Stem cells a novel approach to periodontal regeneration (A review of literature)

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ABSTRACT

In this review of literature, the light will be concentrated on the role of stem cells as an approach in periodontal regeneration. (J Bagh Coll Dentistry 2014; 26(3):89-97).

INTRODUCTION

Recent exciting discoveries place dentists at the forefront of engaging their patients in potentially life-saving therapiesderived from a patient's own stem cells located in deciduousand permanent teeth. Adult stem cells, including dental stemcells, have the potential, like bone marrow-derived stem cellsand adipose-derived stem cells, to cure a number of diseases.

In medicine, stem cell-based treatments are being usedand investigated for conditions as diverse as Parkinson's disease, neural degeneration following brain injury, cardiovasculardisease and autoimmune diseases. Stem cells will be usedin dentistry for the regeneration of dentin and/or dental pulp, biologically viable scaffolds will be used for the replacementof orofacial bone and cartilage, and defective salivary glandswill be partially or completely regenerated.

Dental stem cells can be obtained from the pulp of theprimary and permanent teeth, from the periodontal ligament, and from associated healthy tissues. Exfoliating/extracted deciduousteeth and permanent teeth extracted for orthodontictreatment, trauma or dental implant indications are all readilyavailable sources of dental stem cells. The harvest of these dentalstem cells results in minimal trauma. Dental professionals have the opportunity to make their patients aware of these new sources of stem cells that can be stored for future use as new therapies are developed for a range of diseases and injuries[1] .

Historical Review

In 2000, the National Institutes of Health(NIH) released two studies of research on human teeth detailing the discovery of adult stem cells in impacted third molars and even more resilient stem cells in deciduous teeth.

Dentistry and medicine are evolving into new forms, in which care is being delivered with increasing frequency through biologically based approaches. The first wave of this paradigm shift in health care is likely more imminent than anyone is willing to predict at present, and its impact will eventually be felt in every medical and dental office and setting [2] .

No longer will it be necessary to rely on the chance offending a cellular match from a donor, and devastating andformerly incurable diseases could potentially be treated.

This is no longer science fiction. Some stem cell therapies have already been approved or are being reviewed by the U.S.Food and Drug Administration (FDA), while others are atvarious stages of development. Starting in the 1970s, it was discovered that cells taken from bone marrow post-natally had the ability to differentiate into bone, cartilage and marrow fat cells when they were transplanted [3] .Stem cell research has increased dramatically in recent years as the potential for use of stem cells has become better understood. Degenerative diseases increase in incidence with age, it can be anticipated that the need for viable and improved treatment options for these diseases will increase. Patients are being treated using stem cells for cardiovascular, orthopedic, dental, oncological and other condition. New stem cell therapies will become available in the future, and in the next three years it is anticipated that stem cell product therapies for graft-versus-host disease, damaged heart muscle due to cardiac disease and knee cartilage repair will become available [4].

Stem Cell Types and Sources

Stem cells are defined as cells that have clonegenic and self-renewing capabilities and differentiate into multiple cell lineages [5] .Stem cells are the foundation cells for every organ and tissue in the body, astem cell has two defining characteristics:

(i) The ability for indefinite self - renewal to give rise to more stem cells; and

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(ii) The ability to differentiate into a number of specialized daughter cells to perform specific functions [6].

Multipotent stem cells

Consist of three major types — ectodermal (skin and nerves), mesodermal or mesenchymal (bone, cartilage, muscle and adipose), and endodermal (intestines and other). The two main categories of stem cells are embryonic stem cells and adult stem cells, defined by their source.

Embryonic stem cells

Embryonic stem cells (ESCs) are derived from the cells of the inner cell mass of the blastocyst during embryonic development. ESCs have the capacity to differentiate into any cell type and the ability to self-replicate for numerous generations. A potential disadvantage of ESCs is their ability to differentiate into any cell lineage and to proliferate endlessly unless controlled [7] .The clinically observed teratoma is a tumor that is an example of ESCs growing into a "different and undesired tissue." ESCs can be obtained only from embryos and therefore are associated with ethical issues.

Adult stem cells

Sources of adult stem cells include the umbilical cord, amniotic fluid, bone marrow, adipose tissue, brain and teeth [8] .Adult stem cells are not subject to the ethical controversy that is associated with embryonic stem cells; they can also be autologous and isolated from the patient being treated, where as embryonic stem cells cannot.

Induced pluripotent stem cells (iPS)

The newly discovered iPS cells are adult or somatic stem cells that have been coaxed to behave like embryonic stem cells [6] .iPS cells have the capacity to generate a large quantity of stem cells as an autologous source that can be used to regenerate patient-specific tissues. However, even the authors of these recent reports have cautioned that any carcinogenic potential of iPS cells should be fully investigated before any commercialization can be realized.

Amniotic fluid-derived stem cells (AFSCs)

AFSCs can be isolated from aspirates of amniocentesis during genetic screening. An increasing number of studies have demonstrated that AFSCs have the capacity for remarkable proliferation and differentiation into multiple lineages such as chondrocytes (for cartilage), adipocytes (for fat), osteoblasts (for bone), myocytes (for muscle), endothelial cells, neuronlikecells and live cells [9] .The potential therapeutic value of AFSCs remains to be discovered.

Umbilical cord blood stem cells (UCBSCs)

UCBSCs derive from the blood of the umbilical cord [10] .There is a growing interest in their capacity for self-replication and multilineage differentiation, and UCBSCs have been differentiated into several cell types that resemble cells of the liver, skeletal muscle, neural tissue, pancreatic cells, immune cells and mesenchymal stem cells [11] .Several studies have shown the differentiation potential of human UCBSCs in treating cardiac [12] and diabetic diseases in mice [13] .The greatest disadvantage of UCBSCs is that there is only one opportunity to harvest them from the umbilical cord at the time of birth. Similarly, amniotic stem cells can be sourced only from amniotic fluid and are therefore subject to time constraints.

Bone marrow-derived stem cells (BMSCs)

BMSCs consist of both hematopoietic stem cells that generate all types of blood cells and stromal cells (mesenchymal stem cells) that generate bone, cartilage, other connective tissues and fat. BMSCs are currently the most common commercially available stem cell [4] .They can be isolated from bone marrow aspiration or from the collection of peripheral blood-derivedstem cells following chemical stimulation of the bone marrow, by means of subcutaneous injection, to release stem cells [14].

Adipose-derived stem cells (ASCs)

ASCs are typically isolated from lipectomy or liposuction aspirates. They have been differentiated into adipocytes, chondrocytes, myocytes, and neuronal and osteoblast lineages, and may provide hematopoietic support. ASCs express some, but certainly not all, of the cell markers that bone marrow MSCs express. ASCs have an advantage in that adipose tissue is plentiful in many individuals, accessible and replenishable, the ability to reconstitute tissues and organs using ASCs versus other adult stem cells has yet to be comprehensively compared and documented.

Dental stem cells (DSCs)

Dental stem cells (DSCs) can be obtained from the pulp of the primary and permanent teeth, from the periodontal ligament, and from other tooth structure. Periodontal ligamentderived stem cells are able to generate periodontal ligament and

cementum [15] .Extracted third molars; exfoliating/extracted deciduous teeth; and teeth extracted for orthodontictreatment, trauma or periodontal disease are all sources of dental stem cells from the dental pulp. The dental pulp offers a source of stem cells postnatally that is readily available, with a minimally invasive process that results in minimal trauma.

Exfoliating or extracted deciduous teeth offer extra advantages over other teeth as a source of stem cells. Stem cells from deciduous teeth have been found to grow more rapidly than those from other sources, and it is believed that this is because they may be less mature than other stem cells foundin the body. Additional advantages of sourcing stem cells from exfoliating deciduous teeth are that the cells are readily available, provided they are stored until they may be needed later in life; the process does not require a patient to sacrifice a tooth to source the stem cells; and there is little or no trauma. The structures of interest to the dental profession are the enamel; dentin; dental pulp; cementum; periodontal ligament; craniofacial bones; temporomandibular joint, including bone, fibrocartilage and ligaments; skeletal muscles and tendons; skin and subcutaneous soft tissue; salivary glands; and so forth. Without exception, neural crest-derived and/ormesenchymal cells form all these dental, oral and craniofacial structures during native development. Several populations of adult stem cells have been explored for the regeneration of dental, oral and craniofacial structures, including bone morrow stem cells(BMSCs), adipose-derived stem cells (ASCs) and dental stem cells(DSCs), [16] which despite important differences between them, are likely the subfamily of mesenchymal stem cells [17].

Adult dental pulp stem cells (DPSC)

An increasing number of studies have indicated dental pulp is a highly vascularized tissue and contains several niches of stem cells. The DPSC have multipotency, being capable of differentiating into odontoblasts, osteoblasts, adipocytes, chondrocytes, or neural cells. The regenerative capacity of the human dentin/pulp complex implies that dental pulp may contain the progenitors that are responsible for dentin repair [8] .First identified adult DPSC in human dental pulp in 2000 and found DPSC could regenerate a dentin-pulp-like complex, which is composed of mineralized matrix with tubules lined with odontoblasts, and fibrous tissue containing blood vessels in an arrangement similar to the dentin-pulp complex found in normal human teeth. The same group further verified that DPSC posses striking features of self-renewal capability and multiline age differentiation by finding that DPSC were capable of forming ectopic dentin and associated pulp tissue in-vivo and differentiating into adipocytes and neural-like cells [18].

Stem cells from dental follicle (DFSC)

The dental follicle is a mesenchymal tissue that surrounds the developing tooth germ. During tooth root formation, periodontal components, such as cementum, periodontal PDL, and alveolar bone, are created by dental follicle progenitors [19]. Stem cells from dental follicle have been isolated from follicle of human third molars and express the stem cell markers: Notch1, STRO-1 and nestin. DFSC were found to be able to differentiate into osteoblasts/cementoblasts, adipocytes, and neurons [20] .In addition, immortalized dental follicle cells were transplanted into immunodeficient mice and were able to recreate a new PDL-like tissue after 4 weeks. These cells may be a useful research tool for studying PDL formation and for developing regeneration therapies.

Periodontal ligament stem cells (PDLSC)

The PDL is a specialized connective tissue, derived from dental follicle and originated from neural crest cells. Recent studies have shown that mesenchymal stem cells (MSC) obtained from PDL – PDLSC are multipotent cells with similar features of the BMMSC and DPSC, capable of developing different types of tissuessuch as bone and tooth associated tissues. It was reported that PDLSC could differentiate into cells that can colonize and grow on biocompatible scaffold, suggesting an easy and efficient autologous source of stem cells for bone tissue engineering in regenerative dentistry. Orciani et al. [21] verified the osteogenic ability of PDLSC and pointed out that differentiating cells were also characterized by an increase of Ca2+ and nitric oxide production. The authors demonstrated that local re-implantation of expanded cells in conjugation with a nitric oxide donor could represent a promising method for treatment of periodontal defects. Besides osteogenic ability, differentiation of PDLSC to the cementoblastic lineage was also emphasized. The conditioned medium from developing apical tooth germ cells was shown to be able to provide a cementogenic micro-environment and induce differentiation of PDLSC along the cementoblastic lineage. When transplanted into immunocompromised mice, the induced PDLSC showed tissue regenerative capacity to produce cementum/PDL -like structures, characterized by a layer of cementum -

like mineralized tissues connected with PDL - like collagen fibers. There is evidence that human PDL,with its mesodermal derivatives, produced neural crest- like cells. Such features suggest a recapitulation of their embryonic state. The human PDL reveals itself as a viable alternative source for possible primitive precursors to be used in stem cell therapies.

Stem cells from Human Exfoliated Deciduous teeth (SHED)

The discovery of stem cell in deciduous teeth [22].Sheds light on the intriguing possibility of using dental pulp stem cells for tissue engineering. The obvious advantages of SHEDs are:

a) Higher proliferation rate compared with stem cells from permanent teeth; because they are less mature than other stem cells found in the body.
b) Easy to be expanded *in-vitro*.
c) High plasticity since they can differentiate into neurons, adipocytes, osteoblasts and odontoblasts.
d) Readily accessible in young patients.
e) Especially suitable for young patients with mixeddentition.
f) The process does not require a patient to sacrifice a tooth to source the stem cells.
g) There is little or no trauma.

Stem cell properties of adult human periodontal ligament cells

Stem cell properties include self-renewal, multipotency, and stem cell marker expression. PDL cells obtained from extracted human molars were highly proliferative and clonogenic. Further analysis revealed that PDL cells, including cell populations, expressed the stem cell markers CD105, CD166, and STRO-1, CD146/ MUC18. Under defined culture conditions, periodontal ligament stem cells (PDLSCs) differentiated into cementoblast- like cells, adipocytes, and collagen-forming cells; *in vivo*study indicated that PDLSCs also showed the capacity to form cementum /PDL like tissues [23, 24] .

Human induced pluripotent stem cells (iPS), which have similar properties to human embryonic stem (hES) cells, have been generated from dental tissue exfoliated deciduous teeth, apical papilla and dental pulp stem cells by viral vectors reprogramming [25]. Human gingival fibroblast- and periodontal ligament fibroblast-derived induced pluripotent stem cells showed similar characteristics to human embryonic stem cells.

These induced pluripotent stem cells showed differentiation potential to form embryoid bodies *in vitro* and expressed genes for endoderm, ectoderm and mesoderm. Teratoma formation following implantation into mouse testes was observed. Induced pluripotent stem cells may be a potential autologous stem cell source for future regenerative therapy [26].

The Ability of Human Stem Cells to Regenerate Periodontal Tissues

It has been demonstrated that PDL may contain progenitor cells capable of differentiation into cementoblasts in vitro. Taken together, the results of these studies demonstrated the capacity of multipotent stem cells from human PDL, or periodontium-derived stem cells (pdSCs), to generate a cementum-like tissue in vivo, thus representing a new therapeutic option for periodontal regeneration.

Periodontal diseases that lead to the destruction of periodontal tissues including periodontal ligament (PDL), cementum, and bone are a major cause of tooth loss in adults and are a substantial public-health burden worldwide.

The periodontium is a topographically complex organ consisting of epithelial tissue and soft and mineralized connective tissues. Several diseases affect the composition and integrity of periodontal structures causing destruction of the connective tissue matrix and cells, loss of fibrous attachment and resorption of alveolar bone [27]. These changes often lead to tooth loss. The ultimate goal of periodontal treatment is to prevent further attachment loss and regenerate the periodontal supporting tissues lost because of the disease. Currently, a great improvement has been made on the understanding of cellular and molecular events involved in the formation and regeneration of periodontal tissues, and tissue engineering based approaches have emerged as prospective alternatives to conventional treatments.

A method to isolate and expand a stem cell population from periodontal granulation tissue has been described recently [28]. These pdSCs were positive for the neural stemness markers Nestin& Sox2 & can differentiate into various cell types of the neuronal lineage, including glial cells. However, whether pdSCs are also capable of differentiation into the osteogenic lineage & regenerating periodontal tissue in vivo is unknown.

At test sites where collagen sponges with pdSCs were transplanted, a reformation of PDL-like tissue, elements of bone, and osteocytes lacunae in the bone tissue could be seen after 6

weeks. Some putative transplanted cells were observed to attach onto root dentin surfaces. Blood vessels and collagen fibers could also be shown in the PDL tissue. In the regenerated PDL tissues, immature thin fibers were obliquely arranged parallel to the bone surfaces and not in a perpendicular direction. Such a fibril anchoring was never observed in the control sites. These observations were consistent for all four rats sacrificed 6 weeks postsurgery.However a "functional periodontium was not evident. Down-growth of junctional epithelium was observed to a slight degree over the investigation period. In several, new formation could be observed. Periodontium –derived ligament stem cells (PDL stem cells), which have been isolated from root surface of extracted teeth, were first described by Seo et al. [(15)] .In contrast, the current authors used human adult pdSCs that had been isolated from patients who suffered from a chronic type of periodontitis with a severe degree of inflammation. The stemness of these cells was verified .Several studies demonstrated that mesenchymal stem cells are capable of differentiating into osteoblast-like cells, cementoblast-like cells, and adipocytes. These data are in agreement with results demonstrating that human adult pdSCs isolated from granulation tissue and subsequently expanded ex vivo are capable of differentiating into the osteogenic lineage [(28)].

The investigation of the regenerative capacity of human stem cells in animal model prerequisites the necessity of immunocompromised animals to avoid the rejection of the stem cell grafts. In a pilot study Zhao et al. [(29)] demonstrated that cementoblasts have a marked ability to induce mineralization in periodontal wounds while implanted dental follicle cells seem to inhibit periodontal sites of the current animal model, a cementum layer was observed on the root surfaces. This may suggest that this layer was comparatively immature and newly deposited onto previously denuded root surfaces. Obviously, even in this case a "functional periodontium" seemed not to be regenerated. Conceptually, the delivery of pdSCs to the denuded area in periodontal defects may serve as a viable approach to promote ideal periodontal tissue regeneration. When implanted into immunocompromised rats in association with a conductive carrier material, sphere-expended human pdSCs possessed the potential to develop periodontal tissues. Of particular importance was the observation that the human pdSCs could produce both mineralized and soft connective tissues with many morphologic features similar to cementum –like layers containing inserted Sharpey fibers. This strongly implies that this tissue is of a periodontal nature. This in vivo study clearly showed that human adult pdSCs transplanted into an athymic rat model were able to regenerate tissue element at different levels. However, prior to the ultimate use of pdSCs in human trials, further in vivo animal studies should be conducted to optimize the cells regenerative capacity.

According to recent estimates, 80–90 percent of human beings have at least one impacted “third molar” that must be removed surgically, and a large number of teeth are routinely extracted because of periodontitis or orthodontic reasons. On the other hand, deciduous teeth are routinely lost in childhood and are generally discarded. While orthodontic treatment and extraction of wisdom teeth are common in the young, there is a portion of the aged population whose third impacted molars were not removed at the correct stage, especially in rural regions and developing countries. As dental stem cells share properties with mesenchymal stem cells, dentistry should be at the forefront of stem cell translational and clinical research because of the huge numbers of patients involved and the accessibility of teeth, with the result that no major surgery is required to obtain cells. There is also considerable interest in the wider potential of these cells to treat disorders involving mesenchymal (or indeed non-mesenchymal) cell derivatives, such as in musculoskeletal disease or other life-threatening diseases cells.

Rationale of PDLSCs used for the periodontal tissue regeneration

Even though bone marrow mesenchymal stem cells can contribute to the regeneration of new cementum, bone and periodontal ligament in beagle dog [(30)] , PDLSCs are the most promising candidates for the periodontal regeneration by comparing periodontal ligament cells (PDLCs), iliac bone marrow mesenchymal stromal cells, and alveolar periosteal cells Three layered cell sheets of each cell source supported with woven polyglycolic acid were transplanted autologously to the denuded root surface of beagle dog. After eight weeks, significantly more periodontal regeneration was observed as newly formed cementum and well-oriented PDL fibers in PDLC group than in the other groups. Nerve filament was observed in the regenerated PDL tissue only in the PDLC group, as well as the largest amount of alveolar bone regeneration [(31)]. More studies in beagle dog showed the formation of cementoblasts by seeding of the autologous

periodontal ligament cells [32].In an extreme experiment, extracted dog's premolar teeth were maintained in a dry environment for a month after isolation and proliferation of the PDL cells. Cultured autologous PDL cells were found to assist the re-establishment of periodontal architecture of autotransplanted teeth that is devoid of viable periodontal cells [33]. To identify the cell source of PDL regeneration, researchers extracted first molars from the maxilla of 10 lacZ transgenic ROSA26 mice and transplanted them into the maxillary first molar socket of 10 wild type ROSA26 mice. After 2 weeks, no donor cells from lacZ transgenic mice were detected in the periodontal ligament space. This experiment indicated that periodontal tissue regeneration was induced by host cells, which replaced the donor periodontal tissue cells after allogenic tooth transplantation [34].

Nanomaterials scaffold

The goal of periodontal tissue regeneration consists of establishing reparative pathways in order to treat degenerative, injury, and trauma in periodontal ligament and related bone. Despite the fact that the periodontal ligament cells, mesenchymal stem cells or periodontal ligament stem cells can turn into a population of differentiated cells in certain environments, those cells cannot reconstruct three dimensional tissues without proper scaffold materials.

The synthetic polymer, polysaccharide hydrogel, bio-ceramics, biomimetic peptides and collagen were investigated as transplant scaffold for tissue regeneration. Scaffold materials with nanoscale topography, such as in the form of nanoparticles, nanoporous and nanofibers, show very different mechanical properties and unique biocompatibility to cell behaviors compared to flat bulk materials. The recent development in biomaterial has brought nanotechnology in improving dental implant surface modification [35,36].

Three-dimensional porous nano-hydroxyapatite/chitosan scaffolds were prepared through a freeze-drying process. Human periodontal ligament cells were seeded onto the scaffolds, and then these scaffolds were implanted subcutaneously into athymic mice. The expression of type I collagen and alkaline phosphatase were up-regulated in HA/chitosan scaffold. After implanted *in vivo*, human periodontal ligament cells proliferated and grew in the scaffold with surrounding tissue [37]. Using a self-assembling bioactive matrix. Dr. Snead's group demonstrated the ability to induce ectopic formation of enamel at chosen sites adjacent to a mouse incisor cultured *in vivo* under the kidney capsule. The resulting material revealed the highly organized, hierarchical structure of hydroxyapatite crystallites similar to native enamel [38].

In a de novo test autologue PDL cell sheets were transplanted into a delayed replanted avulsed tooth in canine replantation model [39]. The cell sheet containing original extracellular matrix showed a successful deliveryof PDL and formation of new PDL tissue for 8 weeks. However, without proper three dimensional extracellular matrix supports, PDL tissue was unable to be fully regenerated.

Tissue Engineering With Stem Cells

Stem cells from a tiny amount of tissue, such as the dental pulp, can be multiplied or expanded to potentially sufficient numbers for healing large, clinically relevant defects. Stem cells differentiating into multiple cell lineages offer the possibility that a common (stem) cell source can heal many tissues in the same patient, as opposed to harvesting healthy autologous tissue to heal like tissue. Finally, stem cells can be seeded in biocompatible scaffolds in the shape of the anatomical structure that is to be replaced [40].

The fundamental reasons for the effectiveness of stemcells are as follows:

- Unlike end-lineage cells, stem cells can be expanded ex vivo (outside the body). Thus a small number of stem cells can be sufficient to heal large defects or to treat diseases. In contrast, a large number of end-lineage cellsneed to be harvested for tissue regeneration, necessitating donor site trauma and defects.
- Stem cells may elaborate and organize tissues in vivo, especially in the presence of vasculature.
- Stem cells may regulate local and systemic immune reactions of the host in ways that favor tissue regeneration.
- Stem cells may provide a renewable supply of tissueforming cells.

Applications of dental stem cells

Stem cells prove to be a better option as stem cell therapy could potentially lead to the regeneration of tooth roots, with PDL that can remodel with host bone, which would be functionally superior to titanium dental implants .(ibid).

Banking teeth and dental stem cells offers patients a viable alternative to using more invasive or ethically problematic sources of stem cells, and harvesting can be done during routine

procedures in adults and from the deciduous teeth of children. Now, dental professionals have the opportunity to make their patients aware of these new sources of stem cells that can be conveniently recovered and remotely stored for future use as new therapies are developed for a range of diseases and injuries.

Dental applications under investigation:

a) Craniofacial regeneration
b) Cleft lip and palate
c) Tooth regeneration
d) Pulp regeneration
e) Periodontal ligament regeneration
f) Enamel and dentin production.

Stem Cell Handling and Cryopreservation

Stem cells are released from small amounts of tissue, in the case of dental stem cells from dental pulp. The tissue is placed in an enzyme solution that releases the stem cells, which are then cultured to multiply. This can be accomplished using serum-free medium, removing the need for use of animal serum. Differentiation then occurs and the cells are transplanted either alone or with a scaffold or other biomaterials, depending on the application.

Cryopreservation

Stem cells must be derived from living tissue and must be preserved. This is achieved by cryopreservation. The cells are rapidly cooled to subzero temperatures as low as −196° Celsius, stopping any cellular or biochemical activity. Rapid freezing is necessary to prevent ice from forming around or inside the cells and to prevent dehydration, as these would cause cell damage and death. Extracted permanent and deciduous (including exfoliating) teeth can be preserved for future use with cryopreservation. Research has demonstrated that stem cells derived from the dental pulp of extracted third molars retain the ability to differentiate into multiple cell types following thawing after cryopreservation using liquid nitrogen [41] Stem cells derived from the periodontal ligament are viable following cryopreservation [24]. After two years of cryopreservation, stem cells have been able to differentiate and to proliferate, and it has been concluded that Dental Stem Cells DSCs can undergo long-term cryopreservation [42].

Periodontal ligament (PDL) is the most crucial tissue to support the tooth and provide anti-shock function.Although the metal implantscan be used to replaceteeth rootsand support artificial crowns, the dental implant survival rate for16 years is as low as 82.94%. Consideringthe biological and technicalcomplications, the cumulativeimplant success rate is 51.97% [43]. The most failurereason is related to absence of osseo- integration in early healing stageor due to the occurrence of periimplantitis in long-term follow-up.Both of them are closely associatedwith the poor periodontal tissueregeneration. So, it is extremelydemanded to properly regeneratePDL after implantation.Since the initial conception of tissue engineering published in Science[44], in the light of recent improvements in nanotechnologyand stem cell biology, the tissue regeneration in periodontology has become well understood and applied in clinic trials. Tissue engineeringis the use of a combinationof functional cells, engineering andmaterials methods, and suitable biochemical and physio-chemical factors to improve or replace biologicalfunctions. The main goal of periodontaltissue regeneration is the optimizationand enhancement of thebiological mechanisms of periodontalwound healing in order to maximizethe extent of the restored periodontalapparatus, i.e. alveolar bone, PDL and cementum [45]. Even though several regenerativeapproaches, such as guidedtissue regeneration, topicalapplicationof enamel matrix derivative, various growth factors, have been proposed in order to treat periodontal disease [46], the periodontal tissue regeneration was limited using these treatments and the efficacy is unclear. Mesenchymal stem cell (MSC)-mediated tissue regeneration is a promising approach for regenerative medicine for a wide range of applications.

CONCLUSIONS

a) Among all the dental-derived stem cells identified, PDLSCs are unique population capable of forming an ectopic cementum/ PDL -like structure.
b) With the addition of 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.
c) Both dental and non-dental derived stem cells might be potentially applied in regenerative periodontal therapies.
d) Agencies around the world are now funding stem cell research, and growing numbers of scientists are entering this field. The result should be a global collaboration focused on delivering clinical outcomes of immense benefit to the world's population. It is only the beginning of a very long road of work and

discovery, but one thing is certain the research on stem cells – the precursors for life is vital and must go on.

e) Hence to conclude: ***"Pro-life paves the path for life"*** [47].

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