal defects,[85,86] has led researchers to further explore biologically-based regenerative therapy strategies involving growth factors, gene therapy and replacement therapy with stem cells.

Growth factors

In another approach to induce periodontal regeneration, polypeptide growth factors have been locally applied to the root surface in order to facilitate the

cascade of wound healing events that lead to new cementum and connective tissue formation. Among the myriad growth factors currently characterized and available, epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), PDGF and TGF-β have been proposed to be of potential use in relation to their regulatory effects on immune function, cells of epithelium, bone and soft connective tissues. Two of these growth factors, PDGF and IGF-I, enhance regeneration in beagle dogs and monkeys with periodontal disease.[87,88] Another promising group of polypeptide growth factors is the bone morphogenetic proteins (BMP) which offer good potential for stimulating bone and cementum regeneration.[89]

Enamel matrix proteins

Enamel matrix proteins, produced by Hertwig's epithelial sheath, are known to play an important role in cementogenesis, as well as in the development of the periodontal attachment apparatus.[90,91] There is some evidence that these proteins also play a role in regeneration of periodontal tissues after periodontal therapy.[92] *In vitro* studies have demonstrated that EMD addition to cultures of periodontal fibroblasts results in enhanced proliferation, protein and collagen production as well as promotion of mineralization.[93,94] As no specific growth factors have been identified in EMD preparations,[93] it is postulated that EMD acts as a matrix enhancement factor, creating a positive environment for cell proliferation, differentiation and matrix synthesis.[95]

New perspectives for periodontal regeneration

There is no doubt that the desired clinical endpoint is predictable regeneration of the periodontal tissues damaged by inflammation to their original form and function. To date, this has been elusive. In order for regeneration to occur there will need to be the coordinated recruitment of specialized cells to the area and deposition of specific matrix molecules consistent with both soft and hard connective tissue formation and this will be largely driven by soluble cytokines and growth factors.

To understand the rational basis of regenerative procedures, more information is needed on the variety of molecular and cellular processes associated with the formation of each periodontal component. In particular, very little is known about cementogenesis and the mechanisms necessary for reattachment. While the use of growth factors shows some promise in this area, these suffer from being very broad in their range of activity and thus lack a degree of tissue specificity. Therefore, it seems reasonable to continue to probe local factors (both cellular and molecular) which may be specific for the regeneration of the periodontal tissues. To this end efforts to characterize stem cell populations in the periodontal ligament have become important.

Stem cells in human periodontal ligament

The presence of multiple cell types (fibroblasts, cementoblasts and osteoblasts) within the postnatal periodontal ligament has led researchers to speculate that these cells may share common ancestors. The possibility that progenitor cells might exist in the postnatal periodontal ligament has been recognized for some time but until recently had never been formally proven.[96] These cells are believed to provide a renewable cell source for normal tissue homeostasis and periodontal wound healing.[61,97]

Cell kinetic studies in mice have indicated that a group of progenitor cells exhibiting some classical features of stem cells exist in the periodontal ligament.[67,98,99] Recently, multipotent stem cell populations, termed periodontal ligament stem cells (PDLSCs), have been isolated from the periodontal ligament of extracted human third molar teeth.[10] These PDLSCs give rise to adherent clonogenic clusters that resemble fibroblasts and are capable of developing into adipocytes, osteoblast- and cementoblast-like cells *in vitro*, and demonstrate the capacity to produce cementum- and periodontal ligament-like tissues *in vivo*.[10,70,100] PDLSCs express an array of cementoblast and osteoblast markers as well as the BMSSC associated markers, STRO-1 and CD146 antigens, which are also present on dental pulp stem cells.[101,102] The similarity between PDLSCs, dental pulp stem cells and BMSSCs suggests that PDLSCs represent another MSC-like population. Further work is now focusing on identifying markers uniquely expressed by PDLSCs to discriminate these cells from other types of MSC-like cells identified in dental tissues.[65] However, this is likely to be a complex task as earlier studies have indicated that there is considerable heterogeneity amongst cells of the periodontal tissues with regenerative capacity.[103]

The first reported isolation and identification of mesenchymal stem cells in human periodontal ligament was in 2004.[10] Since then, there has been considerable activity trying to understand the function of these cell populations and their interactions with each other with a view to laying the fundamental groundwork for clinical applications in regenerative periodontics. A number of studies have now been carried out to confirm the presence of MSC-like cells in the periodontal ligament. These have not been limited to human but also include mouse, rat and sheep.[69–71,102–105] All of these studies have confirmed the multipotent nature of periodontal ligament stem cells, and while the initial studies indicated this to include an ability to

differentiate into osteoblast, cementoblast or lipidogenic phenotypes at least one recent study has indicated an ability of these cells to differentiate into neuronal precursors.[105] Importantly cryo-freezing does not seem to alter the properties of PDLSCs[68] and this will have significant relevance should "banking" of these cells become a clinical necessity.

Potential applications of stem cells in periodontal therapy

Identification of stem cells in postnatal dental tissues has presented exciting possibilities for the application of tissue engineering as well as gene and cell-based therapies in reconstructive dentistry. The use of stem cells with these technologies may constitute novel strategies for regenerative periodontal therapy.

Tissue engineering

Tissue engineering is a specialized field of science based on principles of cell biology, developmental biology and biomaterials science to fabricate new tissues to replace lost or damaged tissues.[106,107] Successful tissue engineering requires an appropriate extracellular matrix or carrier construct which contains regulatory signals and responsive progenitor cells. A potential tissue engineering approach to periodontal regeneration involves incorporation of progenitor cells and instructive messages in a prefabricated three-dimensional construct, which is subsequently implanted into the defect site (Fig 6).[58] This strategy eliminates some of the limitations associated with conventional regenerative procedures because of direct placement of growth factors and progenitor cells into the defect site overcomes the normal lag phase of progenitor cell recruitment to the site.

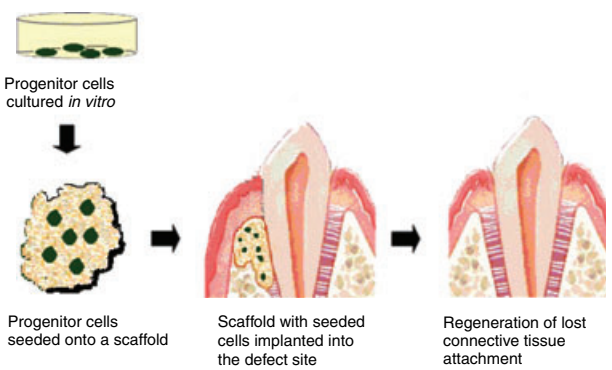

Fig 6. Schematic representation of periodontal tissue engineering. An engineered matrix (left) with necessary cells and instructive messages seeded *in vitro*, and then (right) transferred into a periodontal defect to promote regeneration. Rapid formation of an epithelial seal should be encouraged to minimize salivary and microbial contamination during wound healing.

Table 5. Requirements for successful tissue engineering

Biochemical features
• Space maintenance
• Barrier or exclusionary features
Biological functions
• Biocompatibility
• Incorporation of cells (with appropriate phenotype for ongoing periodontal regeneration)
• Incorporation (and bioavailability) of instructive messages

(Adapted from reference 58.)

The technical requirements for successful cell-based tissue engineering can be divided into two main categories (Table 5): engineering issues related to maintenance of an *in vivo* cell culture in the defect (e.g., biomechanical properties of the scaffold) and biological functions of the engineered matrix (including cell recruitment, neovascularization and bioavailability of growth factors).[108]

With respect to the biochemical features of the matrix scaffold, these compounds should act in a manner consistent with the principles of membrane-based guided tissue regeneration and have similar design features as listed in Table 4.[80] In particular, these properties should include: ease of handling, rigidity to withstand soft tissue collapses into the defect, and ability to maximize cell colonization and tissue ingrowth of desired type.[109] It is also important that unwanted epithelium is not totally excluded, but rather encouraged to form a biological seal over the scaffold and onto the tooth in the vicinity of the cemento-enamel junction, protecting the regenerating events occurring beneath.[110]

The concept of cell transplantation into periodontal defects was first described over 15 years ago.[111] Since then, other studies have attempted to induce periodontal regeneration using implantation of cultures of periodontal ligament fibroblasts and alveolar bone cells.[112] While these investigations met with some success, overall the treatment strategies were somewhat limited due to the heterogeneous nature of the crude cell preparations used in these studies. More recently, the use of purified stem cells for tissue engineering approaches to facilitate periodontal regeneration has been investigated. Transplantation of autologous bone marrow MSCs in combination with atelocollagen into class III defects in dogs has been shown to regenerate cementum, periodontal ligament and alveolar bone.[113] *Ex vivo* expanded PDLSCs co-transplanted with hydroxyapatite/tricalcium phosphate ceramic (HA/TCP) particles into nude rats are capable of forming cementum/periodontal ligament-like structures.[68] One novel report has shown that stem cells isolated from the root apical papilla of human teeth and PDLSC can be combined to regenerate the

root/periodontal structure respectively.[114] In this study a root-shaped scaffold structure was prepared into which stem cells isolated from the root apical papilla from porcine teeth were seeded. Gelfoam containing porcine PDLSC was then wrapped around the artificial root construct and then placed into a prepared bony socket in the mandible of mini-pigs. After a three-month healing phase this biologically created "root" was restored with a porcelain crown. Collectively, these findings demonstrate the feasibility (and potential) of using a combination of MSC-like cell populations for functional tooth regeneration.

Gene and cell-based therapy

The inherent proliferative and pluripotent capabilities of stem cells may offer lifelong opportunities for treatment of some important human diseases, including periodontitis, by repairing, replacing or regenerating damaged tissues. Stem cells may act as suitable vehicles for the delivery of therapeutic genes in gene therapy, and as therapeutic agents *per se* in cell-based therapy.

Gene therapy is a new approach for the treatment of human diseases. It relies on genetic engineering, which involves molecular techniques to introduce, suppress or manipulate specific genes, thereby directing an individual's own cells to produce a therapeutic agent. In the context of periodontal regeneration, gene therapy seeks to optimize the delivery of agents such as growth factors to periodontal defects so that the limitations associated with topical application (e.g., short duration of action) can be overcome.[115] Two major strategies for delivering therapeutic transgenes into human recipients are: (1) direct infusion of the gene of interest using viral or non-viral vectors *in vivo*; and (2) introduction of gene into delivery cells (often a stem cell) outside the body *ex vivo* followed by transfer of the delivery cells back into the body.[30]

The use of both *in vivo* and *ex vivo* gene delivery strategies via adenoviral (Ad) vectors encoding growth promoting molecules such as platelet-derived growth factor (PDGF) and bone morphogenetic protein-7 (BMP-7) has been investigated for its potential in periodontal regeneration by Giannobile and colleagues.[116–119] Recent findings in rats have revealed sustained transgene expression for up to 10 days at Ad-BMP-7 treated sites,[119] and enhanced bone and cementum regeneration at Ad-BMP-7 and Ad-PDGF treated sites beyond that of control vectors.[118] The introduction of transgenes into dental stem cells may offer an alternative to conventional methods because stem cells have the potential to provide a sustained source of growth factors for regeneration. However, much work is still needed to optimize the number of cells that are virally transduced to express specific genes, in order to maximize the duration and extent of gene expression, and ultimately to determine the success of gene transfer techniques in periodontal regeneration. Further research is also needed to address potential risks of viral recombination and immune responses towards viral antigens which could potentially hinder the progress of gene therapy in treating periodontal diseases.[120]

Current challenges and future directions for research

In view of the gaps and deficiencies in our knowledge of periodontal development and its applications to periodontal therapy, many challenges need to be overcome before stem cell-based treatment can become a clinical reality. This section highlights the main biological, technical and clinical challenges in the area, which could form the basis for further research.

Biological challenges

Despite biological evidence showing that regeneration can occur in humans, complete and predictable regeneration still remains an elusive clinical goal, especially in advanced periodontal defects. Periodontal regeneration, based on replicating the key cellular events that parallel periodontal development, has not been possible because of our incomplete understanding of the specific cell types, inductive factors and cellular processes involved in formation of the periodontium.[58] Furthermore, most basic discoveries on periodontal stem cells have emerged from cell culture and animal models which does not always translate to the human situation. Thus, not all findings in animal models can be directly extrapolated to humans. In addition, the molecular pathways that underlie stem cell self-renewal and differentiation are also largely unknown.[121] Further research is needed to elucidate the cellular and molecular events involved in restoring lost periodontal tissues before a reliable biologically-based therapy can be developed. In light of these concerns, the isolation and characterization of stem cells from periodontal tissues may provide a good starting point to investigate the role of stem cells in periodontal wound healing and their potential applications in regenerative therapy, including tissue engineering.

Technical challenges

Biologically, the matrix scaffold should have good biocompatibility for the cellular and molecular components normally found in regenerating tissues.[122,123] There is evidence to suggest that cultured human PDLSCs in a suitable scaffold and implanted into surgically-created periodontal defects can result in the formation of a periodontal ligament-like structure.[68] However, the optimal mechanism of propagation and

incorporation of these cells into a carrier scaffold still needs further refinement.[124,125] In addition, further studies are needed to understand the conditions that induce lineage-specific differentiation and efficacy of *in vitro* expanded stem cells derived from regenerating periodontal defects.[7,126] Possible karyotypic instability and gene mutations can limit the usefulness of cell lines after prolonged culture.[127] There are also difficulties in providing clinical-grade stem cell lines using animal-free media to prevent cross-infection in humans.[128] Thus, refinement of current techniques to facilitate laboratory handling of these cells and to maximize their regenerative potential represents a long-term endeavour if these cells are to be used in clinical periodontics.

Clinical challenges

In addition to biological and technical challenges, there are a number of clinical barriers in MSC-based clinical therapy that must be understood and overcome: immune rejection, tumour growth and efficacy of cell transplantation.

Firstly, it is important to understand how the immune system will respond to human stem cell derivatives upon transplantation. Generally, the immunogenicity of a human cell depends on its expression of class I and II major histocompatibility (MHC) antigens, which allow the body to distinguish its own cells from foreign cells. Human ES cells express a low level of class I MHC antigens, but this expression is up-regulated with differentiation.[129] The use of patient-specific (autologous) adult stem cells from redundant third molar teeth should overcome potential immune rejection.[130,131] However, this approach may be redundant if recent reports are considered which indicate that MSC can suppress the immune system and thus allows the use of either autologous or allogeneic MSC preparations.[132]

Secondly, the prevention of tumour formation following MSC implantation is a major safety consideration as current studies lack sufficient statistical power and long-term follow-up to draw firm conclusions.[133] It is likely that the more specific and extensive the therapeutic application, the longer the stem cells may have to remain *in vitro* to obtain sufficient numbers for therapeutic use. Thus during this extended period in culture there could be a greater likelihood that genetic or epigenetic changes will accumulate. If such changes are not accompanied by an overt phenotypic transformation, they may go undetected and harm the patient. Therefore, it is critical to have a thorough understanding of the rate of genetic change and the type of selective pressures that allows this change to dominate a culture.

Thirdly, it is unclear whether human stem cell derivatives can integrate into the recipient tissue and fulfil the specific functions of lost or injured tissues.[134] Delivery of appropriate cells and molecules to the target site without inducing ectopic tissue formation is of paramount importance for the safety and effectiveness of tissue engineering-based periodontal regeneration. It is hoped that, as our knowledge on progenitor cells, growth factors and delivery systems improves, it will eventually lead to the development of regenerative therapy based on sound scientific principles.

CONCLUSIONS

Regeneration of tissues destroyed by periodontitis has long been an altruistic goal of periodontal therapy. Periodontal regeneration requires consideration of many features that parallel periodontal development, including the appropriate progenitor cells, signalling molecules and matrix scaffold in an orderly temporal and spatial sequence. It is clear that current regenerative procedures are less than ideal but the identification of stem cells in human dental tissues in recent years holds promise to the development of novel, more effective approaches to periodontal regeneration and reconstructive therapy. One way forward is to embrace the field of stem cell-based tissue engineering and adopt an interdisciplinary approach to periodontal regeneration. However, before this is feasible, many biological, technical and clinical hurdles need to be overcome and a thorough understanding of underlying healing processes in periodontal regeneration is required.

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REFERENCES

1. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005;366:1809–1820.
2. Polimeni G, Xiropaidis AV, Wikesjo UM. Biology and principles of periodontal wound healing/regeneration. Periodontol 2000 2006;41:30–47.
3. Rosling B, Nyman S, Lindhe J, Jern B. The healing potential of the periodontal tissues following different techniques of periodontal surgery in plaque-free dentitions. A 2-year clinical study. J Clin Periodontol 1976;3:233–250.
4. Caton J, Nyman S, Zander H. Histometric evaluation of periodontal surgery. II. Connective tissue attachment levels after four regenerative procedures. J Clin Periodontol 1980;7:224–231.
5. Sander L, Karring T. Healing of periodontal lesions in monkeys following the guided tissue regeneration procedure. A histological study. J Clin Periodontol 1995;22:332–337.
6. Xiao Y, Parry DA, Li H, Arnold R, Jackson WJ, Bartold PM. Expression of extracellular matrix macromolecules around

demineralized freeze-dried bone allografts. J Periodontol 1996;67:1233–1244.

7. Ripamonti U, Reddi AH. Tissue engineering, morphogenesis, and regeneration of the periodontal tissues by bone morphogenetic proteins. Crit Rev Oral Biol Med 1997;8:154–163.
8. Stavropoulos A, Kostopoulos L, Nyengaard JR, Karring T. Deproteinized bovine bone (Bio-Oss) and bioactive glass (Biogran) arrest bone formation when used as an adjunct to guided tissue regeneration (GTR): an experimental study in the rat. J Clin Periodontol 2003;30:636–643.
9. Venezia E, Goldstein M, Boyan BD, Schwartz Z. The use of enamel matrix derivative in the treatment of periodontal defects: a literature review and meta-analysis. Crit Rev Oral Biol Med 2004;15:382–402.
10. Seo BM, Miura M, Gronthos S, *et al.* Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004;364:149–155.
11. Bartold PM, Shi S, Gronthos S. Stem cells and periodontal regeneration. Periodontol 2000 2006;40:164–172.
12. Smith A. A glossary for stem-cell biology. Nature 2006;441: 1060.
13. Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. Cell 1997;88:287–298.
14. Vats A, Bielby RC, Tolley NS, Nerem R, Polak JM. Stem cells. Lancet 2005;366:592–602.
15. Pera MF, Cooper S, Mills J, Parrington JM. Isolation and characterisation of a multipotent clone of human embryonal carcinoma-cells. Differentiation 1989;42:10–23.
16. Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al.* Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145–1147.
17. Shamblott MJ, Axelman J, Wang S, *et al.* Derivation of pluripotent stem cells from cultured human primordial germ cells. Proc Natl Acad Sci USA 1998;95:13726–13731.
18. Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. Isolation of a candidate human hematopoietic stem-cell population. Proc Natl Acad Sci USA 1992;89:2804–2808.
19. Slack JM. Stem cells in epithelial tissues. Science 2000;287:1431–1433.
20. Uchida N, Buck DW, He D, *et al.* Direct isolation of human central nervous system stem cells. Proc Natl Acad Sci USA 2000;97:14720–14725.
21. Becker AJ, McCulloch EA, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature 1963;197:452–454.
22. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 1968;6:230–247.
23. Castro-Malaspina H, Gay RE, Resnick G, *et al.* Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. Blood 1980;56:289–301.
24. Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9:641–650.
25. Pittenger MF, Mackay AM, Beck SC, *et al.* Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–147.
26. 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.
27. Wexler SA, Donaldson C, Denning-Kendall P, Rice C, Bradley B, Hows JM. Adult bone marrow is a rich source of human mesenchymal 'stem' cells but umbilical cord and mobilized adult blood are not. Br J Haematol 2003;121:368–374.
28. Baroffio A, Hamann M, Bernheim L, Bochaton-Piallat ML, Gabbiani G, Bader CR. Identification of self-renewing myoblasts in the progeny of single human muscle satellite cells. Differentiation 1996;60:47–57.
29. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 2001;98:2396–2402.
30. Zwaka TP. Use of genetically modified stem cells in experimental gene therapies. In: Regenerative medicine 2006. National Institutes of Health. Bethesda: Department of Health and Human Services, 2006:45–52.
31. Hu Y, Liao L, Wang Q, *et al.* Isolation and identification of mesenchymal stem cells from human fetal pancreas. J Lab Clin Med 2003;141:342–349.
32. Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells. J Cell Biol 2001;153:1133–1140.
33. Zannettino AC, Paton S, Arthur A, *et al.* Multipotential human adipose-derived stromal stem cells exhibit a perivascular phenotype in vitro and in vivo. J Cell Physiol 2008;214:413–421.
34. 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.
35. Collett GD, Canfield AE. Angiogenesis and pericytes in the initiation of ectopic calcification. Circ Res 2005;96:930–938.
36. Prockop DJ, Sekiya I, Colter DC. Isolation and characterization of rapidly self-renewing stem cells from cultures of human marrow stromal cells. Cytotherapy 2001;3:393–396.
37. Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM. Purification and *ex vivo* expansion of postnatal human marrow mesodermal progenitor cells. Blood 2001;98:2615–2625.
38. Gronthos S, Simmons PJ. The growth factor requirements of STRO-1-positive human bone marrow stromal precursors under serum-deprived conditions in vitro. Blood 1995;85:929–940.
39. Friedenstein AJ, Ivanov-Smolenski AA, Chajlakjan RK, *et al.* Origin of bone marrow stromal mechanocytes in radiochimeras and heterotopic transplants. Exp Hematol 1978;6:440–444.
40. Owen ME, Cave J, Joyner CJ. Clonal analysis in vitro of osteogenic differentiation of marrow CFU-F. J Cell Sci 1987;87:731–738.
41. Bennett JH, Joyner CJ, Triffitt JT, Owen ME. Adipocytic cells cultured from marrow have osteogenic potential. J Cell Sci 1999;99:131–139.
42. Kuznetsov SA, Krebsbach PH, Satomura K, *et al.* Single-colony derived strains of human marrow stromal fibroblasts form bone after transplantation in vivo. J Bone Miner Res 1997;12:1335–1347.
43. Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. J Cell Sci 2000;113:1161–1166.
44. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 1997;276:71–74.
45. Narayanan AS, Bartold PM. Biochemistry of periodontal connective tissues and their regeneration: a current perspective. Connect Tissue Res 1996;34:191–201.
46. MacNeil RL, Somerman MJ. Development and regeneration of the periodontium: parallels and contrasts. Periodontol 2000 1999;19:8–20.
47. Lindskog S. Formation of intermediate cementum. I: early mineralization of aprismatic enamel and intermediate cementum in monkey. J Craniofac Genet Dev Biol 1982;2:147–160.
48. Slavkin HC, Bringas P Jr, Bessem C, *et al.* Hertwig's epithelial root sheath differentiation and initial cementum and bone formation during long-term organ culture of mouse mandibular

first molars using serumless, chemically-defined medium. J Periodont Res 1989;24:28–40.

49. MacNeil RL, Thomas HF. Development of the murine periodontium. II. Role of the epithelial root sheath in formation of the periodontal attachment. J Periodontol 1993;64:285–291.
50. Hammarstrom L, Alatli I, Fong CD. Origins of cementum. Oral Dis 1996;2:63–69.
51. Yamamoto T, Hinrichsen KV. The development of cellular cementum in rat molars, with special reference to the fiber arrangement. Anat Embryol (Berl) 1993;188:537–549.
52. Yamamoto T, Domon T, Takahashi S, Wakita M. Comparative study of the initial genesis of acellular and cellular cementum in rat molars. Anat Embryol (Berl) 1994;190:521–527.
53. Ten Cate A. Formation of supporting bone in association with periodontal ligament organisation in the mouse. Arch Oral Biol 1975;20:137–138.
54. Ten Cate AR. The role of epithelium in the development, structure and function of the tissues of tooth support. Oral Dis 1996;2:55–62.
55. Clark R. The molecular and cellular biology of wound repair. 2nd edn. New York: Plenum, 1996.
56. Wikesjo UM, Crigger M, Nilveus R, Selvig KA. Early healing events at the dentin-connective tissue interface. Light and transmission electron microscopy observations. J Periodontol 1991;62:5–14.
57. Wang HL, Greenwell H, Fiorellini J, *et al.* Periodontal regeneration (American Academy of Periodontology Position Paper). J Periodontol 2005;76:1601–1622.
58. Bartold PM, McCulloch CA, Narayanan AS, Pitaru S. Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. Periodontol 2000 2000;24:253–269.
59. Aukhil I. Biology of wound healing. Periodontol 2000 2000;22:44–50.
60. Thesleff I, Nieminen P. Tooth morphogenesis and cell differentiation. Curr Opin Cell Biol 1996;8:844–850.
61. Gould TR, Melcher AH, Brunette DM. Migration and division of progenitor cell populations in periodontal ligament after wounding. J Periodont Res 1980;15:20–42.
62. Gould TR. Ultrastructural characteristics of progenitor cell populations in the periodontal ligament. J Dent Res 1983;62:873–876.
63. Lin WL, McCulloch CA, Cho MI. Differentiation of periodontal ligament fibroblasts into osteoblasts during socket healing after tooth extraction in the rat. Anat Rec 1994;240:492–506.
64. McCulloch CA, Melcher AH. Cell migration in the periodontal ligament of mice. J Periodont Res 1983;18:339–352.
65. Chen SC, Marino V, Gronthos S, Bartold PM. Location of putative stem cells in human periodontal ligament. J Periodont Res 2006;41:547–553.
66. Melcher AH, Cheong T, Cox J, Nemeth E, Shiga A. Synthesis of cementum-like tissue in vitro by cells cultured from bone: a light and electron microscope study. J Periodont Res 1986;21:592–612.
67. McCulloch CA, Nemeth E, Lowenberg B, Melcher AH. Paravascular cells in endosteal spaces of alveolar bone contribute to periodontal ligament cell populations. Anat Rec 1987;219:233–242.
68. Seo BM, Miura M, Sonoyama W, Coppe C, Stanyon R, Shi S. Recovery of stem cells from cryopreserved periodontal ligament. J Dent Res 2005;84:907–912.
69. Nagatomo K, Komaki M, Sekiya I, *et al.* Stem cell properties of human periodontal ligament cells. J Periodontal Res 2006;41:303–310.
70. Gronthos S, Mrozik K, Shi S, Bartold PM. Ovine periodontal ligament stem cells: isolation, characterization, and differentiation potential. Calcif Tissue Int 2006;79:310–317.
71. Jo YY, Lee HJ, Kook SY, *et al.* Isolation and characterization of postnatal stem cells from human dental tissues. Tissue Eng 2007;13:767–773.
72. Smith B, Cafesse R, Nasjleti C, Kon S, Castelli W. Effects of citric acid, and fibronectin and laminin application in treating periodontitis. J Clin Periodontol 1987;14:396–402.
73. Dreyer WP, van Heerden JD. The effect of citric acid on the healing of periodontal ligament-free, healthy roots, horizontally implanted against bone and gingival connective tissue. J Periodont Res 1986;21:210–220.
74. Garraway R, Young WG, Daley T, Harbrow D, Bartold PM. An assessment of the osteoinductive potential of commercial demineralized freeze-dried bone in the murine thigh muscle implantation model. J Periodontol 1998;69:1325–1336.
75. Karring T, Nyman S, Lindhe J. Healing following implantation of periodontitis affected roots into bone tissue. J Clin Periodontol 1980;7:96–105.
76. Nyman S, Karring T, Lindhe J, Planten S. Healing following implantation of periodontitis-affected roots into gingival connective tissue. J Clin Periodontol 1980;7:394–401.
77. Nyman S, Gottlow J, Karring T, Lindhe J. The regenerative potential of the periodontal ligament. An experimental study in the monkey. J Clin Periodontol 1982;9:257–265.
78. Nyman S, Lindhe J, Karring T, Rylander H. New attachment following surgical treatment of human periodontal disease. J Clin Periodontol 1982;9:290–296.
79. Gottlow J. Guided tissue regeneration using bioresorbable and non-resorbable devices: initial healing and long-term results. J Periodontol 1993;64:1157–1165.
80. Scantlebury TV. 1982-1992: a decade of technology development for guided tissue regeneration. J Periodontol 1993; 64:1129–1137.
81. Lundgren D, Laurell L, Gottlow J, *et al.* The influence of the design of two different bioresorbable barriers on the results of guided tissue regeneration therapy. An intra-individual comparative study in the monkey. J Periodontol 1995;66:605–612.
82. Magnusson I, Batich C, Collins BR. New attachment formation following controlled tissue regeneration using biodegradable membranes. J Periodontol 1988;59:1–6.
83. Kwan SK, Lekovic V, Camargo PM, *et al.* The use of autogenous periosteal grafts as barriers for the treatment of intrabony defects in humans. J Periodontol 1998;69:1203–1209.
84. Haney JM, Nilveus RE, McMillan PJ, Wikesjo UM. Periodontal repair in dogs: expanded polytetrafluoroethylene barrier membranes support wound stabilization and enhance bone regeneration. J Periodontol 1993;64:883–890.
85. Karring T, Cortellini P. Regenerative therapy: furcation defects. Periodontol 2000 1999;19:115–137.
86. Needleman IG, Worthington HV, Giedrys-Leeper E, Tucker RJ. Guided tissue regeneration for periodontal infra-bony defects. Cochrane Database Syst Rev CD001724. 2006.
87. Lynch SE, de Castilla GR, Williams RC, *et al.* The effects of short-term application of a combination of platelet-derived and insulin-like growth factors on periodontal wound healing. J Periodontol 1991;62:458–467.
88. Rutherford RB, Ryan ME, Kennedy JE, Tucker MM, Charette MF. Platelet-derived growth factor and dexamethasone combined with a collagen matrix induce regeneration of the periodontium in monkeys. J Clin Periodontol 1993;20:537–544.
89. Ripamonti U, Reddi AH. Periodontal regeneration: potential role of bone morphogenetic proteins. J Periodont Res 1994;29:225–235.

90. Ten Cate AR. The role of epithelium in the development, structure and function of the tissues of tooth support. Oral Diseases 1996;2:55–62.
91. Hammarström L. Enamel matrix, cementum development and regeneration. J Clin Periodontol 1997;24:658–668.
92. Venezia E, Goldstein M, Boyan BD, Schwartz Z. The use of enamel matrix derivative in the treatment of periodontal defects: a literature review and meta-analysis. Crit Rev Oral Biol Med 2004;15:382–402.
93. Gestrelius S, Andersson C, Lidstrom D, Hammarstrom L, Somerman M. In vitro studies on periodontal ligament cells and enamel matrix derivative. J Clin Periodontol 1997;24:685–692.
94. Van der Pauw MT, Van den Bos T, Everts V, Beertsen W. Enamel matrix-derived protein stimulates attachment of periodontal ligament fibroblasts and enhances alkaline phosphate actvity and transforming growth factor beta1 release of periodontal ligament and gingival fibroblasts. J Periodontol 2000;71:31–43.
95. Haase HR, Bartold PM. Enamel matrix derivative induces matrix synthesis by cultured human periodontal fibroblast cells. J Periodontol 2001;72:341–348.
96. Melcher AH. Cells of the periodontium: their role in the healing of wounds. Ann R Coll Surg Engl 1985;67:130–131.
97. Pitaru S, McCulloch CA, Narayanan SA. Cellular origins and differentiation control mechanisms during periodontal development and wound healing. J Periodont Res 1994;29:81–94.
98. McCulloch CA. Progenitor cell populations in the periodontal ligament of mice. Anat Rec 1985;211:258–262.
99. Lekic P, McCulloch CA. Periodontal ligament cell population: the central role of fibroblasts in creating a unique tissue. Anat Rec 1996;245:327–341.
100. 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.
101. 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.
102. Trubiani O, Di Primio R, Traini T, *et al.* Morphological and cytofluorimetric analysis of adult mesenchymal stem cells expanded ex vivo from periodontal ligament. Int J Immunopathol Pharmacol 2005;18:213–221.
103. Ivanovski S, Haase HR, Bartold PM. Isolation and characterisation of fibroblasts derived from regenerating human periodontal defects. Arch Oral Biol 2001;46:679–688.
104. Luan X, Ito Y, Dangaria S, Diekwisch TG. Dental follicle progenitor cell heterogeneity in the developing mouse periodontium. Stem Cells Dev 2006;15:595–608.
105. Techawattanawisal W, Nakahama K, Komaki M, Abe M, Takagi Y, Morita I. Isolation of multipotent stem cells from adult rat periodontal ligament by neurosphere-forming culture system. Biochem Biophys Res Commun 2007;357:917–923.
106. Vacanti CA, Langer R, Schloo B, Vacanti JP. Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation. Plast Reconstr Surg 1991;88:753–759.
107. Narem R, Sambanis A. Tissue engineering: from biology to biological structures. Tissue Eng 1995;1:3–13.
108. Brekke JH, Toth JM. Principles of tissue engineering applied to programmable osteogenesis. J Biomed Mater Res 1998;43:380–398.
109. Whang K, Healy KE, Elenz DR, *et al.* Engineering bone regeneration with bioabsorbable scaffolds with novel microarchitecture. Tissue Eng 1999;5:35–51.
110. Vanheusden AJ, Goffinet G, Zahedi S, Nusgens B, Lapiere CM, Rompen EH. In vitro stimulation of human gingival epithelial cell attachment to dentin by surface conditioning. J Periodontol 1999;70:594–603.
111. van Dijk LJ, Schakenraad JM, van der Voort HM, Herkstroter FM, Busscher HJ. Cell-seeding of periodontal ligament fibroblasts. A novel technique to create new attachment. A pilot study. J Clin Periodontol 1991;18:196–199.
112. Lang H, Schuler N, Nolden R. Attachment formation following replantation of cultured cells into periodontal defects – a study in minipigs. J Dent Res 1998;77:393–405.
113. Kawaguchi H, Hirachi A, Hasegawa N, *et al.* Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. J Periodontol 2004;75:1281–1287.
114. Sonoyama W, Liu Y, Fang D, *et al.* Mesenchymal stem cell-mediated functional tooth regeneration in Swine. PLoS ONE. 2006;1:e79.
115. Ramseier CA, Abramson ZR, Jin Q, Giannobile WV. Gene therapeutics for periodontal regenerative medicine. Dent Clin North Am 2006;50:245–263.
116. Anusaksathien O, Webb SA, Jin QM, Giannobile WV. Platelet-derived growth factor gene delivery stimulates ex vivo gingival repair. Tissue Eng 2003;9:745–756.
117. Jin QM, Anusaksathien O, Webb SA, Rutherford RB, Giannobile WV. Gene therapy of bone morphogenetic protein for periodontal tissue engineering. J Periodontol 2003;74:202–213.
118. Jin Q, Anusaksathien O, Webb SA, Printz MA, Giannobile WV. Engineering of tooth-supporting structures by delivery of PDGF gene therapy vectors. Mol Ther 2004;9:519–526.
119. Dunn CA, Jin Q, Taba M. Jr, Franceschi RT, Rutherford RB, Giannobile WV. BMP gene delivery for alveolar bone engineering at dental implant defects. Mol Ther 2005;11:294–299.
120. Imperiale MJ, Kochanek S. Adenovirus vectors: biology, design, and production. Curr Top Microbiol Immunol 2004;273:335–357.
121. Brivanlou AH, Gage FH, Jaenisch R, Jessell T, Melton D, Rossant J. Stem cells. Setting standards for human embryonic stem cells. Science 2003;300:913–916.
122. King GN, King N, Cruchley AT, Wozney JM, Hughes FJ. Recombinant human bone morphogenetic protein-2 promotes wound healing in rat periodontal fenestration defects. J Dent Res 1997;76:1460–1470.
123. Bhatnagar RS, Qian JJ, Wedrychowska A, Sadeghi M, Wu YM, Smith N. Design of biomimetic habitats for tissue engineering with P-15, a synthetic peptide analogue of collagen. Tissue Eng 1999;5:53–65.
124. Jin QM, Zhao M, Webb SA, Berry JE, Somerman MJ, Giannobile WV. Cementum engineering with three-dimensional polymer scaffolds. J Biomed Mater Res A 2003;67:54–60.
125. Zhao M, Jin Q, Berry JE, Nociti FH Jr, Giannobile WV, Somerman MJ. Cementoblast delivery for periodontal tissue engineering. J Periodontol 2004;75:154–161.
126. Ripamonti U, Crooks J, Petit JC, Rueger DC. Periodontal tissue regeneration by combined applications of recombinant human osteogenic protein-1 and bone morphogenetic protein-2. A pilot study in Chacma baboons (Papio ursinus). Eur J Oral Sci 2001;109:241–248.
127. Maitra A, Arking DE, Shivapurkar N, *et al.* Genomic alterations in cultured human embryonic stem cells. Nat Genet 2005;37:1099–1103.
128. Martin MJ, Muotri A, Gage FH, Varki A. Human embryonic stem cells express an immunogenic nonhuman sialic acid. Nat Med 2005;11:228–232.
129. Draper JS, Pigott C, Thomson JA, Andrews PW. Surface antigens of human embryonic stem cells: changes upon differentiation in culture. J Anat 2002;200:249–258.
130. Bradley JA, Bolton EM, Pedersen RA. Stem cell medicine encounters the immune system. Nat Rev Immunol 2002;2:859–871.

131. Ivanovski S, Gronthos S, Shi S, Bartold PM. Stem cells in the periodontal ligament. Oral Dis 2006;12:358–363.

132. Nasef A, Mathieu N, Chapel A, *et al*. Immunosuppressive effects of mesenchymal stem cells: involvement of HLA-G. Transplantation 2007;84:231–237.

133. Yu J, Thomson JA. Embryonic stem cells. In: Regenerative medicine 2006. National Institutes of Health. Bethesda: Department of Health and Human Services, 2006:1–12.

134. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 2000;28:875–884.

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