PERIODONTAL LIGAMENT STEM CELLS - A REVIEW

Shetty, Thomas, Aga, & Nayak.

ABSTRACT

It is astonishing how quickly the field of genetics has progressed in the past 50 years since the structure of the DNA molecule was first reported. Another major discovery that may revolutionize health care practices has been the derivation of human embryonic stem cells. These cells can be coaxed into developing specific tissues or organs to replace ones that have been lost to disease.
The identification of putative mesenchymal stem cell populations within the periodontium has stimulated interest in the potential use of stem cell-based therapies to treat the damaged caused by trauma or periodontal disease. Here we will discuss the, stem cell like properties and characteristics of stem cells residing within the periodontal ligament and speculate on their future clinical utility.

INTRODUCTION

The concept that stem cells may reside in the periodontal tissues was first proposed almost 20 years ago by Melcher, who queried whether the three cell populations of the periodontium (cementoblasts, alveolar bone cells and periodontal ligament fibroblasts) were ultimately derived from a single population of ancestral cells or stem cells1, 2. The putative presence of stem cells within the periodontal ligament has since been repeatedly referred to in the literature. However, little direct evidence has been provided to support this concept. The most compelling evidence that these cells are present within the periodontal tissues has been provided by the in vivo and histological studies of McCulloch and coworkers.3-6
Since periodontal regeneration is essentially a re-enactment of the development process including morphogenesis, cytodiferentiation, extracellular matrix production and mineralization, such processes support our concept that some mesenchymal stem cells remain within the periodontal ligament and are responsible for tissue homeostasis, serving as a source of renewable progenitor cells generating cementoblasts, osteoblasts and fibroblasts throughout adult life. In the event of injury to the periodontium these mesenchymal stem cells could be activated towards

terminal differentiation and tissue repair or regeneration. 7
The identification of putative mesenchymal stem cell populations within the periodontium has stimulated interest in the potential use of stem cell-based therapies to treat the damaged caused by trauma or periodontal disease. Here we will discuss the stem cell, like properties and characteristics of stem cells residing within the periodontal ligament and speculate on their future clinical utility.

Periodontal Ligament Stem Cells (PDL SCs) 8

Periodontal ligament is a specialized connective tissue that connects cementum and alveolar bone to maintain and support teeth in- situ and preserve tissues homeostasis. PDLSCs have the capacity to generate cementum / periodontal ligament like structure and contribute to periodontal tissue repair. PDLSC differentiate into cementoblasts like cells, adipocytes and collagen forming cells, when transplanted into immunocompromised rodents.
The findings in a recent study by Seo et al (2004)9 suggest that periodontal ligament contains stem cells that have the potential to generate cementum/periodontal ligament like tissue in vivo. Transplantation of these cells, which can be obtained from an easily accessible tissue resource and expanded ex vivo, might hold promise as a therapeutic approach for reconstruction of tissues destroyed by periodontal diseases.

Identification of periodontal stem cells1

Mesenchymal stem cells were first, identified in aspirates of adult bone marrow. Friedenstein and colleagues developed clonogenic clusters of adherent fibroblastic-like cells or fibroblastic colony-forming units with the potential to undergo extensive proliferation in vitro and to differentiate into different stromal cell lineages.10-17
Using the above criteria, recently, cells have been identified that could be classified as mesenchymal stem cells, derived from adult periodontal ligament (periodontal ligament stem cells).
Seo BM et al (2004) showed that the periodontal ligament stem cells exhibited the capacity to generate clonogenic adherent cell colonies when plated under the same growth conditions as described for bone marrow stromal stem cells18.
Interestingly, the incidence of fibroblastic colony-forming units (aggregates of 50 cells or more) derived from periodontal ligament was greater than that recorded for bone marrow (170 for periodontal. ligament stem cell., and 14 for bone marrow stromal stem- cells per 105 cells plated). Whether this represents a propensity for stem cells to be present within this tissue remains to be established.

Growth potential of periodontal ligament stem cells1

In the past, cloning of periodontal ligament fibroblasts has met with little success and resulted in the need to resort to viral transfection to immortalize clonogenic cell lines.19 However, now specific culture conditions have been established to obtain some clones with high proliferative capacity as well as determine that the majority of individually isolated colonies (> 80%) fail to proliferate beyond 20 population doublings. Thus, the high proliferating periodontal ligament

stem cells are representative of only a minor proportion of the cells which can be expanded in vivo over successive cell passages. As detailed above, the periodontal ligament stem cell cultures exhibit approximately 30% higher rates of proliferation compared to the growth of cultured bone marrow stromal stem cells. It appears that these cells maintain this capacity of higher growth potential beyond 100 population doublings before in vitro senescence is noted. This compares to an in vivo senescence rate of approximately 50 population doublings for bone marrow stromal stem cells. It has yet to be established whether this property of periodontal ligament stem cells is similar to that of dental pulp stem cells, where there are increased levels of the cell cycle activator, cyclin-dependent kinase 6, and the mitogen, insulin-like growth factor-2, both known mediators of cell cycle progression from G1 to the start of DNA synthesis.20,21

Notwithstanding the high proliferative potential of the periodontal ligament stem cells, these cells still undergo senescence and thus are considered to have a finite lifespan. This appears to be a feature of most postnatal stem cells that markedly separates them from embryonic stem cells, which are virtually immortal.

Burns JS et al (2005) has indicated that human peripheral fat-derived mesenchymal stem cells have a higher proliferation rate than bone marrow stromal stem cells and are prone to spontaneous immortalization following extensive cell passage. The immortal nature of embryonic stem cells is related to their high expression of the enzyme telomerase, which is important for maintaining telomere lengths and chromosomal stability during cellular division22. Telomerase activity is absent in many mesenchymal stem cells. This may be important. for prolonging cellular senescence by regulating a number of key cell cycle regulators, which permit progression within the cell cycle from G1 to S phase, leading to an increased proliferation potential and survival rate.

Recently, Simonsen JL et al (2002) demonstrated that if bone marrow stromal stem cells are induced to express active telomerase their lifespan was increased almost threefold23 . Thus, the potential exists to develop strategies to genetically manipulate ex vivo expanded mesenchymal stem cells, such as periodontal ligament stem cells, to enhance and regulate their growth properties with a view to clinical applications. Nonetheless, such an approach should be viewed with caution since a recent study has indicated that some over- expressing-telomerase bone marrow stromal stem cell clones can develop into tumors24.

Characterization and origin of periodontal stem cells1

During embryogenesis, the periodontal ligament is formed by cells residing within the dental follicle. These cells are considered to be derived from the ectomesenchyme. Whether they are similar to the mesenchyme from which bone marrow stromal stem cells are derived is unclear, but it is interesting to note that the putative stem cell marker, STRO-1, used to isolate and purify bone marrow stromal stem cells, is also expressed by human periodontal ligament stem cells and dental pulp stem cells. Thus, such expression can be used to isolate human mesenchymal stem cells using immunomagnetic or fluorescence activated cell selection. In addition, periodontal ligament stem cells also share a common expression of the perivascular cell marker CD 146 with bone marrow stromal stem cells. A proportion of these cells also coexpress alpha-smooth muscle actin and/ or the pericyte-associated antigen, 3G5. These observations imply a perivascular origin for these cells and such an origin is indeed consistent with the earlier findings of McCulloch et al (1987)25 who demonstrated the presence of progenitor cells residing within the perivascular spaces of mouse periodontal ligament. Therefore, despite the different embryonic

origins of' bone marrow stromal stem cells and periodontal ligament stem cells, these independently unique stem cell populations appear to reside in the common milieu of the perivascular niches within their respective tissues.

Previous studies have analyzed the phenotypic characteristics of batch cultures, single clone cultures of cells and mesenchymal stem cells expanded in vitro from dental pulp, periodontal ligament, cementum, alveolar bone and bone marrow. Of particular significance has been the failure of these studies to detect the hematopoetic markers CD14 (monocyte/macrophage), CD45 (common leukocyte antigen) and CD34 (hematopoietic stem/progenitor cells/endothelium) in periodontal ligament stem cells or bone marrow stromal stem cells. However, many mature mineralized tissue markers, including alkaline phosphatase, type 1 collagen, osteonectin, osteopontin, osteocalcin and bone sialoprotein are expressed by these cells. In addition, it seems these cells have the potential to express a variety of antigens associated with endothelium (CD106), perivascular tissue (α-smooth muscle actin,

CD146,3G5), as well as general soft connective tissues proteins such as type I and III collagens . The expression of common proteins implicates the existence of common molecular pathways regulating cementum and bone formation, as proposed by Thesieff and colleagues 26.
A significant challenge confronting the characterization of periodontal ligament stem cells has been finding specific markers associated with either periodontal ligament or cementum. One possibility may be to screen these cells for the presence of the cementum-specific protein cementum attachment protein or cementoblastoma protein or periodontal ligament associated proteins (PLAP-I;).
As the periodontal ligament shares some morphological and functional features with tendon, such as dense collagen bundles and the ability to absorb mechanical forces of stress and strain, P Mark Bartold et al (2006) examined the expression levels of scleraxis, a tendon-specific transcription factor, in human cultured periodontal ligament stem cells. Using semiquantitative reverse transcription- polymerase chain reaction, these studies showed that periodontal ligament stem cells expressed measurably higher levels of scleraxis transcripts when compared with bone marrow stromal stem cells. These data imply that periodontal ligament stem cells represent a unique population of postnatal stem cells distinct from bone marrow-derived mesenchymal stem cells1.
To better characterize the periodontal ligament stem cells, studies are now underway to establish the genotypic and protein expression profiles of the periodontal ligament stem cells using EDNA microarray and proteomic technologies. It is hoped that this analysis will help facilitate the identification of key, molecules that distinguish these cells as well as other molecules which might participate in the development of bone, cementum and periodontal ligament.

Differentiation potential of periodontal ligament stem cells1

Gronthos S (1994) have demonstrated that human bone marrow stromal stem cells can, in the presence of inductive media containing ascorbic acid, dexamethasone and an excess of inorganic phosphate, be induced to form mineralized deposits in vitro, which are physiologically similar to hydroxyapatite in vivo27.
In accord with these findings, the capacity to form mineralized deposits in vitro has been demonstrated for a subpopulation of cells derived from primary explants of periodontal ligament

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More recently, Seo BM et al (2004) have demonstrated that human periodontal ligament stem cells exhibited a similar capacity to form Alizarin Red-positive mineralized deposits in vitro under the same conditions . The multipotential capacity of periodontal ligament stem cells has also been demonstrated by their ability to form Oil-red 0-positive lipid-containing clusters of fat cells when cultured in the presence of adipogenic inductive medium18.
The next step in characterizing the periodontal ligament stem cells has been to determine their capacity to form an organized, functional tissue following implantation in vivo. In general, it appears that these cells require a suitable scaffold, such as hydroxyapatite/tricalcium phosphate, to induce the formation of bone, dentin and cementum in vitro . Thus, when periodontal ligament stem cells are incorporated into a hydroxyapatite/tricalcium phosphate scaffold and implanted subcutaneously into immunocompromised mice, a typical cementum/periodontal ligament-like structure forms. In addition, these xenografts formed a type 1 collagen-positive periodontal ligament-like tissue within the transplants connecting with the newly formed cementum, morphologically similar to Sharpey's fibers.
Using human-specific antimitochondria antibodies, the cells responsible for the regeneration of these tissues in the xenografts have been clearly identified as being of human origin.
Interestingly, ex vivo expanded periodontal ligament derived fibroblastic colony-forming unit clones demonstrate a degree of hetereogencity in their morphological cha.-acteristics, differentiation potential and proliferative capacities. This indicates that within the total fibroblastic colony forming unit population there is a mixture of stromal progenitor cells at various stages of development that are most likely maintained by a minor population of multipotential, mesenchymal stem cells with the capacity for self-renewal, as previously described for the bone marrow stromal stem cell system.

Periodontal therapies for periodontal regeneration1

Once tissue destruction has occurred, one of the major goals of periodontal therapy is to regenerate the affected tissues to their original architecture and function. Many surgical procedures have been advocated for periodontal regeneration. Most recently, synthetic barrier membranes have been used to encourage appropriate progenitor cell population of the wound site . This procedure has demonstrated potential for regeneration of the root surface cementum, alveolar bone and periodontal ligament. Unfortunately, the clinical results using this method vary greatly and are often unpredictable.
With improving understanding of the molecular processes associated with tissue repair and regeneration, polypeptide growth factors applied to root surfaces have been used to facilitate periodontal regeneration. To date, these have included epidermal growth factor, fibroblast growth factor, insulin like growth factor, platelet-derived growth factor, tumor-derived growth factor and bone morphogenetic proteins28. Combinations of growth factors such as those present in platelet rich plasma preparations may also be useful in promoting periodontal regeneration. However, the current literature concerning the clinical outcomes of using such combinations is still scant.
At the same time that polypeptide growth factors were being considered for periodontal regeneration, another approach was being developed based on our understanding of tooth root formation and, in particular, cementum formation. While the precise molecular mechanisms of cementum formation are still unclear, one theory (yet to be fully accepted) suggests that a special

matrix is deposited on the newly formed dentin surface that is instrumental in permitting the attachment and differentiation of progenitor cells into cementoblasts. Extracts of this matrix have been applied to root surfaces at the time of periodontal surgery with the aim of inducing periodontal regeneration through the recreation of the molecular events of cementogenesis. Whether these proteins act as instructional messengers, similar to growth factors, for cells to undergo the processes of regeneration, or merely as a scaffold permitting regeneration to proceed is unclear. Nonetheless, clinical results have been encouraging and these proteins appear capable of promoting regeneration of periodontal tissues, albeit not in a completely predictable or consistent manner29,30.

Thus, key factors in attaining successful periodontal regeneration are the correct recruitment of cells to the site and the production of a suitable extracellular matrix consistent with the periodontal tissues. Since cell seeding to enhance regeneration of other tissues (skin, cartilage, bone, cardiovascular components, pancreas, etc.) has been used successfully, it seems logical that autologous periodontal ligament stem cells cultured within a suitable delivery scaffold, in conjunction with the growth and differentiation factors present in an autologous blood clot, will lead to new periodontal tissue attachment via a tissue engineering approach.

Cell seeding and tissue engineering1

Tissue engineering, aimed at developing techniques for the fabrication of new tissues to replace damaged or diseased tissues, is based on principles of cell biology, developmental biology and biomaterials. Recent advances in growth factor biology and biodegradable polymers have set the stage for successful tissue engineering of cartilage, bone and other tissues, of which the periodontium could be considered a prime candidate for such procedures. Studies to date have shown that periodontal ligament cells can he transplanted into periodontal defects with no adverse immunologic or inflammatory consequences31-33. More recently, cementoblasts as well as various periodontal cells transfected with vectors for over expression of various growth factors have been investigated in periodontal tissue engineering models34. A tissue engineering strategy for periodontal regeneration that exploits the regenerative capacity of stem cells residing within the periodontium is an attractive thesis. By using such an approach the need for recruitment of cells to the site is negated and the predictability of the outcome may be enhanced.

Future Implications35,36:

Researchers and physicians are working to design stem cells therapies that are- more effective, reduce invasiveness and the risk to the patients and to avert donor cell rejection by patient's immune system. In future it may be possible for a person to use a sample of his or her own stem cells, to regenerate tissue, which will reduce / even eliminate danger of rejection.

The possible ways are by collecting healthy adult stem cells from a patient and manipulating them in the laboratory to create new tissue. Then the new tissue is transplanted back into patients body where it would work to restore lost function. Therapeutic cloning, as told earlier, might enable the creation of embryonic stem cells that are genetically identical to the patients. This is achieved by manipulating existing stem cells within the body to perform therapeutic tasks. E.g. By designing a drug that would direct a certain type of stem cell to restore a lost function inside the patient's body (gene therapy). Transplanted skeletal or dental stem cells may one day be used

to repair craniofacial bone or even repair or regenerate teeth.
Some potential uses may be: Gene therapy & Sequencing genomes, Genetic engineering of organisms, food crops etc. Therapeutic cloning technology may be used in humans to produce whole organs from single cell or to produce healthy cells that can replace damaged cells.

Conclusion8,1

The prospect of improved regeneration is not only the promise held out by stem cell research, critical studies of unique aspects of early human development are now within reach with the use of embryonic stem cells. Although our understanding of the molecular pathways underlying mesenchymal stem cells differentiation is expanding, translation of this knowledge into tissue engineering strategies remains in its infancy. In the context of orofacial tissue engineering, populations of stem cells that form bone, cementum; dentin and even periodontal ligament have been identified. This has unlocked a new direction of research to restore the form and function of the oral cavity using autologous cells, thereby preventing histocompatibility mismatch and transmission of disease.
With the first reports of adult human stem cell populations residing in the periodontal ligament beginning to emerge, the next phase will be to determine the clinical utility of these cells. Accordingly, further studies are now required to determine the efficacy of ex vivo expanded stem cells to repair periodontal defects. A significant issue will he finding ways to identify and maintain multipotential stem cells in vitro. The growth and differentiation conditions that induce lineage specific commitment will need to be established. In addition, suitable carriers and inductive factors able to help implants integrate into the surrounding environment for the reconstruction of functional complex organ systems will need to be developed. It is expected that a multilevel approach involving cell biologists, matrix biologists, pharmacologists, biomaterials scientists/engineers and nanotechnologists will be required to address many of these issues

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AUTHOR: DR. NEETHA .R. SHETTY
ASSISTANT PROFESSOR
DEPT. OF PERIODONTICS
MCODS, MANGALORE
MOBILE: 9880858025

CO- AUTHORS: DR. BIJU THOMAS
PROF AND HEAD
DEPT. OF PERIODONTICS
ABSMIDS, MANGALORE

DR. ASKAR AGA
PG, DEPT. OF PERIODONTICS
ABSMIDS, MANGALORE

DR. DILIP NAYAK
PROF AND HEAD
DEPT. OF PERIODONTICS
MCODS, MANGALORE

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