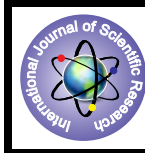


Scope of Stem Cells in Periodontal Regeneration



Medical Science

KEYWORDS : Periodontium, Mesenchymal Stem cells, Tissue engineering, Nanofibrous scaffolds, Growth factors

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ABSTRACT

Periodontal disease is responsible for the loss of tooth supporting periodontal structures such as alveolar bone and the periodontal ligament. Attempts to restore the damaged periodontal tissues based on the use of graft materials, barrier membranes and growth factors have not been successful due to their lack of significant regenerative potential. Recently, studies demonstrating the regenerative potential of dental and non dental mesenchymal stem cells have opened a new window in the field of periodontal regenerative therapy with a potential to overcome the drawbacks of conventional techniques. This review article aims to explore the types of stem cells, cell delivery systems such as nanofibrous scaffolds and tissue engineering techniques that have been investigated and the current challenges associated with the stem cell based periodontal regenerative therapies.

Introduction

The normal periodontium is a complex tissue comprising of hard tissues (cementum and alveolar bone) and soft tissues (gingival and the periodontal ligament). Periodontitis is a destructive process which results in virtually irreparable tissue loss. In the early stages of periodontitis, the tissues may show some reparative capacity however the advanced stages require surgical intervention. Regenerative procedures such as graft placement including autogenous bone grafts (Osseous coagulum, cancellous bone marrow transplants), allografts (decalcified freeze dried bone graft) and alloplastic materials (calcium phosphate biomaterials, bioactive glass and polymers), guided tissue regenerative techniques and root surface modification have variable clinical outcomes depending upon the degree of the damage. Recently, the concepts of tissue engineering and gene based therapy are emerging as effective alternatives to the conventional regenerative techniques.

The periodontal ligament contains a variety of cells including osteoblasts, cementoblasts, fibroblasts, endothelial cells, defense cells, nerve cells and epithelial cells. The ligament also contains a small population of the undifferentiated cells expressing cell surface markers and regenerative potential similar to the mesenchymal stem cells (1). Studies have shown that these periodontal ligament cells possess stem cell properties such as self renewal and, upon stimulation the ability to differentiate into different lineages. Recent experiments including ex vivo expansion of the stem cells from periodontal ligament and their transplantation in the surgical defects in animal models have demonstrated the regenerative potential of these cells (2). Development of new therapeutic approaches based on the knowledge of complex molecular and cellular biologic interactions could offer predictable alternatives to the existing therapies for periodontal regeneration. The aim of this article is provide an insight into the regenerative potential of these pluripotent cells and their possible clinical implications in periodontal regenerative therapy.

Dental Stem Cells

Stem cells are specialized entities capable of self renewal and differentiation into different cell types in response to appropriate stimuli. In 2000, Gronthos et al isolated MSC (mesenchymal stem cell) like cells from the human dental pulp. Since then, MSC like cells have been isolated from the dental follicle, periodontal ligament, apical papilla and from the pulp of the exfoliated deciduous teeth. These cells isolated from several locations have expressed cell markers similar to the Bone Marrow Stem Cells (BMSCs) and have also demonstrated ability to differentiate into several other cell types.

Dental Pulp Stem cells: These were the first stem cells to be

identified from the dental tissues. Studies have shown that these cells have fibroblast like morphology with expression of cell markers such as STRO-1, CD 146/MUC 18 similar to the Bone Marrow Stem Cells as well as embryonic markers such as Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-81 (2,3). When DPSCs were transplanted into immune compromised mice, they generated a dentin like structure lined with human odontoblast like cells that surrounded a pulp like interstitial tissue (4). DPSCs, when cultured in appropriate media are capable of differentiating into osteogenic, adipogenic, chondrogenic, neurogenic and myogenic cells (5). This differentiating and regenerative potential of DPSCs has established their promising role in regenerative and reparative medicine.

Periodontal Ligament Stem Cells: Periodontal ligament is a highly vascular complex connective tissue containing fibers and cellular elements. The regenerative potential of the periodontal ligament was first described by Nyman et al wherein preference was given to the periodontal ligament cells to repopulate the wound area adjacent to the root surface deprived of cementum and periodontal ligament in animal models (6). The formation of cementum and adherent connective tissue demonstrated that the periodontal ligament may contain cells having regenerative potential. The presence of slowly dividing 'progenitor' cells in the paravascular sites in the periodontal ligament was initially investigated by C.A.G McCulloch et al. Subsequently, several studies have been carried out demonstrating the regenerative potential of these cells. PDLSCs express stem cell markers such as STRO-1, CD 146/MUC 18, CD 105 and CD 166 similar to the mesenchymal stem cells derived from other human tissues such as bone marrow (1,2). Under appropriate cultural conditions, PDLSCs can differentiate into cementoblast like cells, osteoblasts, adipocytes and collagen forming cells (1,2). Postnatal stem cells can be recovered from cryopreserved human periodontal ligament, providing a practical approach to utilization of stem cells in regeneration of damaged periodontium (7). Since these cells can be obtained from an accessible tissue resource and expanded ex vivo, development of systematic techniques could offer innovative alternatives to the existing therapies of periodontal regeneration.

Dental Follicle Stem Cells: Dental follicle is a loose connective tissue of ectomesenchymal origin which surrounds the unerupted portion of the tooth. Handa et al were first to report the existence of progenitor stem cells in bovine dental follicle. Research has indicated that DFSCs are responsible for the development of the periodontium including cementum, periodontal ligament and alveolar bone (8). A recent study has shown that epithelial mesenchymal transformation does not occur in the Hertwig's root sheath in rat cementogenesis and that the dental follicle is the origin of the cementoblasts (9). Dental follicle

originates from the ectomesenchymal progenitor cells derived from the migratory cranial neural crest cell during the cap stage of tooth development. Apart from its role in tooth development, Dental Follicle also helps in tooth eruption (Cahill and Marks, 1980). It is retained as a sac of connective tissue surrounding the unerupted portion of the impacted tooth. Dental Follicular cells isolated from impacted third molar teeth have fibroblast like properties with expression of putative stem cell markers Notch-1 and Nestin (10). On isolation and expansion under favorable culture conditions, these cells are capable of differentiation into cementoblast like cells. Some studies have shown that these cells can also differentiate into neurons and adipocytes. Thus DFSCs can be used as a potential source for periodontal regenerative therapy.

Stem Cells from Human Exfoliated Deciduous Teeth: Stem cells can also be retrieved from another easily accessible source that is the exfoliated deciduous tooth. These cells are highly proliferative and clonogenic with capacity to differentiate into a variety of cell types including odontoblasts, adipocytes and neural cells (11). These cells have shown higher proliferation rate and a higher number of population doublings compared to Bone Marrow Stem Cells (BMSCs) and Dental Pulp Stem Cells (DPSCs) (11). Studies have shown that these highly proliferative cells possess the ability to differentiate into odontoblasts like cells in vitro (11). However these cells are unable to regenerate a complete dentin- pulp like complex like the Dental Pulp Stem Cells. Also they do not differentiate directly into osteoblasts but they induce bone formation by locally recruiting host odontogenic cells. These cells being capable of extensive proliferation and multipotential differentiation can be ideal resources for the repair of damaged oral tissues.

Application of Stem Cells in Periodontal Tissue Engineering

Tissue engineering is an interdisciplinary field that applies the principles of cell biology, biomaterials, bioengineering and biochemical expertise towards the development of biological substitutes that restore, maintain or improve the tissue function (12). Tissue engineering requires that the damaged tissue should be regenerated without significant fibrous tissue or scar formation with low risk of immune rejection reactions and disease transmission. Several in vitro and in vivo studies have demonstrated the ability of dental and non dental stem cells to regenerate the damaged periodontium. These techniques can be further refined for the application of this promising technology in periodontal regeneration.

Application of human periodontal ligament cells in the form of cell culture sheets in animal models has resulted in regeneration of periodontal ligament like tissues. In a study carried out by Masateru et al, HPDL cell sheets were transplanted into mesial dehiscence model in athymic rats resulting in the regeneration of periodontal ligament like tissue including an acellular cementum like layer with fibrils anchoring into this layer (36). Transplantation of autologous periodontal ligament cells combined with hydroxyapatite/ tricalcium phosphate into artificially created periodontal defects in miniature pigs resulted in regeneration of periodontal tissues including new bone, cementum and periodontal ligament (Liu Y, 2008) (37). Another study carried out by Akizuki et al demonstrated that application of periodontal ligament cell sheets reinforced with hyaluronic acid carrier to the periodontal defects in beagle dogs resulted in healing of the periodontal tissues with bone, cementum and periodontal ligament formation (38). A combination of stem cells from the apical papilla (SCAP) and periodontal ligament stem cells (PDLs) applied on a hydroxyl apatite/ tricalcium phosphate carrier were able to form cementum with Sharpey's fibres attached to the cementum (Sonoyama, 2008) (24). Studies have shown that non dental stem cells such as bone marrow mesenchymal stem cells (Kawaguchi H, 2004) and adipose derived stem cells (Tobita M, 2008) can regenerate cementum, alveolar bone and periodontal ligament like structures after expansion in vitro followed by transplantation in vivo (19, 20).

Periodontal regeneration can be carried out mainly by employing several methods such as tissue recombination techniques

involving reciprocation of the cellular events that occur in the periodontal development based on the understanding of the cell types, inductive factors and cellular processes involved in the formation of the periodontium, use of appropriate scaffolds or biomaterials containing the regulatory signals and progenitor cells or gene therapy based delivery of growth factors to the periodontal defects (13).

Tissue recombination technique replicates the interactions between the dental epithelium and the ectomesenchyme that occur during the development of the periodontium in the final stages of odontogenesis (13, 14). A study carried out by A. Ohazama et al demonstrated that recombinations between non dental cells (embryonic stem cells, neural stem cells and adult bone marrow stem cells) derived mesenchyme and embryonic oral epithelium of mice resulted in the development of tooth structures and bone tissue when transferred to the adult renal capsular tissues (15). The transfer of embryonic tooth primordia into the adult jaw resulted in the development of tooth structures indicating that an embryonic primordium can develop in its adult environment (15). However the periodontal tissues formed using recombination techniques are formed in association with other dental tissues and this may pose a problem while transplanting these tissues in the periodontal defects (13). The use of human embryonic tissues may therefore limit the application in periodontal regeneration.

Tissue engineering also requires an appropriate three dimensional scaffold or a carrier construct containing growth factors, cell regulatory signals and progenitor cells. Scaffolds allow the cells and the growth factors to be delivered and immobilized in a given location and serve as a template for tissue regeneration (16).

The three dimensional scaffold materials should have the following properties (16,17):

- 1) It should be highly porous with an interconnected pore network for cell growth and transport of growth factors.
- 2) Biocompatible and bioresorbable with controlled degradation and resorption rate.
- 3) Suitable surface chemistry for cell attachment, proliferation and differentiation.
- 4) It should be free from transmittable disease and should not evoke an immunologic or an exuberant inflammatory response.
- 5) Sufficient rigidity and strength to prevent the soft tissue collapse into the defect.

Synthetic polymer, bioceramics, biomembranes, polysaccharide hydrogel, Titanium mesh and collagen have been investigated as scaffold materials for tissue transplantation. Human periodontal ligament cells seeded in a porous Chitosan/Collagen scaffold prepared using freeze drying process and implanted subcutaneously in athymic mice demonstrated substantial proliferation of the ligament cells and the surrounding tissues into the scaffold (Y. Zhang, 2006) (22). Incorporation of the periodontal ligament cells with collagen sponge in class III furcation defects in dogs, demonstrated greater periodontal regeneration as compared to guided tissue regeneration techniques utilized in periodontal reconstruction (Suaid FF, 2012) (23). Stem cells from the apical papilla in miniature pigs were loaded into a root shaped hydroxyl apatite/tricalcium phosphate block containing an inner post channel space to allow installation of a porcelain crown (W. Sonoyama, 2008) resulting in significant regeneration of the root and periodontal structure (24).

Recently, the combination of nanofibrous materials with stem cells and growth modulatory factors has demonstrated successful results in animal models. The microporous nature of nano fibrous scaffolds accelerate the cell adhesion compared to smooth surface scaffolds and also allow the attachment of bioactive molecules such growth factors and drugs (18). Nanofibers are known to enhance cell adhesion possibly by increasing protein adsorption, integrin expression or by altering the signal pathways (21). Nanofibers structurally mimic the native extra-

cellular matrix thereby altering the cellular responses (similar to ECM cell interactions) such as adhesion, proliferation, differentiation and tissue regeneration (21). In a study carried out by Iwata et al, periodontal ligament cell sheets supported with woven polyglycolic acid were transplanted to the dental root surfaces with three wall periodontal defects resulted in regeneration of new bone and cementum with well oriented collagen fibers in the defects (25). Periodontal Ligament cells cultured in gelatin nanofibrous membranes fabricated by electro spinning of the aqueous gelatin solution demonstrated improved cell attachment, growth and proliferation (Zhang, 2009) (26). Collagen and Poly caprolactone fibers were dissolved and the solution was electro spun into a nanofibrous mesh (Jae Jun Lee, 2009) (27). The osteoblastic

cells cultured on the membrane exhibited enhanced initial adhesion, growth and penetrative abilities. Incorporation of metronidazole antibiotic in poly caprolactone nanofibers resulted in sustained drug release over a prolonged period of 19 days when evaluated for treating periodontal diseases (Zamani, 2010) (28). A study by Inanc et al demonstrated increased cell adhesion and osteogenic differentiating properties of periodontal ligament cells when cultured on the electro spun poly (DL-Lactic-co-glycolic acid) PLGA nanofiber membrane scaffolds (29). Self assembled peptide nanofibers induced the periodontal ligament fibroblasts to produce type I and type III collagen fibers indicating its use in periodontal tissue regeneration (Kumada Y and Zhang S, 2010) (30).

Combination of growth factors such as Epidermal growth factor, Fibroblast growth factor, Insulin-like growth factor (IGF), Platelet derived growth factor (PDGF) and Transforming growth factor- β (TGF- β) have been known to induce periodontal healing and regeneration. Application of PDGF and IGF-1 to the roots of periodontitis affected teeth in beagle dogs showed enhanced regeneration of the periodontal tissues (31). A family of polypeptide growth factors, the Bone Morphogenetic proteins can also induce osteogenesis and cementogenesis in the periodontal tissues (U. Ripamonti, 1994) (32). These factors combined with the progenitor cells in a suitable 3D tissue construct can be efficiently utilized for regeneration of the damaged periodontium.

Gene therapy is another new approach which is being investigated for the regeneration of periodontal tissues. In periodontal regenerative techniques, gene therapy can ensure the controlled delivery of growth promoting factors and signal molecules for extended periods of time in vivo. The delivery of transgenes can be carried out either by infusion of the gene using viral vectors such as Adenovirus or in biological microparticles like liposomes in vivo or by introduction of the gene into delivery cells (stem cells) outside the body followed by transfer of these genetically modified cells back into the patient's body (Zwaka, 2006) (33). In a study by Q.M Jin, dermal fibroblasts were transduced with Adenovirus encoding Bone Morphogenetic Protein 7 (BMP 7) followed by transplantation into large osseous defects in rat models (34). The osseous defects treated with the gene therapy demonstrated greater amount of bone and cementum formation compared to the control groups. The growth factors administered locally in the periodontal defects have a short half life due to rapid proteolytic activity. This drawback can therefore be overcome by the application of gene based therapy (Lin NH, 2009) (35).

Challenges in Periodontal Regeneration based on Stem Cell Therapy

The application of stem cell therapy for periodontal regeneration is based on the principles of tissue engineering involving the use of appropriate cell sources, scaffolds for cell expansion and inductive growth factors. Predictable and realistic approach based on sound biologic principles

still remains a distant goal because of the lack of thorough understanding of the processes involved in periodontal regeneration and difficulties in its clinical application. The current research on stem cells has been restricted to in vitro and animal studies which may not be applicable in human situation. Further investigations are required to improve our knowledge of the molecular and cellular pathways associated with stem cell expansion in vitro and differentiation in vivo. An incomplete understanding of the cell types, cell sources, growth factors and the cellular process has led to the difficulties in obtaining appropriate clinical grade cell lines for transplantation into the periodontal defects.

A suitable scaffold material allowing the incorporation of the progenitor cells and growth factors for complete periodontal regeneration needs further development. There are difficulties encountered in the expansion of stem cell lines free from contamination and gene mutations. Karyotypic instability and gene mutations may develop after prolonged culture. Further studies are needed to improve the understanding of the microenvironment that can induce stem cell differentiation and growth.

The major clinical challenges that need to be overcome to ensure the effectiveness of stem cell therapy are the possibilities of Immune rejection reaction and oncogenesis (Lin et al 2009) (35). Immune rejection is the most important host response towards the cell component of the engineered tissue containing allogenic or ex vivo expanded autologous cells. However a study by Gang Ding et al demonstrated that a sheet of periodontal ligament stem cells can repair allogenic bone defects in animal models without immunological rejection due to low immunogenicity and immunosuppressive function possessed by these cells. Autologous stem cells harbored from the periodontal ligament of third molar teeth and expanded ex vivo can overcome the possibility of immune rejection.

Prevention of oncogenesis or tumor formation is another important aspect which needs consideration during therapeutic application of the Stem cell based regeneration. If the stem cells remain in vitro for long duration of time for application in complex clinical situations, there are greater chances of accumulation of the genetic and epigenetic factors (Lin et al 2009) (35). Therefore, an improvement in the knowledge of the factors responsible for the gene mutations in a cell culture is necessary to prevent the hazardous outcomes of this therapy.

Furthermore, issues such as sustained delivery of the growth factors for a prolonged period of time and the regeneration of the periodontal tissues without scar or ectopic tissue formation (35) need to be addressed before this technique is utilized successfully for periodontal repair.

Conclusion

Periodontitis is a complex inflammatory disease resulting in the loss of the supporting periodontal tissues. A wide range of surgical techniques, graft materials, growth factors and barrier membranes have been used over the past several decades for the repair of the damaged periodontal tissues. However, the overall effects of these techniques have been unpredictable. Stem cell technology has the potential to overcome the limitations associated with the existing periodontal therapies and may provide a new direction for predictable and efficient tissue regeneration of the periodontium. Periodontal regeneration using stem cell therapy requires a complex interplay of many factors such as appropriate progenitor cells, matrix scaffolds, growth factors and signal molecules. A thorough understanding of the process of periodontal tissue formation and repair can result in the development of a revolutionary standardized therapy based on scientific principles.

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