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Potential of PDL Stem Cells in Periodontal Regeneration- Review

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Abstract: *Periodontal regeneration refers to healing after periodontal surgery that result in the restoration of the lost periodontium and attachment apparatus including cementum, alveolar bone and periodontal ligament. Although, regenerative techniques have been available for several years, and some have shown promising results, none of the techniques are without problems and none have proven to be 100% effective. Stem cells are the latest entrant in periodontal regeneration. Amongst which, Dental Mesenchymal Stem Cells are currently the focus of research as these cells can be harvested with considerable ease and are associated with minimal donor morbidity. Periodontal ligament stem cells possess characteristics of mesenchymal stem cells and are a promising tool for periodontal regeneration. Recently, great progress has been made in Periodontal ligament stem cells transplantation. The present review updates the potential of Periodontal ligament stem cells and discusses standard criteria for culture and biological safety for application of Periodontal ligament stem cells.*
Keywords: *Periodontal regeneration; Tissue engineering; Mesenchymal stem cells; Dental mesenchymal stem cells; Periodontal ligament stem cells.*

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I. Introduction

Periodontitis is an inflammatory disease of the periodontium which manifests clinically as loss of supporting periodontal tissues including periodontal ligament and alveolar bone. For decades periodontists have sought various forms of treatment to repair the damage which occurs during periodontitis.[1]

Conventional periodontal therapy often results in residual pockets usually inaccessible to adequate cleaning, which deterioates the long-term prognosis of the treated teeth. It largely involves repair of the gingival connective tissues and the coronal portion of periodontal ligament with virtually no repair of cementum or alveolar bone. The events do not constitute regeneration but repair of the periodontium. [2]

Periodontal regeneration includes regeneration of alveolar bone, cementum, Periodontal ligament and gingiva. Periodontal defects may be morphologically characterized as suprabony or intrabony, as furcation or gingival recession defects, or their combinations.[3]

The application of various regenerative biomaterials, such as bone autografts, allografts, cell barrier membranes used in guided tissue regeneration procedures, application of growth factors (e.g. enamel matrix proteins), or their combinations, have been pursed with various degree of success to regenerate lost tooth support. However current regenerative procedures that are used either alone or in combination have limitations in attaining complete and predictable regeneration, especially in advanced periodontal defect.[4]

Tissue engineering as proposed by Langer, et al. comprises of multiple progenitor cells, signaling molecules and conductive extracellular matrix scaffold, along with an adequate blood supply. According to Hynes, et al. the most critical component of tissue engineering is the choice of stem cell population.[5]

Stem cells are undifferentiated embyonic or adult cells that continously divide. A fundamental property of stem cells is self-renewal or the ability to go through numerous cycles of cell division while maintaining the undifferentiated state. Stem cells can be either embryonic or adult (postnatal). Adult stem cells exist throughout the body in different tissues, including bone marrow, brain, blood vessels, liver, skin, retina, pancreas, peripheral blood, muscles, adipose tissue and dental tissues.[6] Hematopoietic stem cells from the bone marrow were the first type adult stem cells to be identified.[7]

The search for more acccessible mesenchymal stem cells (MSCs) than those found in bone marrow has propelled interest in dental tissues, which are rich sources of stem cells. uptill now, five different human dental stem cells have been reported: Dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (SHED), periodontal stem cells (PDLSCs), stem cells from apical papilla (SCAP) & dental follicle progenitor cells (DFPCs).[8]

Treatment Of Periodontal Disease

Conventional periodontal therapy (most mechanical and surgical periodontal procedures) largely involves repair of the gingival connective tissues and the coronal portion of periodontal ligament with virtually no repair of cementum or alveolar bone. These events do not constitute regeneration but repair of the periodontium. There are several drawbacks associated with conventional regenerative techniques which have been described in (Table 1).

A number of different procedures have been described to promote true and predictable regeneration of the periodontium since the 1980s. To describe the latest trends, principles of these different treatment approaches include the use of graft materials to compensate for the bone loss incurred as a result of periodontal disease, use of barrier membranes for guided tissue regeneration, and use of bioactive molecules.[3]

Bone Grafts	autogenous bone grafts-donor site morbidity and complications; limited graft availability [13-15]
	xenografts and alloplasts associated with only osteoconductive property and prone to fibrous encapsulation [16]
EMD	limited predictability and high degree of variability in results [17,18]
PRP	limited predictability and high degree of variability in results [17,18]
GTR	resorbable membrane are collapsible hence placed along with bone grafts [19]
	non-resorbable membrane prone to infection and and require surgical re-entry procedure [19]
	significant results in case of narrow 2/3 walled defects, circumferential defects, class 2 molar furcations [20]
	no effect in class 3 molar furcations [21]

(Table 1): Limitations of conventional regenerative techniques.[5]

Periodontal Regeneration

In light of the clinical unpredictability of currently available treatment modalities to treat all types of periodontal defects, tissue engineering has emerged as an alternative approach to alleviate the shortcomings of conventional therapeutic options, by regenerating living and functional dental structures.[9]

Tissue engineering is a contemporary area of science based on the principles of cell biology, developmental biology and biomaterials science to develop new procedures and biomaterials to replace lost or damaged tissues. The main requirements for producing an engineered tissue are appropriate progenitor cells, signaling molecules, an extracellular matrix or carrier construct and an adequate blood supply.[10]

Tissue engineering is generally considered to consist of three key element (Fig 1)[11].

- Stem cells/progenitor cells
- Scaffolds or extra cellular matrix
- Signaling molecules.

Steps involved in regeneration of tooth are:

1) Harvesting and expansion of adult stem cells.
2) Seeding the stem cells into scaffold which provides optimized environment.
3) Cells are instructed with targeted soluble molecular signals spatially.
4) Confirming the gene expression profile of the cells for next stage in odontogenesis.

One of the most critical factors in tissue engineering is the choice of scaffold and optimal stem cell population to employ.[10]

Stem Cells

They are defined as clonogenic cells, which are capable of both self-renewal and multi-lineage differentiation. According to their origin and differentiation potential, stem cells are classified as:

• Embryonic stem cells
• Adult stem cells
• Induced pluripotent stem cells.[5]

Embryonic Stem Cell: Embryonic stem cells are capable of multipotential differentiation but clinical feasibility is limited due to ethical issues. The inner cell mass (the part that would form fetus) of the embryo is used to form embryonic cell lines. Embryonic stem cells has a potential to differentiate into germ layers namely ectoderm, endoderm and mesoderm. Tumorigenesis and immune rejection is common with embryonic stem cells.[12]

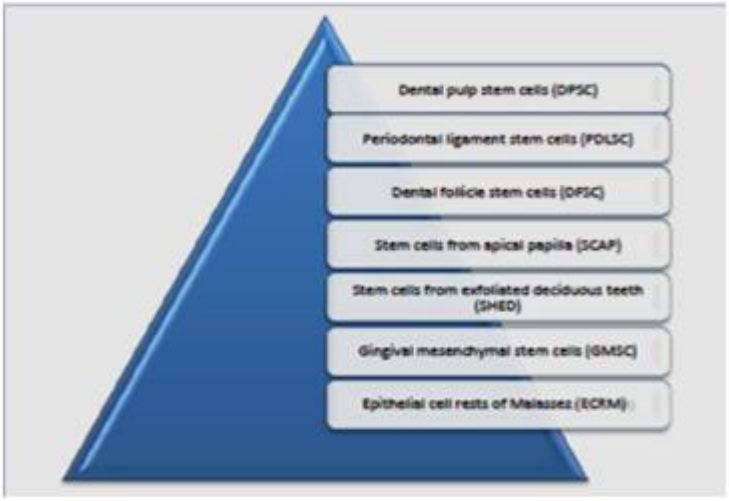
Adult Stem Cells: Adult stem cells are multipotent stem cells. They have been harvested from different kind of tissues like bone marrow, umbilical cord, amniotic fluid, brain tissue, liver, pancreas, cornea, dental pulp, and adipose tissue. Adult stem cells are comparatively easier to isolate and do not have any ethical issues. Immune rejection and teratoma formation is also rare with adult stem cells. Adult stem cells are commonly used in current day practice.[12]

The adult stem cells are basically undifferentiated cells found among differentiated cells in a tissue organ. They are also referred to as Mesenchymal Stem Cells (MSC). Mesenchymal stem cells (MSCs) are spindle shaped cells with the potential for clonogenic proliferation. MSCs were initially reported as fibroblast-like cells that could be isolated from bone marrow via their adherence to plastic in culture and subsequently confirmed as a population (the colony-forming unit-fibroblast) of bone marrow- derived non hematopoietic cells.

In **2006, the International Society for Cellular Therapy (ISCT**) proposed the minimal characterization criteria for human MSCs, including their propensity for adherence to plastic when maintained under standard culture conditions and their ability to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro.

Due to their extensive distribution in many adult tissues, including those of dental origin, MSCs have become an attractive target for use in periodontal regeneration.[3]

The various dental MSCs that has been found till date to be useful in periodontal regeneration as illustrated in (Table 2) include[5]:



(Table: 2) The different dental mesenchymal stem cells.

Periodontal Ligament Stem Cells

The concept that the stem cells may reside in the periodontal tissues was proposed approximately 20 years ago by **Melcher**, who queried whether the three cell populations of the periodontium (cementoblasts, osteoblasts, and periodontal ligament fibroblasts) were derived from a single population of ancestral cells or stem cells. 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**.[15]

Identification of Periodontal Stem Cells:

Mesenchymal stem cells were first, identified in aspirates of adult bone marrow by **Friedenstein and colleagues** by their capacity to form 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.[15-18] 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).[15]

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 cells.[19] 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 10^5 cells plated). Whether this represents a propensity for stem cells to be present within this tissue remains to be established.[15]

Characterization And Origin Of Periodontal Stem Cells

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. 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.[15]

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)[20] 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.

The presence of MSCs in PDL is also supported by the findings of **Trubiani et al**. who isolated and characterized a population of MSCs from PDL, which expressed a variety of stromal cell markers (CD90, CD29, CD44, CD105, CD166, and CD13). These cells are found to express markers like STRO-1,CD146, α-smooth muscle actin and/or the pericyte-associated antigen, 3G5, an array of cementoblastic /osteoblastic markers including alkaline phosphatase, matrix extracellular phosphoglycoprotein phosphoglycoprotein (MEPE), bone-sialoprotein, osteocalcin and TGFβ receptor type-1 and general soft tissue proteins such as type I and type III collagen.[21]

Differentiation Potential Of Periodontal Ligament Stem Cells

Ex vivo expanded PDLSCs formed mineralized nodules, cementum-like tissue on the surface of the hydroxyapatite/tricalcium phosphate ceramic particle carrier, along with condensed collagen fibers resembling sharpey's fibers, in the presence of calcium in extracellular matrix. In general, PDL stem cells require a suitable scaffold such as hydroxyapatite /tricalcium phosphate to induce the formation of bone, cementum, and bone in vivo. When ex vivo expanded PDLSCs are implanted in vivo with a suitable scaffold, atypical cementum/PDL like structure forms.[2]

Regenerative Potential Of Pdl Stem Cells

Nakahara et al. reported that autologous periodontal ligament derived cells were required for the regeneration of periodontal tissues with collagen sponge scaffold in dogs.[22] **Mizuno et al.** attempted to regenerate periodontal tissue defects by grafting autologous cultured membrane derived from the periosteum.[23] Findings of **Lin et al**. provide the first evidence that stem cells participate in the healing of regenerating periodontal defects in humans and offer support for the use of stem-cell based tissue engineering in regenerative periodontal therapy.[24] **Gomez et al.** demonstrated the use of human periodontal ligament cell sheet technique which can be applied for regeneration of periodontal ligament–cementum complex in clinical settings.[25]

PDL tissues are clinically accessible in routine clinical practice like tooth extraction, possibly providing a readily available source of stem cells for clinical periodontal regenerative therapy.[19] However, little is known about the characteristics of PDL progenitor/stem cells because PDL is composed of heterogeneous cell populations, and thus far, no highly purified PDLSC clone has yet been established from human PDL tissue.[26]

Trubiani et al.[27] suggested that PDLSCs had regenerative potential when seeded onto three dimensional biocompatible scaffold, thus encouraging their use in graft biomaterials for bone tissue engineering in regenerative dentistry, whereas **Li et al.**[28] have reported cementum and periodontal ligament-like tissue

formation when PDLSCs are seeded on bioengineered dentin. However, there are a few limitations associated with the application of dental MSCs as described in (Table 3)[5]:

Biological	Technical	Clinical
Molecular pathways responsible for stem cell proliferation and differentiation are unknown	Culture mediums are not well developed enough to mimic in vivo conditions to ensure safe and consistent stem cell proliferation and differentiation. Stem cell line production for human trials could be hampered by the use of xenogenic products in culture mediums as they could be a potential source of pathogens. Mesenchymal stem cells have a limited life span unlike embryonic stem cells which are immortal An ideal biocompatible scaffold and transport mechanism is still under research.	Integration of the human stem cell derivatives with the recipient tissue and their ability to carry out the desired functions in humans is still under speculation

(**Table 3):** Limitations in the application of Dental MSC for periodontal regeneration.[5]

Factors That Influence Stem Cell Properties of PDLSCS

Various factors have been shown to regulate stem cell properties of PDLSCs, including tissue origin, age of donor, inflammatory condition, culture method, and growth factors.[13]

1. **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.[29]

PDLSCs derived from deciduous teeth (d-PDLSCs) had greater proliferation, stronger adipogenic potential, and osteogenic potential than PDLSCs derived from permanent teeth,[30,31]but Song and colleagues' findings did not agree with this.[32] Jin's group discovered that PDLSCs derived from resorbed primary teeth expressed increased RUNX2, which upregulated RANKL and downregulated OPG at both the mRNA and protein levels. Thus, d-PDLSCs from resorbed primary teeth may cause unexpected activation of osteoclasts when used in periodontal regeneration but this requires confirmation.[33]

Lee's group revealed that PDLSCs obtained from periodontal ligaments of supernumerary teeth had better colony-forming efficiency than BMMSCs and could differentiate into adipocytes and osteoblasts.[32]

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, and the duration of such inhibitory effects remains to be investigated.[13]

1. **Donor Age:** Donor age also affects stem cells. PDLSCs obtained from aged donors had less regenerative capacity compared with those from young donors.[34] **Zhang and coworkers** compared biological features of PDLSCs obtained from donors at different ages and found that proliferation and migration ability and differentiation potential of PDLSCs decreased as donor age increased. Moreover, PDLSCs in aged groups (older than 41 years) expressed less Stro-1 and CD146 than young donors and failed to form cementum-PDL-like structures in vivo, indicating that the number and regenerative ability of stem cells decreased with increasing donor age.[35]
2. **Culture Methods and Conditions:** Improvements have been made in cell culture methods and conditions to expand PDLSCs rapidly without losing their stemness. For primary culture of PDLSCs, both outgrowth and enzymatic dissociation methods were feasible. However, PDLSCs cultured by enzyme digest methods had greater proliferation rates, better colony-forming efficiency, and stronger differentiation capacity than outgrowth PDLSCs.

Two media are extensively used to culture MSCs and PDLSCs: Dulbecco's minimum essential medium (DMEM) and α-minimum essential medium (α-MEM) containing L-glutamine and L-ascorbicacid- 2-phosphate. Both α-MEM and DMEM can maintain stem cell phenotypes (expression of Stro-1, CD146, CD105,

and CD44) of PDLSCs within passage. However, PDLSCs cultured in α-MEM had greater proliferation rates and stronger osteogenic potential than PDLSCs cultured in DMEM.[36]

Primary cultures of PDLSCs yielded small cell number[37](average 1,250 cells), which is less than needed to generate a cell sheet for periodontal regeneration (at least 4 × 106 cells).[38] **Iwata and colleagues** reported that PDLSCs seeded at a low density (50cells/cm2) proliferated far more rapidly than those seeded at a relatively high density (500 and 5,000 cells/cm2). [37]

3. Growth Factor: As the periodontium is comprised of cementum, alveolar bone, and the functional periodontal ligaments between them, so use of various growth factors to induce PDLSCs differentiation into different directions is of interest.

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.[39-41] 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.[42-44]

Fibroblast growth factor 2 (FGF-2) promoted proliferation of PDLSCs but reversed the beneficial effects of BMP-2 and VEGF on osteogenic differentiation.[41] Unexpectedly, sequential use of FGF-2 followed by BMP-2 resulted in more bone formation than use of BMP-2 or FGF-2 alone. Similarly, sequential use of FGF-2 followed by TGF-β1 also promoted fibroblastic differentiation of PDLSCs.[45]

Future Trends

From a biological perspective, in order for periodontal regeneration to occur, the availability of appropriate cell types, together with a favorable local environment promoting cell migration, adhesion, proliferation and differentiation, all need to be precisely coordinated both temporally and spatially.

Thus, the knowledge of the presence of PDL stem cell and their inherent potential to regenerate various desired tissues at site of destruction is of great importance. Use of tissue engineering strategies in this respect that explore the regenerative capacity of stem cells residing within the periodontium, grown in a three-dimensional construct and subsequently implanted into the defect may help to overcome many limitations with current regeneration modalities. The plausibility of a stem cell-based tissue engineering approach to achieving periodontal regeneration is supported by animal studies demonstrating that PDL cells cultured in vitro can be successfully reimplanted into periodontal defects in order to promote periodontal regeneration.[14]

II. Discussion

Recently, information about PDLSCs has expanded, but no standard protocol for PDLSC culture and identification is available and this leads to studies that cannot be compared. A consensus about isolating, culturing, identifying, and using PDLSCs is needed. To maximize the therapeutic effects of PDLSCs, studies are needed to differentiate biological properties of PDLSCs obtained from different tissues and methods are required for reducing inflammation.

First, young patients (younger than 30 years of age) with a healthy periodontium are suitable candidates because the detrimental effects of age and inflammation on stemness and immunomodulation of PDLSCs can be avoided or at least alleviated. The decision between permanent or primary teeth for sourcing PDLSCs is unclear. Considering primary culture of PDLSCs, an enzyme digest method with type I collagenase and dispase is better than the outgrowth method. Moreover, hypoxia (2% O2), α-MEM, and a low seeding density (50cells/cm2) enhance proliferation and maintain stemness of PDLSCs.

Sequential use of growth factors appears promising and effective for improving stem cell properties of $PDLSC_S$, but interactions among various growth factors have not been studied thoroughly.[13]

III. Conclusion

The future dentistry will be more of regenerative based, where patients own cells can be used to treat diseases. Stem cell therapy has got a paramount role as a future treatment modality in dentistry. Regenerative dentistry will have to go in pace with regenerative medicine. Determining the role of local conditions such as the type of scaffold and the presence of the microorganisms should be very carefully analyzed. Longer patient follow up is needed to study the life time of regenerated tissue.

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