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REVIEW ARTICLE

STEM CELLS, A NEW AGE STRATEGY FOR PERIODONTAL REGENERATION: A REVIEW

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ABSTRACT

Stem cells are known for their ability of self-renewal and their potential of differentiation into mature cell types. These are present in almost all the multi-cellular organisms and are primitive cells. These cells have tremendous capacity for regeneration and can be utilized to repair as well as replace the demaged cells and can be used to transform the treatment options for cases like Parkinson's disease, Alzheimer's disease as well as for cancer and paralysis.If left untreated the periodontal disease can eventually lead to disease progression, leading to loss of tooth mainly in patients who are highly susceptible. Recently introduced Stem cells and tissue engineering have tremendous ability for treating the periodontal disease. After suitable stimulation, stem cells can differentiate into numerous cell types, possessing the regenerative potential. In cases of periodontal regeneration, MSCs provide the most promising results and have been tested in vitro as well as in vivo. Hence, this review discusses different stem cells, their uses as well as concise information on the stem cell based therapy used for the periodontal regeneration.

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INTRODUCTION

Stem cells are known for their ability of self-renewal and their potential to differentiation into mature cell types. These are present in almost all the multi-cellular organisms and are primitive cells. These cells have tremendous capacity for regeneration and can be utilized to repair as well as replace the demaged cells and can be used to transform the treatment options for cases like Parkinson's disease, Alzheimer's disease as well as for cancer andparalysis. Adult stem cells and embryonic cells are the two major type of these cells and this classification is based on their potential to differentiate and their origin (Narang, 2012). Recently introduced Stem cells and tissue engineering have tremendous ability for treating the periodontal disease. If left untreated the periodontal disease can eventually lead to disease progression, leading to loss of tooth mainly in patients who are highly susceptible. Thus, it is quite important to look for new regenerative procedures that are actually effective. Recently introduced stem cell therapy, cell combination therapy, tissue engineering, biomaterials and growth factors provide an attractive as well as effective treatment option for this disease. After suitable stimulation, stem cells can differentiate into numerous cell types, possessing the regenerative potential. In cases of periodontal regeneration, MSCs provide the most promising results and have been tested in vitro as well as in vivo (Filippo, 2020). Mesenchymal stem cells are adult type stem cells that are harboured from the sources like umbilical cord, liver, adipose tissue, placenta, synovial membrane, amniotic fluid, amniotic fluid, bone marrow as well as from teeth. These cells have an important place in the regenerative medicine. These have the ability to develop into numerous types of tissues like bone, cartilage, adipose tissues and are useful in patient-specific gene therapy (Narang, 2012). Mesenchymal stem cells like human exfoliated deciduous teeth cells, dental pulp stem cells, periodontal ligament stem cells, stem cells from apical papilla and dental follicle precursor cells are harvested from the dental tissues and are used in the periodontal regeneration. Non-dental stem cells include adipose-derived stem cells (ASCs), Bone marrow derived mesenchymal stem cells (BMMSCs), induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), that are also widely investigated[2]Hence, this review discusses different stem cells as well as some concise information on the stem cell based therapy used for the periodontal regeneration.

Major Sources of stem cells

- Embryonic germ cells: harboured from fetal tissues from gonadal ridge at later stages of development
- Embryonic stem cells: harboured from an embryo that is 4-5 days old, from the inner mass cell of blastocyst
- Adult stem cells: present in adult tissues and are harboured from mature tissues. They keep the usual turnover of regenerative organs, present as a repair system of the body and replenish specialized cells (Pera, 2000).

Dental Mesenchymal stem cells

Dental pulp derived stem cells/ DPSCs: These were identified by Gronthos et al (2000) and were the first recognized human dental stem cells. These cells consists of cells like nerve cells, fibroblasts, vascular cells as well as undifferentiated stem cells. These are present in extremely vascularized areas of the pulp.

These cells can be harboured from an extracted human teeth by numerous isolation methods. As per numerous studies DPSCs have the capability to differentiate into adipocytes, smooth muscle cells, skeletal muscle cells, odontoblast like cells as well as into osteoblasts. Also, various studies suggest that these cells have Mesenchymal stem cell surface marker like CD105, CD10, CD29, CD13, CD44, CD73, CD59, CD90. Also these cells do not express HLA-DR, CD34, CD45 and CD14 (Seyed, 2016). The major characteristic of DPSCs are the polarized cellular bodies and mineral nodules, out-turning into odontoblastic differentiation (Hynes, 2012). The functional and phenotypic properties are quite identical to mesenchymal stem cells harboured from the bone marrow. These cells can bring about specific as well as innate immune responses by interactions with B lymphocytes, T cells, N K cells and macrophages. These cells bring about the immunological effect by triggering the apoptosis of the cells and by stopping the activated T cell proliferation (Ji, 2019). DPSCS decreases Immunoglobulin production by B lymphocytes. These also decrease the production of IL-17 and increase the secretion of IFNϒ (Maioli, 2016). As per a study conducted by Park et al (2011), DPSCs have a very small capability to construct the cementum- like structure in comparison to PDLSCs (Park, 2011).

Stem cells from human exfoliated deciduous teeth/ SHED: Have the capacity to regenerate the bone as well as tissues like dentin. These have good osteoinductiveproperties and also have high proliferation rate. These cells brings about the activation, maturation differentiation of T lymphocytes, showing theimmunomodulatory capabilities. These cells inhibits the Th17 lymphocytes but stimulates T lymphocytes (Yildirim, 2016). These increase the production of IL-10 while decrease the production of inflammatory markers like TNF-α, IFN-ϒ, IL2. Cause the polarization of macrophages of bone marrow towards M2, exerting anti-inflammatory effect and regeneration of periodontium (Silva, 2010). According to a study conducted by Gao X et al (2018), SHEDs cause the reduced cytokine expression , decreased the gingival bleeding and form links joining the alveolar bone and PDL (Gao, 2018).

Dental Follicle Stem Cells (DFSCs): Derived from the neural crest, these cells are seen in the dental follicle of the dental germ. They need a particular environment in order to differentiate into neurons, cardiomyocytes, bone cells, adipocytes, chondrocytes. As per the recent data these also have the capability to differentiate into ductal as well as salivary gland cells (Xu, 2017). These migrate around the tooth bud and differentiate into osteoblasts, cementoblasts, PDLs, leading to formation of periodontium. CD44, CD105, CD13, CD73, CD56, human leukocyte antigen, STRO-1, CD44, Neurogenic locus notch homolog protein 1 (NOTCH-1) are the surface cluster of differentiation markers for these cells. Out of these markers, CD44 and STRO-1 are utilized for the identification of these cells. The expression of TLR2/TLR4 in DFSCs membranes, can be stimulated by Porphyromonasgingivalis and Fusobacteriumnucleatum, causing the Peripheral blood mononuclear cells (PBMCs) proliferation inhibition. Also the secretion of anti-inflammatory cytokines such as IL-10 is increased and the secretion of pro-inflammatory markers is decreased by DFSCs. The process of degradation of the bone is repressed by modulating the chemotaxis and phagocytosis. As per a study conducted by Ma et al (2012), there was an increased migration, proliferation and osteogenic differentiation of PDLSCs on implantation of DFSCs-sEVs (small extracellular vesicles) into the periodontal defects. As per the previous data, sEVs works by decreasing the inflammatory cytokine expression and increasing the osteogenic abilities of bone marrow-derived mesenchymal stem cells (BMMSCs). RANKL- containing sEVs are released from the osteoblasts that will be signed over to osteoclast precursor cells. Later on these gets transformed to osteoclasts and provoke Osteoprotegerin/Receptor activator of the NF-κB ligand/receptor activator of the NF-κB (OPG/RANKL/RANK) signalingpathway, that is quite important for the metabolism of the bone (Ancuta, 2023).

Stem Cells from the Apical Papilla (SCAPs): It was isolated from an immature teeth, from the apical papillary tissue in the year 2006 for the first time. These have high levels of self- renewal capacity, low immunogenicity, multilineage differentiation capacity and high proliferation rate. The surface markers of SCAPs are CD24, CD90, CD166, CD73, STRO-1 and CD146. By an apoptosis independent mechanism, these stops proliferation of T lymphocyte (Ancuta, 2023).

Gingiva-Derived Mesenchymal Stem Cells (GMSCs): Firstly, isolated from the lamina propria of the gingival tissue in the year 2009. In the past few years, these cells have shown to be an area of interest for the cell therapy due to their immunomodulatory properties. Inflammatory environment and GMSCs interact by the expression of TLRs 1, 2, 3, 4, 5, 6, 7 and 10. By making Prostaglandin E2, IL-6 or IL-10, human GMSCs can stop the activity of macrophage M1. Also, by PGE2-associated mechanism, GMSCs remarkably decrease the DC (dendritic cell) activation and maturation that leads to the decreased antigen-presenting capacity of DCs and remarkably reduce the inflammatory response. Human GMSCs upregulates the immunosuppressive factors like IL-10, leading to inhibition of T cell activation (Trubiani).

Periodontal Ligament Stem Cells (PDLSCs): PDLSCs were firstly recognized in the third molar. These cells have the capability to produce cementum, alveolar bone, PDLs, blood vessels and peripheral nerves. The ability for proliferation and renewal is also high. Markers like CD90, CD105, CD13, CD73 and CD44 are expressed by PDLSCs. Hematopoietic markers like CD40, CD80, CD86, CD19, CD14, CD45 are not expressed by these cells. Antigens like sex determining region Y- box (Sox) 2, alkaline phosphatase (ALP), TRA-1-60, TRA1-81 are expressed (Ancuta Go, 2023). IFN-ϒ is generated by the PBMCs (Peripheral blood mononuclear cells) and source PDLSCs to produce soluble factors like hepatocyte growth factor, TGF-β, indoleamine leading to decreased PBMCs proliferation. PDLSCs also bring about the apoptosis and proliferation of neutrophils. PDLSCs decrease the production of glycoprotein 1b of major histocompatibility complex and PGE2 arising from the dendritic cells, inhibiting T lymphocyte proliferation. The proliferation of anti-inflammatory Treg cells are also improved by the PDLSCs and stop the production of pro-inflammatory lymphocytes like Th17, Th2 and Th1. PDLSCs also mediate the immunosuppressive mechanism by stopping the migration, proliferation and differentiation of B lymphocytes. Also, these cells stimulate the CD163, arginase 1, IL-10 and inhibit TNF-α, thus potentiating anti-inflammatory phenotype (Ancuta Go, 2023). Colonies of bipolar fibroblastoid cells consisting of oval nuclei having 2-3 nucleoli is the primary culture of PDLSCs. PDLSCs contain huge cytoplasm, numerous mitochondria, endoplasmic reticulum profiles that were extremely rough, few leftover lysosomal bodies having filament bundles and electron dense material on the Ultrastructural analysis (Trubiani). On stimulation with suitable growth factors, PDLSCs express elastin, fibrogenic like genes and reveal powerful immunofluorescence label for fibronectin (Liu, 2020).

Non- Dental stem cells

Adipose-Derived Stem Cells (ASCs): These cells are procured from Adipose tissues. The markers of these cells are same as of BMSCs like CD73, CD105, CD166, CD44 and CD29. CD34, CD31and CD45 are not expressed by ASCs. ASCs have the ability to differentiate into myogenic cells, neurogenic cells, adipocytes, osteocytes and can improve the regeneration of cementum and periodontal vessel. The process for harvesting the ASCs are easy as compared to BMSCs. ASCs along with IFN-ϒ, IL-6 and TNF-α bring about the expression of factors like GBP4 and IL-1RA, that are immunosuppressive. Differentiation of ASCs in the periodontiumis enabled by insulin-like growth factor binding protein-6, which is secreted by ASCs.[16]Method of harvesting the ASCs is not that invasive in comparison to methods used to harvest BMSCs and can be harvested by liposuction and/or subcutaneous adipose tissue fragments in great numbers.[2]

Bone Marrow-Derived Mesenchymal Stem/Stromal Cells (BMSCs): Surface markers like CD44, CD29, CD73, CD105, CD146, CD90 and STRO-1 are expressed by BMSCs. These cells does not express markers like CD34, CD14 and CD45. These can differentiate into osteoblasts, muscle cells, adipocytes and chondrocytes. Has the

capacity to form Sharpey'sfibers, cementum, alveolar bone. These cells can enhance the odontogenic gene expression and can differentiate into fibroblasts, osteoblasts on local or systemic transplantation (Hasegawa, 2006). These cells regulate the immunomodulation, mediating the proliferation of the T cells. These can be used for the treatment of periodontitis as these cells cause inhibition of TNF- α and IL-1, that are inflammatory markers. However, various clinical studies are required to be conducted to assess their capability to modulate the immunity and inflammation, before applying it for treating periodontitis (Xiao, 2018).

Induced Pluripotent Stem Cells (iPSCs): Application of human iPSCs eliminated the shortcoming related to immune-rejection reaction. By the use of numerous transcriptional markers like Krüppel-like factor 4, Sox2, Oct4, somatic cells are reprogrammed into iPSCs. Markers like TRA160, CD73, CD90, TRA180, CD105, CD146 and CD106 are the special markers, expressed by the iPSCs. Dental tissue derived stem cells like the cells derived from gingiva, apical papilla, dental pulp, buccalmucose can generate the iPSCs (Takahashi, 2006). To implant the stem cells, various carriers can be used but this leads to the decreased viability and proliferation of these cells. Hence, the preferred method is the direct injection method (Shimauchi, 2013)

Application in Periodontal Therapy: PDLSC are the major stem cell candidate for regenerating the periodontium. As per a study conducted by Ding *et al* (2010), these cells have the capability to regenerate the periodntium, stating that the tissue engineered PDLSCs can be used for treating the periodontal defects. The proposed mechanism of action mainly depends on the immunomodulatory property of PDLSCs. PDLSCs produce prostaglandin E2, causing PGE2-induced T-cell anergy and hence have less immunogenicity and good immunosuppression. By cell-to-cell contact, that is mainly mediated by programmed cell death 1 ligand 1(PDL1) and programmed cell death protein 1 (PD1), PDLSCs decrease the activation of B cell. As per a study conducted by Monsarrat et al (2014) BMSCs, PDLSCs as well as cells from the gingiva or periosteumcan attain periodontal regeneration. Chen et al in the year 2016 conducted two studies, First study was conducted on 35 patients for assessing the efficacy and safety of the autologous PDLSCs. Other study was conducted on 80 patients for assessing the safety and efficacy of allogeneic PDLSC cell sheet. Chen et al concluded that both are clinically safe. However, no difference in efficacy was noticed. Various studies used stem cells with scaffolds of collagen, hydrogel, fibrins, gelatin etc. But the main drawback of these combinations is host rejection and complex process of transplantation (Lei).

Stem cell-based tissue engineering for periodontal regeneration: Tissue engineering is a field of science that involves the principle of developmental biology, cell biology and biomaterial science to form new tissues, in order to restore the tissues that are demaged or lost. Its success depends on a carrier or a suitable extracellular matrix consisting of responsive progenitor cells and regulatory signals. Various progenitor cells and instructive messages are incorporated into three dimensional scaffolds, that are then planted at the site that has the periodontal defect. This cell- based tissue engineering have certain technical requirements and these requirements are divided into two major types i.e. Biomechanical properties of matrix and biological functions such as neovascularization, bioavailability of growth factors and cell recruitment. The biomechanical properties include capability of the prepared matrix to maximize the cell colonization, tissue in growth of the required type, ease of handling. Also the prepared matrix should be rigid enough in order to withstand the collapse of soft tissue into the defect. This concept of transplanting the cells was firstly described about 15 years back. Various studies were carried out for successful periodontal regeneration using the alveolar bone cells and periodontal ligament fibroblasts. The crude cell preparations used in these studies had heterogeneous nature, hence the treatment policies were limited. In the past few years purified stem cells are assessed for the periodontal regeneration (Lin, 2008). Atelocollagen along with the autologous bone marrow MSCs showed regeneration of periodontal ligament, alveolar bone and cementum regeneration (Kawaguchi, 2004). Hydroxyapatite/tricalcium phosphate ceramic along with expanded PDLSCs showed formation of periodontal ligament like structures, cementum in rats (Seo, 2005).

Non- scaffold tissue engineering

Primary of two types: cell injections and cell sheets.

Cell injections: It is a treatment option when cells are to be planted directly on the defect site and is quite common in stem cell based tissue engineering (Kinnaird *et al*., 2004). Local injections of PDLSCs or DPSCs has been proved to be productive for the treatment of periodontal disease (Baik *et al*., 2014). As per a study conducted by Du J et al (2014), local injection of BMMSC suspension was proved to be effective for periodontal regeneration in rat periodontitis (Lei).

Cell sheet engineering: It is a different scaffold-free procedure of processing the cells by culturing them in ascorbic acid. Temperature responsive cell culture vessels can also be used. These sheets keeps the cell-cell junctions and extracellular matrix. As per a study conducted by Hasegawa et al., (2005), periodontal ligament cell sheets when planted in the mesial dehiscence of rat modal led to the formation of periodontal ligament like tissues, fibrils and accellular cementum (Lei).

Gene and cell-based therapy: Stem cells can be used as vehicles for the delivery of genes in gene therapy. These can also be used as therapeutic agents in the cell-based therapy. Gene therapy is based on the genetic engineering. It consists of molecular techniques in order to introduce, supress and manipulate the specific genes so that a therapeutic agent can be prepared from the individual's own cells. Two main methods has been introduced in order to deliver the transgenes and these methods include the use of non-viral or viral vectors for directly infusing the genes in vivo, second method consists of introducing the genes into stem cells that act as delivery cells, outside the body and then transplantation of the delivery cells into the human body (ex-vivo method) (Lin, 2008).

Summary

Majority of data on the use of stem cells in periodontal regeneration is based on the studies conducted on animal models and from cell cultures. Thus, we cannot generalise all these findings to human beings as there are only few studies that are conducted on human beings. Also, animal models does not always interpret the human situation. The molecular pathways by which the differentiation as well as the self-renewal of the stem cells occurs, is still not clear. Hence, more research needs to be conducted in order to unfold the molecular and cellular events, associated with restoration of the periodontal tissues.

REFERENCES

Ancuta Go., Liliana F., Karina C., Diana D., Darius S. The Role and Involvement of Stem Cells in Periodontology. *Biomedicines.* 2023; 11: 387.

Filippo C., Giacomo G., Ludovica F., Mario A. Stem cells and periodontal regeneration: present and future. *Plast Aesthet Res.* 2020; 7:41

Gao X., Shen Z., Guan M., Huang Q., Chen L., Qin W. Immunomodulatory Role of Stem Cells from Human Exfoliated Deciduous Teeth on Periodontal Regeneration. Tissue Eng. 2018; 24: 1341–1353.

Hasegawa N., Kawaguchi H., Hirachi A., Takeda K., Mizuno N. Behavior of Transplanted Bone Marrow-Derived Mesenchymal Stem Cells in Periodontal Defects. *J. Periodontol.* 2006; 77: 1003–1007.

Hynes K., Menicanin D., Gronthos S., Bartold PM. Clinical utility of stem cells for periodontal regeneration. *Periodontol* 2000. 2012; 59(1):203-27.

Ji L., Bao L., Gu., Zhou Q., Liang Y. Comparison of Immunomodulatory Properties of Exosomes Derived from Bone

Marrow Mesenchymal Stem Cells and Dental Pulp Stem Cells. Immunol. Res. 2019; 67: 432–442.

Kawaguchi H., Hirachi A., Hasegawa N. Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. *J Periodontol.* 2004; 75:1281– 1287.

Lei Hu., Yi Liu., Songlin W. Stem cell-based tooth and periodontal regeneration. Molecular Laboratory for Gene Therapy and Tooth Regeneration, Beijing Key Laboratory of Tooth Regeneration and Function Reconstruction, Capital Medical University School of Stomatology, Beijing, China

Lin N., Gronthos S., Bartold P. Stem cells and periodontal regeneration. *Australian Dental Journal.* 2008; 53: 108–121.

Liu J., Zhao Z., Ruan J., Weir MD., Ma T., Ren K., Schneider A., Oates T.W. Stem cells in the periodontal ligament differentiated into osteogenic, fibrogenic and cementogenic lineages for the regeneration of the periodontal complex. *J Dent.* 2020; 92: 103259

Maioli M., Basoli V., Santaniello S., Cruciani S., Delitala A.P., Pinna R., Milia E., Grillari-Voglauer R., Fontani V., Rinaldi S. Osteogenesis from Dental Pulp Derived Stem Cells: A Novel Conditioned Medium Including Melatonin within a Mixture of Hyaluronic, Butyric, and Retinoic Acids. *Stem Cells Int.* 2016: 2056416.

Narang S, Sehgal N. Stem cells: A potential regenerative future in dentistry. *Indian J Hum Genet.* 2012;18:150-4.

Park J.Y., Jeon S.H., Choung P. Efficacy of Periodontal Stem Cell Transplantation in the Treatment of Advanced Periodontitis. *Cell Transplant.* 2011; 20: 271–285.

Pera M.F., Reubinoff B., Trounson A. Human embryonic stem cells. *J Cell Sci.* 2000; 113:5-10

Seo BM., Miura M., Sonoyama W., Coppe C., Stanyon R., Shi S. Recovery of stem cells from cryopreserved periodontal ligament. *J Dent Res.* 2005;84:907–912.

Seyed H.B., Wichaya W., Justin R., Sasan G., Jamie C., Marcelo F., Luciano C.A., Giuseppe I. Potential for Stem Cell-Based Periodontal Therapy. *J Cell Physiol.* 2016; 231(1): 50–61.

Shimauchi H., Nemoto E., Ishihata H., Shimomura M. Possible Functional Scaffolds for Periodontal Regeneration. *Jpn. Dent. Sci. Rev.* 2013; 49: 118–130.

Silva S., Ramos R.N., Almeida D.C., Bassi E.J., Gonzales R.P., Miyagi S., Maranduba C.P. Mesenchymal Stem Cells Derived from Human Exfoliated Deciduous Teeth (SHEDs) Induce Immune Modulatory Profile in Monocyte-Derived Dendritic Cells. *PLoS ONE* 2014; 9: e98050.

Takahashi K., Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell. 2006; 126: 663–676.

Trubiani O., Pizzicannella J., Caputi S., Marchisio M., Mazzon E., Paganelli R., Paganelli A., Dio. Periodontal Ligament Stem Cells: current knowledge and future perspectives. Stem Cells and Development.1-25.

Venkataiah V.S., Handa K., Njuguna M.M., Hasegawa T., Maruyama K., Nemoto E. Periodontal Regeneration by Allogeneic Transplantation of Adipose Tissue Derived Multi-Lineage Progenitor Stem Cells in Vivo. Sci. Rep. 2019; 9: 921.

Xiao L., Zhou Y., Zhu L., Yang S., Huang R. SPHK1-S1PR1-RANKL Axis Regulates the Interactions Between Macrophages and BMSCs in Inflammatory Bone Loss. J. Bone Miner. Res. 2018; 33: 1090–1104.

Xu Q.L., Furuhashi A., Zhang Q.Z., Jiang C.M., Chang T.H. Induction of Salivary Gland-Like Cells from Dental Follicle Epithelial Cells. *J. Dent. Res.* 2017; 96: 1035–1043.

Yildirim S., Zibandeh N., Genc D., Ozcan E.M., Goker K., Akkoc T. The Comparison of the Immunologic Properties of Stem Cells Isolated from Human Exfoliated Deciduous Teeth, Dental Pulp, and Dental Follicles. Stem Cells Int. 2016: 4682875.

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