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Research Article

PERIODONTAL REGENERATION BY STEM CELLS THERAPY

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ABSTRACT

PERIODONTAL REGENERATION of lost tissues due to periodontal disease is primarily based on a range of surgical procedures, the use of a variety of grafting materials, barrier membranes and growth factors. However, because the predictability of these techniques can be variable, the application of this technology may frequently be restricted to specific case types. Recently, reports have begun to emerge demonstrating that populations of adult stem cells reside in the periodontal ligament of humans and other animals. With the better understanding of molecular biology, stem cell research is being utilized to find biological solutions for biological problems. Stem cells are uncommitted entities capable of both self-renewal and differentiation

into multiple cell lineages and hence these cells may prove to be instrumental in the regeneration of periodontium which has been destructed by several suggested complex mechanisms. These pluripotent stem cell populations persist in multiple organs and when stimulated they proliferate and differentiate in response to local cues provided by the organs they are recruited to. This concept opens a new dimension for new cell-based therapies for periodontal regeneration. Thus this review provides an overview of adult human stem cells and their potential use in periodontal regeneration.

Keywords: Periodontal Regeneration, Pluripotent stem cell, Embryonic Stem cells, Adult stem cells, Mesenchymal stem cells.

INTRODUCTION

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both. Various surveys have shown that the majority of adults in the population suffer from moderate periodontitis, and significant number of people being affected by severe generalized periodontitis at some stage in their lives.[1] The significant burden of periodontal disease and its impact on general health and patient quality of life point to the need for more effective management of this condition.[2] Several procedures have been attempted to achieve periodontal regeneration, including root surface conditioning, bone graft placement, guided tissue regeneration and growth factor application. However, current regenerative procedures that are used either alone or in combination have limitations in attaining complete and predicable regeneration, especially in advanced periodontal defects.[3, 4].Recent advances in stem cell biology and regenerative medicine have presented opportunities for tissue engineering as well as gene-based approaches in periodontal therapy.[5, 6] The following discussion reviews our current understanding of stem cells and their potential application in regenerative periodontal therapy. Major progress in stem cell biology in the dental context is highlighted and the challenges in translating stem cell research into clinical practice are identified.

Stem cells

The term ''stem cell'' first appeared in the literature during the 19th century. A ''stem cell'' refers to a clonogenic, undifferentiated cell that is capable of self-renewal and multi-lineage differentiation.[7] In other words, a stem cell is capable of propagating and generating additional stem cells, while some of its progeny can differentiate and commit to maturation along multiple lineages giving rise to a range of specialized cell types. A pluripotent stem cell can give rise to cell types from all three germ layers of the body (i.e., ectoderm, mesoderm and endoderm) whereas a multipotent stem cell can produce cell types from more than one (but not all) lineages.

Basically, stem cells can be isolated in the earliest stages of embryogenesis (embryonic stem cells) or in different post-natal tissues (adult stem cells) such as brain, bone marrow, skeletal muscle, skin and liver.[8]

Embryonic stem cells (ESCs) were first reported by Damjanov & Solter from teratocarcinomas of mice & humans,[9] containing derivates of all three embryonic germ layers. Later, Evens & Kaufman[10] obtained mouse embryonic stem cells, and Thompson and coworkers[11] isolated human embryonic stem cells. These human embryonic stem cells derived from 4–5 day-old embryo prior to its implantation in the uterus; this stage of embryo is known as blastocysts. The blastocysts are constituted by a specialized compartment called the inner cell mass (ICM), which is a group of approximately 30 cells.(FIG-1)

These cells are plated onto mitotically inactivated mouse fibroblast feeder cells, and cultured with medium containing serum alone or associated with basic fibroblast growth factor. After 6 months or more in culture, the original 30 cells of ICM yield several colonies of ESC.[12] These human ESC colonies were reported to be pluripotent in that they can differentiate into hundreds of others cell types in the adult body.[13](FIG 2) Another important characteristic of human embryonic stem cells is that they express immortality, probably due to their high expression of telomerase, a ribonucleoprotein that adds telomere repeats to chromosome ends, thereby maintaining their chromosomal length. [11]

Studies have reported that ESCs cultured in vitro, and under appropriate conditions, can give rise to several types of mature cells, including nerve, muscle, bone and pancreatic islet.[14](FIG 2) These results show a positive indication for use of ESCs in regenerative treatments. However, use of human embryos for stem cell research remains a challenge due to ethical questions.

Adult stem cells populations (ASCs) may be an alternative to promote tissue regeneration in humans. ASCs are undifferentiated cells found among differentiated cells in a tissue, and their primary roles are to maintain and repair the tissue in which they are found.[8, 12] When compared to ESCs, the adult stem cells present some drawbacks. First, these cells are more difficult to be isolated, because each tissue consists of a number of ASCs located in specific regions not accurately established yet.[8, 12] Second, ASCs cultured in vitro can give rise to a subset of lineages (multipotential).[8, 12, 13] Third, these adult cells are exposed to environmental toxins resulting in genetic mutations.[8] Finally, they are considered to have a finite lifespan in vitro because of the absence of telomeraseactivity.[15]

Adult tissues such as brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, and liver have been reported to contain stem cells. Some studies have suggested that these cells might generate specific cell types that can be used to treat diseases such as Parkinson[16], type I diabetes [17] and coronary diseases.[18] Based on findings obtained in medical fields, new studies in dentistry have been designed to identify and characterize dental-derived stem cells populations, aiming at developing more predictable regenerative approaches for lost tissues as consequence of disease and/or trauma.(FIG 3)

Another population of adult non-haematopoietic stem cells also resides in the bone marrow microenvironment.[19, 20] These are termed bone marrow stromal stem cells (BMSSCs) or mesenchymal stem cells (MSCs) and their biological properties are less well understood. In recent years, human MSCs have been identified in many tissues throughout the adult body. However, the primary source of MSCs is the bone marrow where they exist at a low frequency (one per 34 000 nucleated cells), which declines with age.[21] MSC-like cell populations have also been identified in other tissues, including adipose tissue, muscle, peripheral blood, foetal pancreas and liver. Mesenchymal stem cells have been characterized both morphologically and immuno-phenotypically using various surface markers. While the morphology of MSCs typically falls into one of two types (large and flat or elongated and fibroblastic). Of more relevance to their identification is the expression of a number of phenotypic characteristics of osteoblasts, endothelial, perivascular cells, neural or muscle cells and a range of surface markers (including CD44, CD29 , CD44, STRO-1, CD90, CD105, CD106, CD146, CD140b, CD166, CD271). This broad expression of cell surface molecules suggests a common link between different cellular types since most of these markers are expressed by all distinguishing feature of human MSCs is their ability to form colonies (i.e., they are clonogenic). In addition, under special inductive culture conditions, these cells can differentiate along numerous lineages including those for osteoblasts, adipocytes, myelosupportive stroma, chondrocytes and neuronal cells.[22]

The ability of MSCs to give rise to multiple specialized cell types along with their extensive distribution in many adult tissues (including those of dental origin) have made them an attractive target for use in periodontal regeneration.

Criteria for undifferentiated mesenchymal stem cells are that cells must be negative for hematopoietic progenitor cell markers such as CD14 (monocyte/macrophage), CD45 (common leukocyte antigen) and CD34 (hematopoietic stem/progenitor cells/ endothelium), plus express indicative markers of mesenchymal stem cells, including CD105, CD166, CD29, CD44 and STRO-1.[23]

Bone marrow stromal system of postnatal organisms is an important source of mesenchymal stem cells. Friedesntein et al[24] were the first to recognize that bone marrow stromal system contains an adherent, clonogenic, self-renewing and fibroblast-like population cells (colony forming unit-fibroblastic, CFU-F) denominated bone marrow stromal stem cells (BMSSCs).

Human Dental-Derived Mesenchymal Stem Cells (hMSC)

Recently, mesenchymal stem cell populations derived from dental pulp (DPSCs)[25], exfoliated human deciduous teeth (SHED)[26], and adult periodontal ligament (PDLSCs) [27]have been isolated and identified by their ability to generate clonogenic adherent cell clusters such as BMSSCs. Cloning experiments showed that DPSC, SHED, and PDLSC have a frequency of colony forming cells significantly higher than that of the bone marrow.[28]Besides, proliferation studies demonstrated that multi-colony-derived DPSC, SHED and PDLSC cell cultures exhibited higher rates of proliferation, approximately 30%, 50%, and 30%, respectively, when compared to BMSSCs.[28]Similar to BMSSC, human mesenchymal dental-derived stem cells expressed perivascular cell markers such as CD146 (MUC18), alpha-smooth muscle actin and pericyte antigen associate (3G5), indicating that these different mesenchymal stem cell populations might reside in a common perivascular niche in their respective tissues, although they proceed from different anatomical sites.[27-29] Moreover, analysis of ex vivo expanded DPSC, PDLSC, SHEDs and BMSSSC demonstrated a common expression pattern profile for a variety of antigens associated with bone, dentin, cementum (BMPs, alkaline phosphatase, Type-I collagen, osteonectin, osteopontin, osteocalcin, bone sialoprotein) and fibroblasts (Type-II collagen).[27-29]Although many of these markers were not uniformly expressed, but found in subsets of cells, indicating that DPSC, PDLSC, SHED and BMSSC populations to be heterogenous .[23, 27, 28]

Potential Differentiation of Human Dental-Derived Stem Cells In Vivo And In Vitro Studies Dental pulp stem cells (DPSCs)

Studies by Gronthos et al[19, 23] (2000, 2002) demonstrated that human DPSC cultures are negative for odontoblastic-specific markers, such as dentin sialophosphoprotein (DSPP) and dentin sialoprotein (DSP), suggesting an undifferentiated phenotype of these cells. When 12 single-colony-derived DPSCs strains were transplanted with hydroxiapatite/tricalcium phosphate (HA/TCP) particles subcutaneously into immunocompromised mice, two-thirds of the single-colonies developed a abundant typical dentin/pulp-like complex consisting of a layer of odontoblasticlike cells, aligned around mineralized dentin, while only a limited amount of dentin was detected in the remaining one third. These results imply that single-colony-derived DPSC strains differ from each other with respect to their rate of odontogenesis.[19, 23, 25] However, independent of the density formed, all DPSCs transplants were capable of expressing DSPP identified by immunohistological analysis, indicating that this population of cells might respond to specific environmental signals and differentiate into cells with specific phenotype, in this case, the odontoblasts .[19, 23, 25]

Stem Cells From Human Exfoliated Deciduous Teeth(SHEDs)

Miura et al. [26] (2003) found that SHEDs were capable of differentiating into neural cells, adipocytes, and odontoblasts when cultured in vitro with specific medium such as DPSC. After 12 single-colony-derived SHED clones were transplanted into inmmunocompromised mice, 25% of the clones demonstrated a potential to differentiate into odontoblast-like cells and to form ectopic dentin-like tissue. However, SHEDs were unable to regenerate the dentin-pulp-like complex completely. In addition, transplanted single-colony-derived SHED clones were capable of inducing murine host cells to differentiate into osteoblasts and osteocytes, and to form a significant amount of new bone.

Periodontal Ligament Stem Cells (PDLSCs)

Seo et at [27] (2004) were the first group that isolated PDLSCs from normal impacted third molars. Ex-vivo expanded PDLSCs formed mineralized nodules with the presence of calcium in the extracellular matrix and expressed an array of cementoblastic/osteoblastic markers, including alkaline phosphatase, MEPE (matrix extracellular phosphaglycoprotein), bone sialoprotein, osteocalcin, and TGFβ receptor type I.

Seo et at [27] examined the expression levels of scleraxis, a tendon-specific transcription factor in PDLSCs. They observed that PDLSCs expressed measurably higher levels of scleraxis when compared with BMSSCs. These data imply that PDLSCs represent a unique population of postnatal stem cells distinct from bone marrow-derived mesenchymal stem cells. When ex-vivo expanded PDLSCs were transplanted into immunocompromised mice with the hydroxyapatite/tricalcium phosphate carrier particles, a typical cementum/PDL-like structure was generated, in which a thin layer of cementum-like tissues formed on the surface of the carrier, along with condensed collagen fibers containing sparse cells that resembled PDL structures.

These PDL-like tissues were positive for anti-type I collagen antibody staining and were able to connect with newly formed cementum-like structures that mimicked physiological attachment of Sharpey`s fiber. These results infer that PDLSCs might contain a subpopulation of cells capable of differentiating into cementoblasts/cementocytes and collagen-forming cells in vivo.

To assess whether PDLSCs were able to contribute to periodontal tissue repair, Seo et al [27] transplanted these cells into surgically created defects at the periodontal area of mandibular molars in immunocompromised rats. Transplanted human PDLSCs integrated into the PDL and attached to both the alveolar bone and cementum surfaces. These findings imply a potential functional role of human PDLSCs for periodontal regeneration.

Stem cell applications

A unique concept of dental stem cell banking is also being practiced. Path breaking advances in stem cell research has made it possible to extract valuable stem cells; the building blocks of every human body, from primary teeth (milk teeth) of children and wisdom teeth. These stem cells are carefully preserved at a stem cell center in a special cryogenic storage facility, thus making it possible to use them in the future to cure array of ailments.

Stem cells thus derived from various sources are being utilized in the field of Medicine as well as in the field of Dentistry.

In the field of Medicine, stem cell applications under investigation

S. No.	Clinical Application	Author & Year
1.	Parkinson's disease	Emily J. Schwarz et al &1999 [30]
2.	Neurological diseases	Agnes Arthur etal & 2008[31]
3.	Spinal cord injuries	Dasa C´ı ˇzkova etal, 2005[32]
4.	Bone grafting	Yoichi Yamada etal & 2004[33]
5.	Cardiac disease	Leora B. Balsam etal & 2004[34]
6.	Urological	Leora B. Balsam etal & 2003[35]
7.	Crohn's disease	Yu Oyama et al & 2005[36]
8.	Lupus	Elisabetta Traggiai etal & 2008[37]
9.	Diabetes	Vijayakumar K. Ramiya etal & 2000[38]
10.	Genetic disorders (gene therapy)	Y Verlinsky etal & 2005[39]
11.	Radiation-induced damage	Isabelle M.A. Lombaert etal & 2006[40]

In the field of dentistry, stem cell research is directed towards achieving the following

S. No.	Clinical Application	Author & Year
1	Regeneration of damaged coronal dentine and pulp	Misako Nakashima etal & 2005[41]
2.	Regeneration of resorbed root, cervical or apical dentin and perforations	Peter E. Murray etal &2007[42]
3.	Periodontal regeneration	Naohiko Hasegawa etal & 2006[43]
4.	Craniofacial defects	J.J. Mao, W.V. Giannobile etal & 2007[44]
5.	Whole tooth regeneration	Sonoyama W etal & 2006[45]
6.	Cleft lip and palate	Conejero JA etal & 2006[46]

Potential Clinical Applications for Human Dental-Derived Stem Cells in Periodontal Regeneration

Periodontal regeneration has always remained a challenge as it consists of hard and soft tissues. It is evident, that the ligament complex contains stem cells that can commit to a number of pathways (bone, cementum and ligament). Moreover, the cells respond to inductive factors that include members of the TGF-super-family such as BMP-2, BMP-12, BMP- 7, TGF-a, PDGF and b-FGF.

Literature review shows a limited number of studies on dental-derived stem cells investigating the behavior and characteristics in vitro and their influence on ectopic tissue formation in immunocompromised animals. Among all the dental-derived stem cells identified, PDLSCs are a unique population capable of forming an ectopic cementum/PDL-like structure. However, although cultured PDL stem cells have shown promising results when implanted into surgically created periodontal defects in rats (Seo et al., 2004)[27], only 61% of these cells (PDLSCs) showed ability to form ectopic cementum/PDL-like structure, suggesting that these cellular colonies might contain a mixed population of progenitor cells at various stages of development, and only some cells are capable of self renewing and differentiate into several cell types. Therefore, additional studies are needed to identify which progenitor cells are able to differentiate into periodontal ligament-forming cells, mineral-forming cementoblasts and bone-forming osteoblasts.

SHEDs, another population of dental-derived stem cells, were observed in immunocompromised mice to induce cells to differentiate into osteoblasts and osteocytes, resulting in the synthesis of new bone.[26] However, this cell strain was unable to form periodontal ligament and root cementum. Recently, precursors cells (PCs) isolated from human dental follicle of wisdom teeth were characterized by Morsczeck et al.[47] These cells were able to create in vitro a structure similar to a periodontal membrane composed of fibroblast phenotype cells and calcified structures, full of alkaline phosphatase and bone sialoprotein. PCs transplanted into immunocompromised mice can generate a structure lining the surfaces of the hydroxyapatite particles, consisting of fibrous and rigid connective tissue. Besides, these cells can also express human-specific transcripts concerning bone sialoprotein, osteocalcin and type I collagen. However, these authors found no sign of cementum or bone formation in histological sections of PC-transplants.

Although human BMSSCs have generated positive results regarding the neoformation of periodontal tissues. Kawaguchi et al.[5] (2004) observed that BMSSCs isolated from iliac crest of beagle dogs and autotransplanted into Class III furcation lesions with an atellocolagen carrier, were able to form new cementum, bone and periodontal ligament in practically all root extension. These authors suggest that some factors (adhesion molecules, growth factors, and extracellular matrix macromolecules) present in the lesions might have stimulated the differentiation of transplanted cells into functional and specialized cells. These results, therefore, suggest that both dental and non-dental derived stem cells might be potentially applied in regenerative periodontal therapies. Thus, further studies are needed to investigate how progenitor cells participate in the process of periodontal regeneration, and how the environment of the lesions regulates cell activities.

CONCLUSION

Periodontal regeneration using stem cell therapy and tissue engineering requires consideration of many features that parallel periodontal development, including the appropriate progenitor cells, signalling molecules and matrix scaffold in an orderly temporal and spatial sequence.

This review included studies focusing on characterization of different dental and non-dental derived adult human stem cell populations. The results emphasize the need for further investigation to determine the efficacy of ex vivo expanded stem cells in repairing dental structures and periodontal tissues. Within the limitation of such studies, the PDLSCs showed an ability to form structures similar to root cementum and periodontal ligament. Nevertheless, because these cells are heterogenous in nature due to their capacity to proliferate and differentiate in periodontal tissue forming cells, further studies are needed to investigate how stem cells function in the process of regeneration. Besides other cell

strains such as BMSSCs, SHEDs and precursor cells from human dental follicle of wisdom teeth might be in the future, genetically modified in vitro so that they will be able to differentiate into periodontal tissue cells before they are transplanted in vivo. Subsequent tissue-engineering approaches may then be developed using these progenitor cells within a matrix scaffold, together with the introduction of various signaling molecules in an orderly temporal and spatial sequence.

Recent advancements in scaffold designing, better understanding of growth factor biology and interactions between allogenic stem cells and immune system result in new discipline in dentistry, 'regenerative dentistry'.

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