Review Article

Stem Cells: A Promising Approach for 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. Stem cell biology, an emerging field of research, provides promising methods in vitro as well as in vivo in animal models, which make speculation about a future application in human dentistry reasonable. This opens the way for new cell-based therapies for periodontal regeneration. This review provides brief insights about stem cell basics, the state of art in human dental stem cell research and their potential role in periodontal regeneration.
Key Words: Periodontal Regeneration, Stem Cells

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Introduction

Periodontal regeneration is the reproduction or reconstitution of a lost or injured part of the periodontium so that the form and function of lost structures is restored. Many procedures have been attempted to achieve periodontal regeneration, including root surface conditioning, bone graft placement, guided tissue regeneration (GTR) and growth factor application.

Current regenerative procedures that are used either alone or in combination have limitations in attaining complete and predictable regeneration, especially in advanced periodontal defects and the results in clinical applications vary greatly, depending on the individual anatomy of the defects or the amount of resident vital Periodontal Ligament (PDL). The strategy of periodontal tissue regeneration therapies has been to control inflammation and stimulate stem progenitors to regenerate new periodontal tissues.[1,2]

Recently, numerous studies have emerged from researchers in different fields suggesting the unthinkable – that stem cells isolated from a variety of organs are capable of ignoring their cell lineage boundaries and exhibiting more plasticity in their fates. Plasticity is defined as the ability of post-natal (tissue specific adult) stem cells to differentiate into mature and functional cells of the same or of a different germ layer of origin.

Periodontitis is one of the most prevalent infectious diseases and is characterized by

the destruction of tissues, such as alveolar bone, cementum and the PDL, that surround and support the teeth. The goal of periodontal therapy is the complete regeneration of periodontal tissues, particularly where gross periodontal destruction has compromised the support of the tooth.[3]

In dentistry, the identification of mesenchymal stem cells (MSC)-like populations from both dental and non-dental tissues has presented exciting possibilities for the application of tissue engineering as well as gene-based therapies. Current evidence supports the concept that periodontal regeneration is promoted through the activation of cells in the remaining healthy portion of the PDL as well as in the perivascular region.[1,4]

As these progenitor cells can do elsewhere in the body, the same effect they can reproduce in dental tissues. So the relevance of stem cells from various dental and non-dental sources in periodontal regeneration cannot be ignored.

Periodontal Tissue Engineering:

Tissue engineering is a contemporary area of science based on the principles of cell biology, bioengineering, biomaterials, biochemistry, and biophysics to solve clinical and surgical problems related to tissue loss and organs' functional failures. Tissue engineering using Mesenchymal Stem Cells (MSCs) is a recent therapeutic option with several advantages.[5]

These include high-quality regeneration of damaged tissues without forming fibrous tissue, minimum donor-site morbidity compared to autografts, and low risk of autoimmune rejection and disease transmission. [5,6,7]

Therefore, as undifferentiated cells capable of self-renewing at a high rate of proliferation, and differentiating into multiple cell lineages including mesodermal, endodermal, and ectodermal cells, MSCs represent a valuable resource for tissue engineering. They defined them as a population of postnatal stem cells hierarchically organized with the capacity to differentiate into specialized cells of at least one mesenchymal lineage, such as bone, cartilage, fat, muscle, or neuronal cells. [8-12]

Tissue-engineering strategies have been applied to reconstruct damaged periodontal apparatus, either by reiteration of tooth development based on epithelial–mesenchymal tissue recombination or by seeding of cells on biomaterial scaffolds. The tissue-recombination technique aims to replicate key reciprocal interactions between the dental epithelium and the ectomesenchyme during odontogenesis to regenerate the periodontium.[13-17]

However, the periodontal structures regenerated using tissue-recombination techniques are not formed in isolation from other dental tissues and this may pose problems for implantation into periodontal defects. In addition, there is currently no suitable substitute for the embryonic epithelial compartment of the engineered tooth germ, and the use of human embryonic tissues for periodontal engineering may limit the practical application of this approach.

Stem Cells

The stem cell is the origin of life. As stated first by the great pathologist Rudolph Virchow, "All cells come from cells." The ultimate stem cell, the fertilized egg, is formed by fusion of the haploid progeny of germinal stem cells. The fertilized egg is totipotent; since it forms all the tissues of the developing embryo. In the adult, tissue is renewed by the proliferation of specialized stem cells, which divide to form one cell that remains a stem cell and another cell that begins the process of differentiation to the specialized function of a mature cell type. Stem cells are defined by their potential to self-renew and differentiate into more specialized cell types within a given tissue.[18]

Certain terms need to be known before

understanding stem cells:

- **Progenitor cell:** It is an undifferentiated precursor cell with the capacity to undergo differentiation into specialized cell types; unlike putative stem cells, they do not retain the capacity for self-renewal.
- **Multipotent stem cells:** They are cells that self-renew and differentiate into several different specialized cell types, often within a tissue (e.g., hematopoietic stem cells).
- **Pluripotent stem cells:** They are cells capable of self-renewing and differentiating into any of the three germ layers (endoderm, ectoderm, and mesoderm).
- **Totipotent stem cells:** They are cells derived from the first few divisions of the fertilized egg, which have the potential to give,rise to all the differentiated cells of the fully developed organism.
- **Embryonic stem cells:** They are responsible for the development of an entire organism.
- **Adult stem cells:** They provide a mechanism for maintenance of tissue homeostasis by replacing damaged cells throughout the life of the organism.

Properties of both adult and embryonic stem cells:

Self-renewal: It is defining property of stem cells that allows them to undergo repeated mitotic cell divisions to create at least one daughter cell equivalent to the mother cell that retains latent capacity for differentiation.

Clonogenicity: A stem cell is thought to be "clonogenic," which means that it can proliferate to form a colony of cells.[18-20]

Sources of Dental Stem Cells[18]

The ultimate goal of tooth regeneration is to replace the lost structure. Dental stem cells can be obtained from following tissue:

- Bone marrow stromal cells(BMMSC)
- Human Pulp Tissue (DPSC's, post-natal dental pulp stem cells)
- Exfoliated Deciduous Teeth (SHED)
- Periodontal Ligament (PDLSC)
- Apical Papilla (SCAP)
- Dental Follicle Precursors (DFPC)
- Mesenchymal stem cells from gingival

The PDL seems to possess the ability to re-establish lost periodontal tissues because PDL cells have many osteoblast/cementoblasts–like properties, including the capacity to form mineralized nodules *in vitro,* expression of the bone/cementum–associated markers (alkaline phosphatase [ALP] and bone sialoprotein [BSP]), and response to bone inductive factors, such as bone morphogenetic protein 2, parathyroid hormone, and transforming growth factor-β.[21-23] Nevertheless, considering the complexity of the PDL attachment apparatus and the heterogeneity of its cell populations, the identification of the appropriate cell types necessary for complete regeneration remains one of the key factors in implementing optimal approaches to periodontal regeneration. Heterogeneous MSCs isolated from human PDL harvested from permanent teeth have emerged as a possible cell source for periodontal regeneration. Postnatal PDL stem cells express mesenchymal surface markers, such as Stro-1, CD105 (Endoglin, SH2 antigen), CD146 (MUC 18), and CD166 (ALCAM, SB10 antigen), and have a multipotent capacity to differentiate into adipocyte, osteoblast- like, and cementoblast-like cells in vitro, and to form cementum/PDL–like tissues.[21,22,24] Cells with characteristics of putative MSCs have been found in regenerating periodontal tissues, implying their involvement in periodontal regeneration.[25] Stem cell properties also have been observed in cells derived from other dental tissues, including the pulp of human exfoliated deciduous teeth. *Ex vivo* expanded stem cells from human exfoliated deciduous teeth were found to have a high proliferative capacity and express vascular-related markers, such as basic fibroblast growth factor and endostatin; in addition, these cells

are capable of generating robust amounts of bone *in vivo.*[26-28]

These findings suggest that deciduous teeth may constitute a source of stem cells for potential clinical applications. In the present study, the in vitro biologic properties of highly purified mesenchymal progenitor cell (MPC) subsets harvested from the PDL of deciduous and permanent teeth are comparatively assessed. MPCs have also been isolated from the dental follicle of human third-molar teeth. These fibroblast-like, colony-forming and plastic-adherent cells express stem cell markers (STRO-1 and nestin) and can be maintained in culture for at least 15 passages. STRO- 1-positive dental follicular progenitors have been shown to differentiate into cementoblasts *in vitro*and to form cementum *in vivo.* The finding that immortalized dental follicle cells can generate PDL - like tissue after *in vivo* implantation implies that dental follicular progenitor cells may be a useful research tool for studying PDL formation and for regenerative periodontal therapies.[29, 30]

Potential clinical applications for human dental-derived stem cells in periodontal regeneration

Use of mobilized peripheral blood stem cells has been a recognized therapeutic approach for the reconstitution of hematopoietic bone marrow in cancer patients undergoing myeloablative therapy. The favorable outcome reported for this therapy has led to studies of other stem cell populations, including the dental-derived stem cells, as potential novel cellular-based therapies for a number of diseases and congenital defects.

The presence of different stem cells with respect to their differentiation level, expression of mesenchymal stem cell markers, and multipotency, residing in dental tissues invites further clinical investigation into regeneration of tissues of the orofacial region, including the periodontium, using these cells.

Among all the dental-derived stem cells identified, PDLSCs are a unique population capable of forming an ectopic cementum/PDL-like structure. SHEDs, another population of dental-derived stem cells, were observed in immunocompromised mice to induce cells to differentiate into osteoblasts and osteocytes, resulting in the synthesis of new bone. However, this cell strain was unable to form periodontal ligament and root cementum. These cells were able to create in vitro a structure similar to a periodontal membrane composed of fibroblast phenotype cells and calcified structures, full of alkaline phosphatase and bone sialoprotein.[19,31]

Factors like adhesion molecules, growth factors, and extracellular matrix present in the lesions might have stimulated the differentiation of transplanted cells into functional and specialized cells. These results, therefore, suggest that both dental and non-dental derived stem cells might be potentially applied in regenerative periodontal therapies. Thus, further studies are needed to investigate how progenitor cells participate in the process of periodontal regeneration, and how the environment of the lesions regulates cell activities.[19]

Stem Cell Mediated Gene Therapy

Gene therapy relies on genetic engineering involving molecular techniques to introduce, suppress or manipulate specific genes, thereby directing an individual's own cell 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 the efficacy of topical application of these factors (e.g. a short duration of action) can be overcome.[1,32]

Gene-delivery techniques have been used in periodontal ligament and alveolar bone regeneration in rats, and embryonic stem cells have been shown to express tooth-

initiation genes in mice.[33-35]

Two potential strategies for delivering therapeutic transgenes into human recipients are:

- The direct infusion of the gene of interest using viral or non-viral vectors in vivo.
- The introduction of the gene into delivery cells (often a stem cell) outside the body ex vivo followed by transfer of the delivery cells back into the body.

The inherent proliferative and pluripotent capabilities of stem cells may offer life-long opportunities for treatment by repairing, replacing or regenerating the damaged tissues.

The use of adenoviral vectors to enable the over-expression of growth-promoting molecules, such as platelet-derived growth factor and bone morphogenetic protein-7, has been investigated for its potential in periodontal regeneration.[36, 37]

Sustained release of bone morphogenetic protein-7 and platelet-derived growth factor by transformed cells implanted into experimental periodontal defects results in enhanced regeneration of bone and cementum. The use of such technology with dental stem cells may offer an alternative to conventional methods as a result of their ability to provide a renewable source of growth factor release for regeneration. STRO-1- selected rat dental pulp stem cells transduced with bone morphogenetic protein-2 demonstrate enhanced odontogenic differentiation compared with non-transduced cells.[38]

However, much work is still needed to optimize the number of cells that are virally transduced and to maximize the duration and extent of gene expression. Further research is also needed to address the potential risks of viral recombination and immune responses towards viral antigens, which may ultimately determine the success of gene-transfer techniques in periodontal regeneration. Many challenges remain in bringing stem cell research to clinical practice in humans, and it is important to overcome these challenges to fulfill our goal of treating periodontal diseases with stem cell-based therapy.[39-40]

Potential Challenges

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). The isolation and characterization of stem cells from periodontal tissues have provided a good starting point for understanding the role of progenitor cells in periodontal healing. However, we have incomplete understanding of the way that roots develop, and little is known about the signaling mechanisms that occur during this process.[41]

For periodontal regeneration to occur, we need to replicate the key cellular events that parallel periodontal development and to understand the specific cell types, the inductive factors and the cellular processes involved in formation of the periodontium. Given that the fate of stem cells is influenced by their interaction with the microenvironment (including soluble and immobilized factors, extracellular matrix and signals from neighboring cells), understanding the key components regulating the properties of stem cells may elucidate ways to expand stem cells properly and control their differentiation precisely.[2]

Technical challenges

The technical challenges in stem cell therapy are associated with cell manipulations, scaffold materials and delivery systems. Culture conditions are not sufficiently developed to mimic the cell microenvironment *in vivo* and to ensure that both cell proliferation and differentiation can be performed safely and consistently. Furthermore, as cell culture medium often requires xenogenic products (such as fetal

bovine serum or mouse feeder layers), cell cultures may not be completely free of pathogens and infectious risks are a concern.[42]

The search for the ideal biocompatible scaffolding materials and delivery system is a significant technical pursuit. The ideal matrix scaffold should mimic native extracellular matrix, support cell attachment, allow controlled release of bioactive factors, be conducive to tissue in growth and facilitate laboratory handling.[43]

The establishment of an optimal culture condition free of potential cross-contaminations is relevant in producing clinical-grade human stem cell lines and for performing basic research involving the regulation of their self-renewal and lineage determination. Second, timing is an inherent constraint in cell therapy and tissue engineering. Some autologous construct based approaches may involve weeks to months of *ex vivo* processing. Although the use of stem cells can often minimize the processing time compared with somatic cells, possible karyotypic instability and gene mutations of the cells after prolonged culture can also limit their usefulness.

Clinical challenges

Clinical challenges in stem cell-based periodontal therapy relate to immune rejection after administration, oncogenic properties of stem cells and functional integration of transplanted tissues into the host. It is important to understand how the immune system will respond to stem cells or their derivatives upon transplantation.[44]

Generally, the immunogenicity of a human cell depends on its expression of class I and II major histocompatibility antigens, which allow the body to distinguish its own cells from foreign cells. Human embryonic stem cells express a low level of class I major histocompatibility antigens, but this expression is upregulated with differentiation. A potential solution to this problem lies in the use of autologous stem cells (from cell ⁄ tissue banks) to overcome immune rejection. Furthermore, the production of patient- specific pluripotent stem cells (or induced pluripotent stem cells) from adult somatic cells is now feasible and the differentiation of autologous induced pluripotent stem cells into cell types desired for transplantation is being explored.[45]

Recent findings relating to the immunosuppressive effects of MSCs both *in vitro* and *in vivo* have also raised the possibility of using allogenic stem cells without the need for donor and recipient cross matching.[46]

Finally, it is unclear whether human stem-cell derivatives can integrate into the recipient tissue and fulfill the specific functions of lost or injured tissues. It will be necessary to demonstrate that stem cells develop into stable cells and display the characteristics and functions of normal host cells following their transplantation. It is hoped that, as our knowledge on progenitor cells, growth factors and delivery systems improves, we will make stem cell based therapy a safe and effective approach in periodontal regeneration.[1]

Conclusion

Restoration of tissues destroyed by periodontitis to their original form and function has been a long-standing goal of periodontal therapy. However, our current available regenerative therapies are crude and of poor clinical predictability.

There is need for novel regenerative technologies to be developed based on contemporary understanding. In order for this to become a reality it will be necessary for us to obtain a complete understanding of periodontal development and the progenitor cells involved in this process. Subsequent tissue-engineering approaches may then be developed using these progenitor cells within a matrix scaffold, together with then introduction of various signaling molecules

in an orderly temporal and spatial sequence.

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