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Periodontal Ligament Stem Cells: A Literature Review

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ABSTRACT

Due to specialized property of multipotency of adult postnatal stem cells , these can be experimentally induced to differentiate into various specialized cell lineages. This has generated considerable interest in the arena of stem cell based therapeutics. Periodontal ligament stem cell (PDLSC) are one of these type of cells within the periodontal ligament which represents a significant development in this regard. Achieving predictable periodontal regeneration has long been a challenge, and it is known that cells involved in the mechanisms of periodontal wound healing are of mesenchymal stem cell MSC type. Thus, periodontal ligament stem cell (PDLSC) based therapeutics may be a step towards predictable periodontal regeneration and also these cells may have alternative potential applications in hard tissue and tooth engineering. PDLSC may be isolated, grown under tissue culture conditions, expanded, optionally genetically modified and then collected and transplanted. This paper aims to overview the current knowledge, recent developments and methodology regarding PDLS based applications.

Keywords: Mesenchymal, Periodontal ligament, Stem cells and Tissue engineering

INTRODUCTION

Stem cells show potential for many different areas of health and medical research, and studying them can help us understand how they transform into the dazzling array of specialized cells that make us what we are.[1] Discoveries in stem cell research present an opportunity for scientific evidence that stem cells, whether derived from adult tissues or the earliest cellular forms, hold great promise that goes far beyond regenerative medicine.[2] Stem cell research has become a promising field for tissue regeneration and implementation of regenerative medicine.[3] Since the discovery and characterization of multipotent mesenchymal stem cells from bone marrow, similar populations from other tissues have now been characterized.[4]

Human dental stem cells have been isolated from various sources which include dental follicle progenitor cells (DFPCs), dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAPs).[5] These cells can differentiate into various cell types like chondrocytes, osteoblasts and adipocytes. These cells also generate solid structures of body such as bone, new dental tissue (cementum, dentin, PDL, and dental pulp), cartilage, muscle and can also regenerate nerves.[6] In this article, we will put focus on Periodontal Ligament Stem Cells (PDLSC).

DISCUSSION

Periodontal Ligament Stem Cells (PDLSCs) arise from migrated neural crest cells during tooth development which connects the cementum to alveolar bone, and functions to support the tooth alveolar socket.[7] The concept that stem cells reside in periodontal tissues was first proposed by Melcher.[8] Human PDLSC were first isolated by Seo *et al*. in 2004.[9] PDLSCs in defined culture conditions differentiate into cementoblasts, adipocytes and collagen forming cells. These cells when transplanted generate a cementum/ PDL-like structure that contribute periodontal tissue repair.[10] However, PDLSCs obtained from mature periodontal ligaments possess stem cell properties similar to MSCs rather than neural crest cells. PDLSCs express MSC surface markers (CD105, CD90 and CD73) but lack expression of CD45, CD34, and CD14 or CD11b, CD79a, or CD19 and HLA class II. Moreover, PDLSCs located in the perivascular wall of periodontal ligaments have resemblance with pericytes in morphology, differentiation potential, cell phenotype (expression of pericyte-associated markers CD146, neural/glial antigen-2 and CD140B), and the ability to form capillary-like structures *in vitro*.[7]

When stimulated in an appropriate conditions, PDLSC can attain the formation of new bone, cementum and periodontal ligament. Investigating the potential of PDLSC to undergo cementoblast / osteoblast differentiation shows their ability to form mineralized nodules with high calcium content in the extracellular matrix and the expression of a number osteoblast/cementoblast markers-alkaline phosphatise, matrix extracellular phosphoglycoprotein, bone sialoprotein, osteocalcin, TGF- beta receptor type I.[12]

Immunomodulatory ability of PDLSCs is comparable to bone marrow MSCs. First, PDLSCs possessed low immunogenicity due to the absence of HLAII DR or T cell costimulatory

molecules (CD80 and CD86).[13] Secondly, PDLSCs inhibited proliferation of allogeneic T cells through upregulation of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2). Surprisingly, after osteogenic induction, the inhibitory effect of PDLSCs on T cell proliferation was intact. Third, PDLSCs suppressed B cells proliferation, differentiation, and migration through cell-to-cell contact, which was mediated by programmed cell death protein-1.[14] The low immunogenicity and immunosuppressive effects on T and B cells allow use of allogeneic PDLSCs in periodontal regeneration.After an experimental study in sheep and swine model it was concluded that therapeutic effects of allogeneic PDLSCs were equal to those of autologous PDLSCs.[15]

Culture Methods for PDLSCs

In order to expand PDLSCs rapidly without loosening their stemness, improvements have been made in cell culture methods and conditions. Outgrowth and enzymatic dissociation methods both were feasible for primary culture of PDLSCs. However, enzyme digest methods had greater proliferation rates, better colony-forming efficiency, and stronger differentiation capacity than outgrowth PDLSCs. Furthermore, the success rate of primary culture was greater with type I collagenase and dispase together than with using trypsin and EDTA. Hence, type I collagenase and dispase are recommended for primary culture of PDLSCs.[16]

The culture medium, also affects biological features of PDLSCs. Two media are extensively used to culture PDLSCs: Dulbecco‘s minimum essential medium (DMEM) and α-minimum essential medium (α-MEM) containing L-glutamine and L-ascorbicacid-2-phosphate. Stem cells phenotype can be maintained by both α-MEM and DMEM (expression of Stro-1, CD146, CD105, and CD44) of PDLSCs within passage 8. However, PDLSCs cultured in α-MEM had greater proliferation rates and stronger osteogenic potential than PDLSCs cultured in DMEM. This may be due to more amino acids, vitamins, and nucleotides in α-MEM than in DMEM. Thus, α-MEM is more suitable for PDLSCs culture than DMEM.[17]

An experimental study had shown that hypoxia (2% O_2) enhance expression of pluripotency markers (Oct-4, Sox-2, and c-Myc) and the differentiation potential of PDLSCs Additionally, the osteogenic potential of PDLSCs was promoted under hypoxia (2% O2) via activation of p38 and ERK 1/2 signaling pathways. Thus, hypoxia facilitates the maintenance of multipotency in PDLSCs.[7,18]

Primary cultures of PDLSCs yielded small cell numbers (average 1,250 cells), which is less than needed to generate a cell sheet for periodontal regeneration (at least 4 × 106 cells). Therefore, expanding PDLSCs *in vitro* without losing their stemness is important. Iwata and colleagues reported that proliferation is rapidly in cells which are seeded at a low density (50 cells/cm2) than those seeded at a relatively high density (500 and 5,000 cells/cm2). The colony-forming efficiency of PDLSCs seeded at a low density increased with passage, implying that seeding cells at a low density may exclusively select highly proliferative and replicative PDLSCs.[19]

Factors Influencing Stem Cell Properties of PDLSCs

Tissue origin, age of donor, inflammatory condition and growth factors are various factors have been shown to regulate stem cell properties of PDLSCs.[7]

- **Tissue origin:** PDLSCs were collected mainly from the mid third portion of the root surface after permanent tooth extraction. However, **Wang and colleagues** demonstrated that some PDL tissue remained in the alveolar socket. PDLSCs isolated from the alveolar socket-alveolar bone derived PDLSCs (a-PDLSCs)-were compared with conventional root surface-derived PDLSCs (r-PDLSCs) and had higher proliferative ability, as well as stronger osteogenic and adipogenic differentiation potential than r-PDLSCs.[20]

Permanent teeth (p-PDLSCs) derived PDLSCs have less proliferation capacity and weaker adipogenic potential and osteogenic potential than that derived from deciduous teeth (d-PDLSCs) D-PDLSCs could also form a cementum-PDL structure when implanted in a nude mouse. P-PDLSC transplants formed a more typical cementum/PDL-like tissue and expressed more cementum/PDL-related genes (CP23 and collagen XII) than did d-PDLSCs transplants.52

PDLSCs derived from resorbed primary teeth express increased RUNX2, which upregulated RANKL and downregulated OPG at both the mRNA and protein levels. These imbalances between RANKL and OPG finally led to osteoclast differentiation and root absorption. Thus, d-PDLSCs from resorbed primary teeth may cause unexpected activation of osteoclasts when used in periodontal regeneration.[21]

Recently additional sources of PDLSCs have been identified. PDLSCs obtained from periodontal ligaments of supernumerary teeth had better colony-forming efficiency than BMMSCs and could differentiate into adipocytes and osteoblasts. Furthermore, PDLSCs isolated from periodontal granulation tissue in periodontitis patients expressed Stro-1 and CD146 and improved new bone formation when transplanted in mouse calvarias defects. Even so, the potential risks of infected tissue-derived PDLSCs are a concern because the effects of pathogenic microorganisms on PDLSCs are largely unknown. For instance, LPS from *Porphyromonas gingivalis* (the main pathogen of chronic periodontitis) severely inhibited osteogenic differentiation and promoted expression of proinflammatory cytokines (IL-1β, IL-6, and IL-8) in human PDLSCs.[22]

- **Donor Age:** With age regenerative capacity and ability to form cementum-PDL-like structures *in vivo* of stem cells decreases.[7] Zhang and coworkers concluded that proliferation and migration ability and differentiation potential of PDLSCs decreased as donor age increased.[23]

Soluble factors, especially growth and differentiation factors must be studied in detail as stem cells properties are affected by these. Finally, techniques are needed for the restoration and improvement of PDLSC regenerative capacity in elderly patients.[7]

Growth Factors: Various growth factors have been tested for modification of stem cell properties of PDLSCs.

Recently, efforts to keep PDLSCs undifferentiated at early stages of cell culture have been made to ensure better multipotency of stem cells to differentiate into osteoblasts/cementoblasts and

fibroblasts at later culture stages. The beneficial effects of BMP-4 on PDLSCs may be due to the significant overlap in responsive genes between BMP-4 and Oct-4. BMP-4 may be an effective way to maintain the stemness of PDLSCs during a long-term culture. BMP-2 and -7 and vascular endothelial growth factor (VEGF) have been verified to enhance osteogenic differentiation of PDLSCs and promote the repair of bony defect in animal models. In contrast, transforming growth factor-β1 (TGF-β1) and its downstream protein connective tissue growth factor (CTGF) accelerated fibroblastic differentiation of PDLSCs through upregulation of type I collagen, α-smooth muscle actin, and periostin. Furthermore, fibroblast growth factor 2 (FGF-2) promoted proliferation of PDLSCs but reversed the beneficial effects of BMP-2 and VEGF on osteogenic differentiation.[24,25]

Markers for PDLSC

Fibroblastic morphology and express markers of PDLSCs are similar to those of MSCs. MSCs express at least 95% of CD73, CD90, and CD105 surface antigens and less than 2 % of hematopoietic antigens and endothelial cell lineage markers. Phenotypically, PDLSCs positively express a variety of stromal cell markers, including CD13, CD29 (integrin β1), CD44, CD73 (ecto-5′-nucleotidase), CD90 (Thy-1), CD105 (endoglin), CD106 (vascular cell adhesion molecule; VCAM-1), and CD166. PDLSCs are negative for the following markers: CD11b, CD14 (monocyte and macrophage markers), CD31 (endothelial marker), CD33, CD34 (primitive hematopoietic progenitor and endothelial cell markers), CD45 (pan-leukocyte marker), CD133, CD144, and B cell markers such as CD79, CD19, and HLADR. It has also been shown that PDLSCs include about 3 % STRO-1- and CD146-positive cells (>3 % or >80 %). However, specific markers for identification of PDLSCs have not yet been characterized. Recent studies have demonstrated that PDLSCs express pluripotent stem cell markers including OCT3/4, SSEA4, and SOX2 and the tendon-specific marker, scleraxis.[26]

Clinical Applications of PDLSC

Osteogenic Potential of PDLSC

Lower osteogenic potential has been observed in case of PDLSC isolates than BMSC and also dental pulp derived stem cells.[9] Kim et al, who reported new bone formation by PDLSC in a periimplant defect model, albeit at lower levels than BMSC. For generating graft biomaterials for bone tissue engineering in regenerative dentistry can be envisioned, as these cells are more routinely accessible , however necessary to delineate more refined isolates of pluripotent progenitors using genomic and proteomic marker characterization. In periodontal ligament cell lines, homeobox protein Msx2 expression has been shown to coincide with the suppression of osteoblastic differentiation and mineralization.[27] More recently, the molecular marker periodontal ligament associated protein-1(PLAP-1) /asporin has been identified by Yamada et al. as being specific to periodontal ligament phenotype and to inhibit mineralization.[28]

Periodontal Regeneration by PDLSC

Seo et al demonstrated a cementum/PDL-like complex generated in surgically created periodontal defects by transplanting *in vitro* expanded human PDLSCs in a ceramic particle scaffold. Porcine model study reports transplanting autologous swine PDLSCs, which lead to the generation of a root/periodontal complex capable of supporting a porcelain crown, resulting in normal tooth function.[9] Subcutaneous injection of PDLSC with hydroxyapetite or beta-tricalcium phosphate scaffolds resulted in the generation of cementum and PDL- like structures adjacent to the surface of scaffolds.[29] Besides periodontal regeneration, another potential application of PDLSCs is in the area of hybrid tooth engineering' in combination with other stem and progenitor cell populations and scaffolds.

Ma et al. showed that *in vitro* induction of PDLSC with dentin noncollagenous proteins increased cell differentiation along the cementoblast lineage, denoting a potential inductive role of root surface in the activation of PDLSC differentiation, which can be utilized for bioengineering applications. Recently, a promising novel 3D human PDLSC cell pellet, which self-secretes extracellular matrix (ECM) and has favorable fabrication and handling, demonstrated the formation of aCementum/PDL-like complex on transplantation into immunocompromised mice.[30]

PDLSC transplanted into fenestration defects with fiber-guiding scaffolds produced by the rapid prototyping technique induced the regeneration of PDL-like tissues that showed angulations similar to healthy and mature ligamentous tissues. Moreover, injection of autologous PDLSC with bone grafting materials into the intrabony defects of deep periodontal pockets in 3 periodontitis patients significantly improved the value of clinical index including probing depth, gingival recession, attachment level, and attachment gain, which persisted for up to 72 months.[9,30]

Preservation of PDLSC[31,32]

Human PDLSCs can be recovered from cryopreserved PDLSCs and cryopreservation does not affect the growth capacity of these cells (Seo et al. 2005; Vasconcelos et al. 2012). Preservation techniques such as freezing in liquid nitrogen used for isolation of cryopreserved PDLSC which may be saved for future use. Periodontal ligament, preserved frozen in liquid nitrogen, generated high proliferative PDLSC, although the number of PDLSC colonies derived was decreased in comparison with freshly isolated tissue samples. The cryopreserved PDLSCs maintained their stem cell characteristics such as expression of STRO-1, multipotent differentiation capacity, and ability to form cementum/periodontal-ligament-like tissues (Seo et al., 2005; Vasconcelos et al., 2012). Thus, in future, use of cryopreserved PDLSCs could widen the application arena.

Future Perspectives

Although PDLSC play an important role in the development and regeneration of PDL tissues. however due to isolation difficulty of pure form , they have limited replicative capacity. Subcutaneously transplanted iPSC-derived Neural crest like cells revealed no capacity to form

tumors. Since iPSC-derived Neural crest like cells would work out the rarity and limited replicative capacity of PDLSC and decrease the risk of iPSC-related tumor formation, it is therefore possible that iPSC-derived Neural crest like cell could become the alternatives of PDLSC and the prospective cells for the achievement of complete PDL tissue regeneration.[29]

CONCLUSION

Recent findings demonstrate that periodontal ligament contains a population of multipotent postnatal stem cells that can be isolated and expanded *in vitro*, providing a unique reservoir of stem cells. Hence, it can be concluded that human PDLSC may hold promise as the basis of practical cellular based treatment for periodontal regeneration. Challenges for periodontal regeneration using PDLSC include the inflammatory environment, intrinsically associated with periodontal disease and difficulties in obtaining histological evidence in human clinical trials. Other issues yet to be assessed adequately are proteomic profiling, optimal cell density for implantation, optimal scaffolds for cell delivery, assessment of tissue regeneration by image analysis, and the development of bioreactor technologies that can more efficiently address the clinical need for large scale production of MSC, including PDLSC.

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