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Review Article

Stem Cells in Periodontal Regeneration - A New Era

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Abstract

Regeneration of lost tooth and periodontium remains a great challenge. Periodontists have been striving hard to regenerate the lost periodontium by a variety of surgical procedures, by use of different bone grafts, barrier membranes and growth factors. Recent advances have enumerated the presence of adult stem cells in periodontal ligament of humans and other animals. This has opened the way for new cell-based therapies for periodontal regeneration. For this to become a reality a thorough understanding of adult human stem cells is needed. This review provides an elaborated view of human stem cells and their use in regeneration of the periodontium. The review was conducted through search of articles in various electronic databases such as GOOGLE SCHOLAR, PUBMED, EMBASE, SCIENCE DIRECT and MEDLINE search engines till the year 2018.

Keywords: *Stem Cells; Periodontal Regeneration; Periodontium*

Introduction

The periodontium is an unusually complex tissue comprised of two hard (cementum and bone) and two soft tissues (gingiva and periodontal ligament). After periodontitis has been established, regeneration can be induced only by therapeutic intervention. Among the tissues of the periodontium, the periodontal ligament (PDL) is a highly fibrous tissue and accounts for a high turnover rate [1]. The progenitor cell populations within the periodontal ligament appear to be enriched in locations adjacent to blood vessels and exhibit the classical cytological features of stem cells, including small size, responsiveness to stimulating factors and slow cycle time.

The concept that stem cells may reside in the periodontal tissue was first proposed almost 20 years ago by Melcher [2], who queried whether the three cell populations of the periodontium (cementoblasts, alveolar bone cells and periodontal ligament fibroblasts) were ultimately derived from a single population of ancestral cells or stem cells.

Regeneration of the periodontium is a combination of alveolar, cemental, and PDL regeneration on a previously diseased root surface. Though a variety of treatment modalities (GTR, bone grafts, growth factors, host modulating factors) have been employed for periodontal regeneration, all these have shown limited success, especially in challenging clinical situations.

Recently, efforts have been made on cell-based regenerative approaches using stem cells. Stem cells appear to have a promising therapeutic potential in periodontal regeneration due to their plasticity and ability to differentiate into different cell lineages.

In this review, we have discussed the current and potential applications of stem cells in periodontal regeneration. The review was conducted through search of articles in various electronic databases such as GOOGLE SCHOLAR, PUBMED, EMBASE, SCIENCE DIRECT and MEDLINE search engines till the year 2017.

What is a stem cell?

A stem cell is defined as a clonogenic cell that has the capacity to self-renew itself and has a multi-lineage differentiation [3]. These cells are seen in organisms that have the ability to renew and differentiate into other cell types. Thus they are considered as the repair system of the body. Once a stem cell undergoes division, the new cells can either transform into other cells with specialised functions or remain as a stem cell.

Properties unique to stem cells are:

1. **Self-renewal:** The ability of a stem cell to undergo many cycles of mitotic cell division despite maintaining its undifferentiated state.

2. **Infinite potency:** It is the ability of a stem cell to differentiate into enormous mature cell types.

Haeckel, a German Biologist first introduced the term 'stem cell' in the year 1868 [4]. The term stem cell was coined by Wilson. Alexander Maksimov, a Russian Histologist proposed the term "stem cell" for scientific use. Since then, the stem cells have been a boon to medicine and dentistry.

Types of stem cells: Various types of stem cells are:

1. **Embryonic stem cells:** They are cultures of cells derived from the epiblast tissue of the inner cell mass of the blastocyst.

2. **Somatic/Adult stem cells:** They are found in specialised tissues and organs and are undifferentiated in nature. They are more mature with a finite lifespan and have multipotent differential capacities.

3. **Induced pluripotent stem cells:** These are recently developed somatic cells that are reprogrammed to become pluripotent cells. It represents an alternative to multipotent adult stem cells in periodontal regeneration treatment. They are unique and have proved that cell differentiation is not in one way. They have added advantage that they are easily accessible and patient specific.

Dental stem cells: The various types of human dental stem cells that can be isolated are:

1. Stem cells from Human Exfoliated Deciduous Teeth (SHED).

2. Dental Pulpal Stem Cells (DPSCs).

3. Periodontal ligament stem cells (PDLSCs).

4. Stem Cells from Apical Papilla (SCAP).

SHED: SHED was discovered by Dr. Songoto Shi in 2003. Miura., *et al.* differentiated SHED into DPSCs, neural cells, adipocytes, osteoblast-like and odontoblast-like cells [5]. The neural crest origin of SHED was investigated by Abbas.

Advantages of SHED

1. It is an economical painless technique.
2. There is no risk of tissue rejection and it can be useful for blood relatives.

1/3rd of the root of healthy primary incisors and canines can be used for SHED banking.

The roots of primary molar are not suitable for SHED since it results in the obliteration of the pulp chamber and takes a longer time to resorb.

DPSCs: DPSCs are present inside the dental pulp and are of mesenchymal in origin. *In-vitro*, these stem cells have osteogenic and chondrogenic potential and will differentiate into dentin whereas *in-vivo* it differentiates into dentin-pulp-like complex [6]. The unique feature of DPSCs that make them an ideal candidate for dental tissue regeneration are: Easy surgical access to collection, can be safely cryopressed, generate more typical dental tissue than non-dental stem cells. Recently isolated are the immature DPSCs. They are a sub-population of DPSCs used in the generation of dental pulp organ.

Periodontal ligament stem cells (PDLSCs): These cells are multipotent postnatal stem cells found in the human periodontal ligament. They were first described by Seo., *et al* [7]. They have the capacity to form cemental/periodontal ligament-like structure when transported into rodents which help in periodontal tissue repair. Sources of isolation of PDLSCs are cryopreserved periodontal ligament and they have properties like expression of Mesenchymal Stem Cells (MSC) surface markers, single-colony strain generation, multipotent differentiation and cemental/pdl-like regeneration. They have higher proliferation rate than skeletal stem cells derived from bone marrow. Also, they express scleraxis, a tendon/ ligament specific transcription factor, at higher levels compared to bone marrow/ DPSC. It has also been found that cryopreservation doesn't affect the growth capacity of PDLSC, since the expression of STRO-1 (a multipotent differentiation capacity marker) remains unaltered.

Stem cells from apical papilla (SCAP): These stem cells lie in the immature roots of permanent teeth within the apical papilla. Sonoyama., *et al.* first described SCAP [8]. *In-vivo* the SCAP forms odontoblast-like cells which form the root dentin. In infected immature permanent teeth and teeth with abscess, SCAP helps in apexogenesis. Since the SCAP lies in the apical papilla, they can survive pulpal necrosis due to periapical vasculature. Therefore, after endodontic disinfection, SCAP has the capacity to generate primary odontoblasts that enhances complete root formation due to the survival of Hertwig's Epithelial Root Sheath (HERS).

Aging of stem cell

After 120 days, MSCs start losing their proliferative potential *in-vitro*. Various changes occur in stem cells during culturing which include:

- Gradual decrease in proliferation index
- Shortening of telomere
- Functional impairment
- Typical Hayflick phenomenon of cellular aging.

The number of times a human cell undergoes division until the cell division stops is called hayflick phenomenon [9]. Leonard Hayflick discovered 40 years ago that cultured normal human cells have limited capacity to divide, after which they become senescent, a phenomenon now known as the Hayflick limit.

Applications of stem cells in dentistry: Research of stem cells is aimed towards the regeneration of damaged PDL, pulp, dentin and resorbed roots. Stem cells in the field of tissue engineering have provided faster healing of ulcers and oral wounds. Gene-transfer methods that are used to manipulate salivary protein and oral microbial colonization is also possible with the help of stem cells [10].

Stem cells in osseous regeneration: The recent application of adult mesenchymal stem cells is found in the gingival connective tissues which are shown to have an osteogenic potential and hence regenerate bone in mandibular defects. These stem cells have the property to suppress the inflammatory response by enhancing the recruitment of anti-inflammatory cytokines and regulatory T-cells and also by inhibiting the leucocytic proliferation and inflammatory cytokines. Hence they provide a favourable environment for osseous regeneration [11].

Role of stem cells in medicine: They are widely used in various fields of medicine for regeneration of brain tissue, heart therapies, for bone regeneration and for treating muscular dystrophy. Adipose tissue as well as cartilage can be regenerated using SHED. The first advanced animal study for bone regeneration was done in 2008. This study revealed the reconstruction of large sized cranial defects using DPSCs in rats [12].

Procurement of stem cells from human exfoliated deciduous teeth: There are 3 steps in procuring stem cells from human exfoliated deciduous teeth.

1. **Step 1:** Collection of teeth: The freshly-extracted teeth are transferred in a vial which contains hypotonic phosphate buffered saline solution. The vials are carefully sealed, placed into thermette and placed into an insulated metal transport vessel. This helps in maintaining the sample in a hypothermic state during transportation. This procedure is called sustentation. A time period of 40h should not exceed in harvesting and processing these cells from the exfoliated teeth.

2. **Step 2:** Isolation of stem cells: The tooth surface of the exfoliated teeth is washed three times with Dulbecco's phosphate buffered saline without Ca^{2+} and Mg^{2+}. This is followed by disinfection and again PBSA washing. The pulp tissue is isolated, placed in a sterile petridish and washed with PBSA for three times. Tissue digestion is carried out using Type I collagenase and dispase for 1h at 37°C. The isolated cells pass through a 70 um filter. This is done to obtain single cell suspensions which are then cultured in a MSC medium. After 24h, the isolated colonies are visible.

3. **Step 3:** Stem cell storage: The approaches used for stem cell storage are by means of cryopreservation and magnetic freezing.

Studies done using stem cells

Periodontal regeneration was achieved in animal and human studies using PDLSCs, BMMSCs, and cells derived from gingiva or periosteum [13] (Table 1-10).

Defect	Carrier	Animal model	Cell association	Duration of implant	Outcomes
Bone defects on both sides of the mandible	Platelet-rich plasma	Hybrid dog	Autologous	2, 4 and 8 weeks	Platelet-rich plasma, in combination with bone marrow stem cells, elicited true bone regeneration with the formation of well-formed mature bone and neovascularisation.
Class III furcation defect	Atelocollagen	Beagle dogs	Autologous	1 month	Autotransplantation of bone marrow-derived mesenchymal stem cells into periodontal defects aided regeneration.
Class III furcation defect	Atelocollagen	Beagle dogs	Not defined	4 weeks	These results suggested that transplanted bone marrow stem cells survived within the defect and differentiated into cells that make up the periodontal tissues.

Trans-gingival periodontal defect –alveolar bony defects	Pluronic F-127 gel	New Zealand White rabbit	Autologous	6 weeks	Implanted bone morphogenetic protein-2-expressing bone marrow stem cells generated significantly more bone than unmanipulated bone marrow stem cells
Periodontal fenestration defects	Collagen membrane	Beagle dogs	Autologous	8 weeks	Transplantation of bone marrow stem cells resulted in the formation of cementum, alveolar bone and periodontal ligament, leading to significantly greater periodontal regeneration.
Class III root furcation defect	Calcium alginate gel	Beagle dogs	Not defined	6 weeks	The bone marrow-derived mesenchymal stem cells expressing basic fibroblast growth factor promoted faster regeneration of the periodontal bone tissue
Class II furcation Defect	Platelet-rich plasma and 10% calcium Chloride	Mongrel dogs	Autologous	8 weeks	Implantation of bone marrow stem cells with platelet-rich plasma led to complete filling of the furcation defect with cementum, alveolar bone and periodontal ligament
Bilateral mandibular, alveolar and periodontal defects	Pluronic F-127 gel	Beagle dogs	Autologous	8 weeks	Implantation of the bone morphogenetic protein-2-expressing mesenchymal stem cells resulted in the regeneration of larger volumes of bone than mesenchymal stem cells alone without the negative effects of root ankylosis and resorption. Functional Sharpey's fibers and cementum were also generated
Bone defects on both sides of the mandible	Platelet-rich plasma	Hybrid dog	Allogeneic	2, 4 and 8 weeks	Implanted bone marrow stem cells generated well-formed mature bone with neovascularization when compared with controls

Table 1: *Bone marrow derived mesenchymal stem cells [14-22].*

One-wall intrabony defect	Hydroxyapatite/β-tricalcium phosphate and collagen	Beagle dogs	Autologous	8 weeks	Both the periodontal ligament cells and the bone marrow-derived cell sheets enhanced periodontal regeneration with the formation of new cementum and well-oriented periodontal ligament fibers

Table 2: *Bone marrow- derived cell sheets [23].*

Oro-maxillofacial bone defect	Collagen sponge	Humans	Autologous	Upto 1 year	Implantation of a biocomplex composed of dental pulp stem cells and a collagen sponge scaffold resulted in optimal bone repair and complete regeneration of bone at the injury site
Apical involvement defect	None mentioned	Beagle dogs	Autologous	8 weeks	After 8 weeks of implantation, the defects that received dental pulp stem cells were very similar to those of the negative control, indicating that the dental pulp stem cells did not promote regeneration. Periodontal ligament stem cells were found to have the best regenerative capacity
Bone defects on both sides of the mandible	Platelet-rich plasma	Hybrid dog	Allogeneic	2, 4 and 8 weeks	Implanted dental pulp stem cells generated well-formed mature bone with neovascularization when compared with controls

Table 3: *Dental pulp stem cells [24,25].*

Periodontal lesion of the maxilla and mandibular first molars	Hydroxyapatite and β-tricalcium phosphate	Miniature pig	Autologous	12 weeks	Transplanted green fluorescent protein-labelled cells were identified in the newly formed bone, suggesting that the transplanted cells contributed to new-bone formation

Table 4: *Periodontal ligament stem cells [26].*

Deep intrabony defect	Hydroxyapatite/β-tricalcium phosphate	Human	Autologous	3 - 72 months	This study demonstrated the potential efficacy and safety of implanting autologous periodontal ligament progenitor cells in the treatment of human periodontitis. All three patients treated with periodontal ligament progenitor cells displayed clinical benefits from the treatment

Table 5: *Periodontal ligament progenitor cells [27].*

Mucoperiosteal flaps raised between upper canines and premolars	Extracted autologous tooth roots	Miniature pig	Autologous	2, 4 and 12 weeks	Allogeneic transplantation of the periodontal ligament stem cell sheets enhanced periodontal tissue repair in a manner similar to that seen in autologous transplants.
One-wall intrabony defect	Hydroxyapatite/β-tricalcium phosphate and collagen	Beagle dogs	Autologous	8 weeks	Periodontal ligament- and bone marrow-derived cell sheets enhanced periodontal regeneration with the formation of new cementum and well-oriented periodontal ligament fibers.

Table 6: *Periodontal ligament stem cells and periodontal ligament cell sheets [28, 29].*

Cavity left after extraction of lower incisor	Root-shaped hydroxyapatite/β-tricalcium phosphate block and gel foam	Miniature pig	Not defined	3 months	Implanted periodontal ligament stem cells in combination with stem cells from apical papilla in an attempt to generate a root/periodontal complex led to the formation of bio-roots with significantly better compression strength than defects that did not receive stem cells

Table 7: *Periodontal ligament stem cells with apical papilla [30].*

Apical involvement defect	None mentioned	Beagle dogs	Autologous	8 weeks	The autologous periapical follicular stem cells generated new cementum, alveolar bone and Sharpey's fibers of periodontal ligament.

Table 8: *Periapical follicular stem cells [31].*

Alveolar sockets from tooth removal	Gelatin/chondroitin-6-sulphate/hyaluronan tricopolymer scaffold	Miniature pigs	Autologous	40 weeks	Bone marrow fluid in combination with dental bud cells promotes tooth regeneration

Table 9: *Dental bud stem cells [32].*

Critical-sized bone defects in the parasymphyseal region of the mandible	Hydroxyapatite/β-tricalcium phosphate	Miniature pig	Autologous	2, 4 and 24 weeks	Implanted stem cells derived from miniature pig deciduous teeth differentiated directly into new bone, resulting in the formation of markedly more new bone in the defect site

Table 10: *Stem cells from deciduous teeth [33].*

Dental socket stem cells

Dental sockets have recently been shown to be a potential source for stem/progenitor cells. Tissue samples from extraction socket, 4th and 8th week post-extraction have revealed the presence of osteoblasts and osteoid.

Hurdles in application of stem cells

Challenges in the biological field

The discoveries on periodontal stem cells are basically from animal models and cell cultures. Hence they are not always applicable to the human situation. Thus, not all findings in animal models can be directly extrapolated to humans. The molecular pathways of stem cell differentiation and self-renewal are unknown even today [34]. Hence further research is required to gain more knowledge about the molecular and cellular events that take place in restoring lost periodontal tissues before a biologically-based therapy is developed. Hence the characterization and isolation of stem cells from periodontal tissues provides a starting point to investigate the role of stem cells in periodontal wound healing, regenerative therapy and tissue engineering.

Challenges in the technical field

Prolonged culturing of stem cells leads to instability and gene mutations in cells, thus leading to difficulty in cell manipulation which is an important technical challenge. The matrix scaffold should have good biocompatibility for regenerating tissues at a molecular and cellular level [35]. Human PDLSCs are found to be a suitable scaffold when implanted into surgically-created periodontal defects. This resulted in the formation of a periodontal ligament-like structure [36,37]. In addition to this, further research is needed to understand the lineage-specific differentiation and efficacy of *in-vitro* stem cells derived from regenerating periodontal defects. Karyotypic instability and gene mutations can hinder the usefulness of cell lines after prolonged culturing. Thus, improvement in the current techniques helps to facilitate laboratory handling of these stem cells which serves in maximizing the regenerative potential of these cells to be used in clinical Periodontics.

Challenges in the clinical field

Immune system responds to human stem cell derivatives upon transplantation. Hence it is important to understand the interaction between the two. Immunogenicity of the human cells depends on its expression of major histocompatibility (MHC) antigens which are of two types: class I and II. They allow the body to distinguish foreign cells from its own body cells. Human ES cells express class I MHC

antigens at low levels, but these levels are up-regulated with differentiation [38]. The use of autologous stem cells from third molar teeth overcomes potential immune rejection [39].

There is a major safety consideration regarding prevention of tumour formation following MSC implantation despite insufficient statistical power and long-term follow-up to draw firm conclusions [40]. This is because they are more specific in nature and have extensive the therapeutic use. The longer the stem cells remain *in-vitro*, the greater the numbers for therapeutic use. This extended period in the culture leads to a greater genetic or epigenetic change that accumulate over time. If this is not accompanied by an overt phenotypic transformation, there might be chances of undetected harm to the patient. Therefore, it is important to understand the rate of genetic changes and the type of selective pressure that allows this changes to dominate a culture.

It is unclear if human stem cells can integrate into the recipient tissue and fulfil the specific functions of lost or injured tissues [41]. Eventually further research and long term studies will lead to the development of regenerative therapy.

Future

The future for stem cell-based periodontal regeneration is very promising. However, as with all new technologies, questions often arise at a faster rate than answers. The time is now ripe to move from animal studies to human clinical trials. Various animal studies done using stem cells provide overwhelming evidence to support that mesenchymal stem cells can be used for periodontal regeneration. However, it is evaluative in moving this field towards human clinical utility. Various considerations such as appropriate delivery devices, immunogenicity, autologous cells vs allogeneic cells and cost-effectiveness are all important and should not be overlooked. Furthermore, the next critical phase is the systematic validation of specific mesenchymal stem cells as reliable sources for cytotherapeutic use. Therefore, the establishment of large-scale preparation helps in incorporating the stringent protocols of good manufacturing procedures will be an absolute necessity.

Many researchers have seen promising results in several preclinical animal studies. Based on this, numerous clinical trials are ongoing globally to further validate these findings. The next evolution of stem cells is regenerative dental kits, which will have the ability to deliver stem cell therapies locally as part of routine dental practice. This is aimed mainly at reprogramming dental stem cells into its embryonic state which will expand their potential to differentiate into enormous amount of tissue types. In animal studies, researchers have been successful in making specific dental tissues and tooth-like structures. Further advancement will be regeneration of functional tooth in humans.

Conclusion

Human stem cell research is a newly developing field in medicine and companies developing stem cell therapies face various types of risks out of which some of them are not able to be manageable, thus pushing this venture into a highly speculative enterprise. The latest clinical trials are being performed on human platelet-derived growth factor, recombinant human fibroblast growth factor-2 and tricalcium phosphate (GEM-21) to bring dreams into reality [42]. Hence it is early to conclude if these trials will bring about effective stem cell based therapies in the near future.

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