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A Review of Periodontal Ligament-Derived Mesenchymal Stem Cells Being Used to Regenerate the Periodontal Ligament and Periodontium

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ABSTRACT

Periodontal disease is an inflammatory disease of the tissues making up the periodontium that consists of alveolar bone resorption, recession of the gingiva, as well as damage to the periodontal ligament and cementum caused by accumulation of bacteria in the oral cavity. The method of treatment is dependent upon the depth of pocket formation and stage of disease advancement. When pockets are at a depth between 4 and 5 mm, nonsurgical treatments such as scaling, root planing, and antibiotics are used to treat. Surgical methods are used, however, when pockets are deeper than 5 mm. Both nonsurgical and surgical treatments currently used have limited capabilities to regenerate parts of the periodontium. The discovery of periodontal ligament-derived mesenchymal stem cells and their ability to generate cementoblasts, osteoblasts, adipocytes, chondroblasts, and fibroblasts in vitro that all contribute to the formation of the periodontium. This paper discusses the aims of current and future research on periodontal ligament stem cells and their potential to regenerate the periodontal ligament, as well as the entire periodontium.

Keywords

Mesenchymal stem cells, Periodontium, Regeneration, Stem cells, Periodontal ligament.

Abbreviations

BMSSC: Bone marrow stromal stem cells; cASC: Canine adipose-derived stem cells; DFSC: Dental follicle stem cells; DPSC: Dental pulp stem cells; EGF: epidermal growth factor; FGF: fibroblast growth factor; IGF: insulin-like growth factor; iPSC: Induced pluripotent stem cells; JE: Junctional epithelium; MMP: Matrix metalloproteinase; PDGF: platelet-derived growth factor; PDLSC: Periodontal ligament stem cells; RANKL: Receptor activator of nuclear factor kappa-β ligand; SHED: Stem cells from human exfoliated deciduous teeth; SPCL: starch and poly-ε-caprolactone; TGF-β: transforming growth factor beta.

Discussion

Periodontal Disease

Periodontal disease exists in 47% of adults over 30 in the United States, making it, in addition to tooth decay, one of the largest threats to oral health [1]. These diseases are mainly caused by a microbial infection in the mouth that causes the host to release an immune response, resulting in inflammation of the gums and bone surrounding the tooth surface [2]. It has been found that smoking, hereditary factors, stress, diabetes, and poor oral health all put a person at higher risk of developing periodontal disease. Although these raise the risk, the microbial infection can be prevented by brushing and flossing daily as well as seeing a dental professional yearly [1,3].

In the early stages during which the gums swell and bleed, the disease is referred to as gingivitis. However, as the condition advances, the gum tissue dissociates from the tooth, bone loss occurs, and tooth loss may occur [3]. This advanced stage is referred to as periodontitis and occurs when oral bacteria remain on teeth long enough to form plaque underneath the gums and on the tooth surface. The plaque ultimately advances to form calculus, a hard film that requires removal by a dental professional [1,3]. When the disease progresses, symptoms such as red gums, pain while chewing, loose teeth, and bad breath become more apparent.

The chronic inflammatory response due to the infection drastically affects the structure of the periodontium. First, the periodontium consists of four tissues: gingiva (gums), cementum (tooth surface), alveolar bone (bone socket), and the periodontal ligament (anchors the tooth root to the alveolar bone socket) [4]. Periodontal health remains in homeostasis by immune cells residing in the gingiva at all times. Therefore, the immune response is constantly engaged due to the balance between the host and oral biofilms and acts first with a non-specific immune response [4]. When dental plaque builds up surrounding the givina, inflammation occurs along with aggregation of proteolytic and obligately anaerobic bacteria. An inflammatory response is initiated when these species show potential pathogenic properties in the space lined by epithelium between the tooth and gingiva called the gingival sulcus [4]. The pathogens result in a disruption of homeostasis between the oral microbiome and host, irreversibly harming the four tissues of the periodontium [5]. The treatment and regeneration of this irreversible tissue damage is a highly popular area of current research.

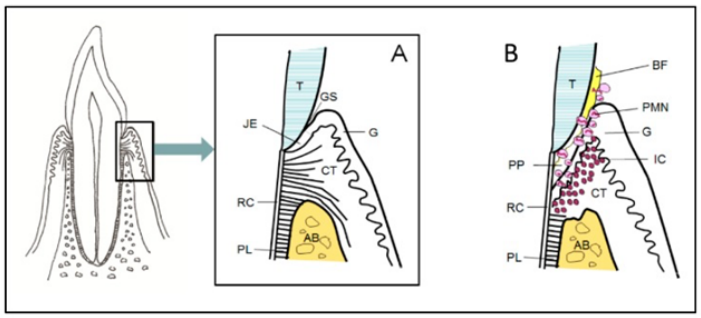

Figure 1: Structure of the periodontium consisting of the junctional epithelium (JE), gingiva (G), periodontal ligament (PL), alveolar bone (AB), connective tissue (CT), gingival sulcus (GS), root cementum (RC), the tooth (T), periodontal pocket (PP), bacterial biofilm (BB), inflammatory cells (IC), and neutrophils (PMN). A shows a healthy periodontium while B shows a periodontium with periodontitis [4].

There is a constant flow of various bacteria and microbes in and out of the oral cavity. Therefore, the structure of the junctional epithelium, shown as JE in Figure 1, acts as a seal between the tooth surface and gingiva in order to protect the underlying tissue from constant microbial invasion. Molecular factors such as cell-to-cell adhesion and chemotaxis act to maintain the homeostasis and aid in the functioning of the JE. However, when there is prolonged inflammation and virulence factors, pocket formation occurs due to the JE moving apically on the root surface and activating collagen destruction [4]. When the periodontal pockets form, tissue degradation occurs through the stimulation of neutrophils, macrophages, and osteoclasts due to the production of proinflammatory cytokines, chemokines, and antimicrobial peptides, while tissue formation is suppressed. The introduction of the collagenolytic matrix metalloproteinases (MMPs) and elastases to the gingival tissues leads to the degradation of the type I collagen that is resident in connective tissue and the periodontal ligament [6]. When the MMPs, elastases, and plasma cells migrate deeper into the connective tissue, the irreversible damage of the alveolar bone and periodontal ligament progresses [7].

Bone resorption is the main irreversible issue caused by periodontal disease. When the inflammatory cascade is activated, the proinflammatory cytokines (IL-1β, IL-6, and TNF-α) and MMP proteins then activate the release of osteoclasts [4]. The proinflammatory cytokines are upregulated due to RANKL expression while osteoblast differentiation and new bone formation is inhibited, which favors osteoclastogenesis and alveolar bone resorption [8]. In addition, the MMP production plays a role in degrading type I collagen, osteoclast migration, as well as osteoclast activation, which all work with the cytokines to cause the characteristic irreversible bone resorption of periodontitis [9].

Periodontitis can be initially diagnosed based on alveolar bone loss and analysis of the periodontal attachment apparatus. As the disease progresses, the stage can be determined by the depth of the periodontal pocket formation. When the pocket formations enlarge, the teeth become loose and will fall out if treatment is ignored [4]. Therefore, the further the disease progresses before diagnosis will require a more invasive treatment method.

Current Treatments

The course of treatment for periodontal disease is dependent upon the stage and grade of the condition, which can be determined by x-rays, mouth examinations, and pocket depth measurements. A healthy mouth typically has pockets between 1 millimeter and 3 millimeters while pockets at 4 millimeters are an indication of early periodontal disease. When diagnosed early, the pockets can be treated nonsurgically through scaling, root planing (demineralization of root surfaces), or a course of antibiotics [10]. However, if the pockets reach a depth of 5 millimeters, the condition is considered more advanced and difficult to clean, which requires surgery as a treatment. There are various options for surgery depending on which sections of the periodontium are most significantly damaged, however, they all provide limited value for complete regeneration of the entire periodontium [10]. For instance, both soft tissue and alveolar bone grafts are options for gum recession and bone resorption. When the gingiva is destroyed, gum recession occurs and a periodontist can obtain a small soft tissue graft from the palate or a donor and place it on the destroyed area in order to reduce recession and cover exposed root surfaces. In contrast to the soft tissue graft, bone grafts are performed to prevent further bone loss and promote future natural bone growth [10]. These bone grafts are made of various materials such as xenografts, allografts, autografts, or alloplastic materials. However, studies found these bone grafts have a very minute osteoinductive capacity due to them eventually becoming engulfed by dense fibrous connective tissue [11]. In addition to grafting, pocket reduction surgery (flap surgery), guided tissue regeneration, and tissue-stimulating proteins are also surgical methods of periodontal treatment. In the case of pocket reduction surgery, a periodontist creates tiny incisions in the pocket in order to lift the gingiva and provide a greater root surface to perform root planing and scaling, ultimately creating easier surfaces to clean both during and after the procedure without regenerating any of

the lost tissues [10]. Guided tissue regeneration is performed by placing a barrier membrane between the existing alveolar bone and root surface in order to keep bacteria, unwanted epithelium, and gingival connective tissue from invading and allow for regrowth due to the regenerative cells in the periodontal ligament, which is located between the tooth root and the bone socket [12]. Although this is proven to effectively promote connective tissue attachment to the root surface, there has been little evidence of new bone and cementum formation [12]. Therefore, this is not true regeneration but has given a promising direction of research into the regenerative cells in the periodontal ligament [13]. In addition to the guided tissue regeneration, tissue-stimulating proteins can be used in conjunction with the flap surgery to induce the growth of bone and tissue by applying a gel containing amelogenin, a protein found in developing teeth in infants, and various other growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), PDGF and TGF-β to the tooth root due to them having roles in immune system regulation, epithelial cells, and bone and soft connective tissues [14,15].

Although bone and soft tissue grafting, root surface conditioning, and guided tissue regeneration are popular courses of treatment for advanced periodontal disease, these methods are not considered to conduct true regeneration of the entire periodontium. In order to be considered full regeneration, the four tissues of the periodontium (alveolar bone, periodontal ligament, gingiva, and cementum) must all regrow [16]. Therefore, research is currently being performed to understand the full developmental pathway of the periodontium in order to create the most effective treatments using growth factors, enamel matrix proteins, gene therapy, and stem cell replacement therapy. Stem cell therapy is a rapidly growing area of interest in periodontal regenerative research due to the discovery of multipotent periodontal ligament stem cells (PDLSC) in the periodontal ligament of the third molar in humans [16].

Periodontium Development and Healing

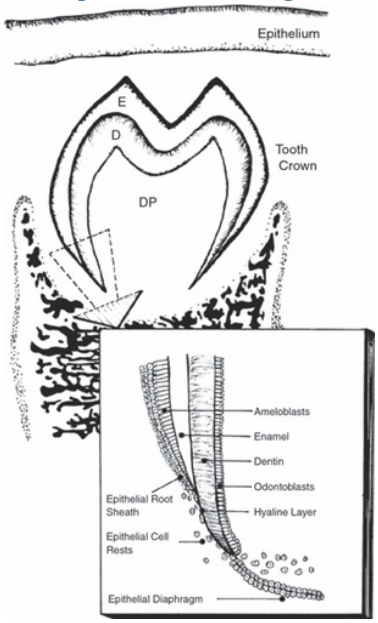

Figure 2: Diagram of root and periodontal development at the end of the crown stage, consisting of enamel (E), dentine (D), and dental papilla (DP) [17].

Children's teeth begin developing around the ages of 6 to 12 month, in which the periodontium development begins at the end of the crown stage when the inner and outer enamel epithelium join together to form the cervical loop as shown in Figure 2. Hertwig's epithelial root sheath then forms from rapid proliferation of cells on the cervical loop and separates the dental papilla from the dental follicle [18]. Odontoblast differentiation then proceeds from the newly made dental papilla, producing root dentine [19]. After root dentine formation, the root sheath cells secrete a matrix of proteins onto the surface of the root dentine [17]. Once the root sheath breaks down, it is believed that follicle cells attach to the protein matrix and differentiate into cementoblasts [20]. These cementoblasts continually deposit cementum, developing the apical portion of the root and also embedding collagen fibers, known as Sharpey's fibres, in the cementum [21]. After Sharpey's fibres formation, the dental follicle generates periodontal ligament fibroblasts and osteoblasts, leading to periodontal ligament formation and alveolar process in the tooth socket [22]. Once Sharpey's fibres insert into the newly-formed alveolar bone process, the periodontal attachment apparatus is officially formed [23].

When the periodontal tissues are damaged through periodontal disease, the wound healing and regeneration can be separated into three stages: inflammation, granulation tissue formation, and the remodeling of newly formed tissues [17,24]. The length of each stage varies depending on the type of tissue damaged, how invasive the injury was, as well as various local and systemic factors [24].

First, an inflammatory response occurs when a blood clot fills the damaged site, bringing oxygen, tensile strength, and growth factors to the matrix. After clot formation, monocytes and neutrophils phagocytose the injured or damaged tissue, epithelial cells migrate to close the wound, and the fibrin clot forms into granulation tissue which then develops into either scar or regenerated tissue [25]. When the tissue heals, it is deemed either repaired or reconstructed. Repaired tissue does not restore complete function of the original tissue. As for reconstructed tissue, the complete function and structure is restored and identical to the original tissue, as is the goal of periodontal regenerative therapy for periodontal disease [17,25].

The goal of periodontal therapy using stem cells is to mimic the spatial and temporal sequences of periodontium development that occur in early childhood [26]. Mesenchymal stem cells are used in periodontal therapy by migrating to the periodontal defect site and, due to growth factor gene expression, differentiating into the various cells needs to generate the periodontal attachment apparatus [27]. These cells needed to construct the entire apparatus are osteoblasts, cementoblasts, and fibroblasts. The generated osteoblasts secrete the matrix needed for alveolar bone differentiation, fibroblasts create extracellular matrix and collagen, and the cementoblasts form the follicular cells surrounding the

tooth root as well as the cementum [28].

After periodontal therapy, the healing process is much more complicated than simple soft tissue healing due to the presence of mineralized tissues. Procedures such as flap surgery and scaling can promote healing of gingival connective tissues and the periodontal ligament. However, the cementum and alveolar bone are not repaired [5]. In order for full periodontal regeneration to occur, each of the four tissues needs to be regenerated by a highly programmed spatial and temporal sequence of specific inductive factors, root surface cells, and the availability of the extracellular matrix [17,29].

Stem Cells

Stem cells are found in various parts of the body and have the ability to generate multiple different types of cells and tissues and have four different sources: embryonic tissue, fetal tissue, postnatal tissue, as well as induced pluripotent stem cells (iPSC) [30]. First, the embryonic stem cells have the ability to differentiate into all three germ layers, but there are multiple ethical issues related to obtaining human embryonic stem cells due to the potential destruction of an embryo. On the other hand, adult and induced pluripotent stem cells have fewer ethical dilemmas [31]. The induced pluripotent stem cells are created by obtaining somatic tissue and reprogramming them into a pluripotent state that basically mimics embryonic stem cells [32]. In contrast to the pluripotent nature of embryonic stem cells and iPSCs, adult mesenchymal stem cells are multipotent, meaning they have the ability to differentiate into a limited amount of cell lineages and are required to be extracted from a certain cell source [32]. However, although adult stem cells have a limited differentiation capacity, they are more easily accessible, immunocompatible, less ethically controversial, and are found in adult dental tissues which provides a high probability for use in periodontal regenerative medicine [33].

Mesenchymal Stem Cells

Multiple types of mesenchymal stem cells have been used to promote regeneration of certain periodontal tissues, however, each of them comes with their own challenges that make the PDLSCs more attractive as the progenitor cell of interest. For instance, bone marrow-derived stem cells have been found to promote periodontal regeneration but are much more difficult to obtain than PDLSCs [34]. In addition, adipose-derived stromal cells are obtained via liposuction and have been mixed with platelet rich plasma, resulting in periodontal ligament and alveolar bone regeneration in mice [35,36]. Although this shows promising regeneration of the two tissues, the PDLSCs have been showing promising results to regenerate all four periodontal tissues. In contrast to the bone marrow and adipose-derived stem cells, intraoral mesenchymal stem cells have been obtained from every tooth tissue other than enamel. These consist of dental pulp stem cells, PDLSC, stem cells from apical papilla, and various others [37]. Of these intraoral stem cells, the periodontal ligament mesenchymal stem cells are of current particular interest.

PDLSCs: Regeneration Potential

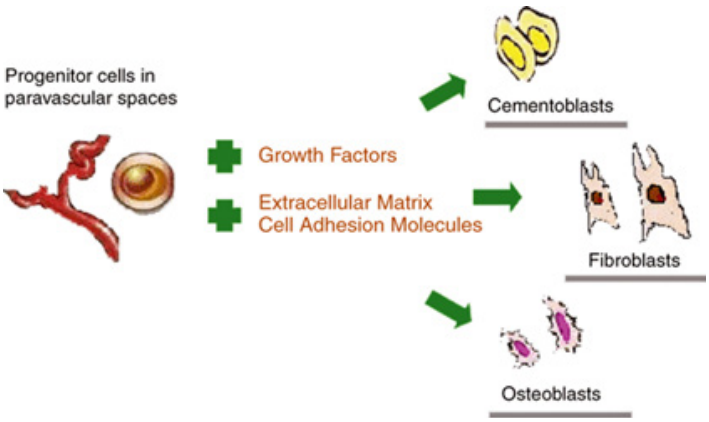

Figure 3: Differentiation potential of progenitor cells found in the periodontal ligament [38].

The periodontal ligament contains stem cells that are typically found in small perivascular niches surrounding blood vessels. They have sparked much interest in the dental field due to their ability to generate synthetic cells such as fibroblasts, odontoblasts, and cementoblasts (Figure 3) [38]. The progenitor stem cell's ability to generate these various cell lines provides the periodontal ligament with a renewable cell source necessary for wound healing and homeostasis maintenance [39]. The PDLSCs have been extracted from human third molars and have given rise to fibroblasts. These fibroblasts were then found to grow into adipocytes, cementoblasts, and osteoblasts in vitro, in addition to growing into cementum and periodontal ligament tissues in vivo [37,40].

As research progressed, the PDLSCs were found to express cementoblast, osteoblast, and BMSSC markers (STRO-1 and CD146 antigens) that are also found on dental pulp cells [41]. Due to the similar markers being expressed by the BMSSC, dental pulp cells, and the PDLSC, a specific marker that distinguishes PDLSC is being researched in order to specifically extract those cells since they have shown a 30% higher proliferation rate than BMSSC [42]. However, the heterogeneity of the periodontium cell population creates a major challenge to this research [41].

The PDLSC mesenchymal stem cells show a strong potential to restore the entire periodontal attachment apparatus and even the periodontium due to their ability to differentiate into cementoblasts and osteoblasts [43]. A recent review showed that when PDLSCs were implanted into periodontal defects, almost three quarters of their studies showed significant improvement [44]. In addition, periodontal ligament cell sheet cultures have been studied in autologous animal models with intra bony defects and in allogeneic miniature swine. The intra bony defect subjects showed regeneration with new bone, periodontal ligament, and cementum formation [45]. The miniature swine showed very similar results with the allogeneic cell sheets and no rejection [46]. These studies and reviews show promising results for future research to be done on human subjects and the complete periodontium restoration.

PDLSCs: Combination with Growth Factors and Space Maintenance Systems

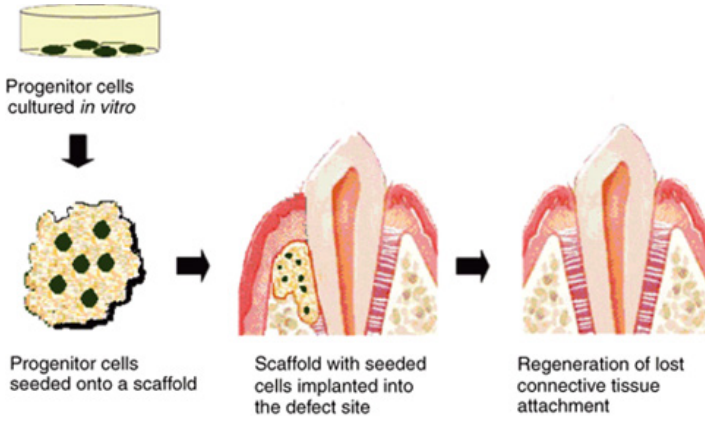

Figure 4: PDLSCs grown in vitro, placed in 3D scaffold, then implanted into the periodontal defect [17].

In order to promote the periodontium regeneration, periodontal tissue engineering methods have been undertaken in order to combine the PDLSCs with growth factors and space maintenance systems [47]. The periodontal regeneration has multiple requirements in order for it to be successful consisting of having enough progenitor cells to differentiate into the four periodontal tissues, appropriate growth and differentiation signals, mechanical support to the periodontal ligament fibers, angiogenic signals for homeostatic maintenance, infection control, as well as a 3D scaffold that can fit snugly into the defect site (Figure 4). These scaffolds have both biochemical features and biological functions that are necessary for their success once implanted. They have to be biocompatible with the appropriate regenerative cells and growth factors while also maintaining space and creating a barrier from invading unwanted microbes and tissues other than epithelium. Instead, the goal is to have the epithelium completely cover the scaffold and the cemento-enamel junction area [47].

Scaffolds with cASCs

The 3D scaffolds provide the ability to perform direct delivery of growth factors and progenitor cells, which is a major limitation in conventional regeneration methods [48]. The idea that regenerative space was necessarily led to the construction of multiple monophasic barrier membranes. This regenerative space would allow for the promotion of the growth of certain periodontal tissues at differing times due to specific growth factors. However, the results were very inconsistent when tested clinically. Due to the unpredictable results, biphasic and triphasic constructs were created in order to create an environment that mimicked the spatial and temporal sequences in natural periodontal development [48]. These biphasic constructs consisted of the composite foam consisting of a starch and poly-ε-caprolactone (SPCL) in the fibre mesh of the construct. The silanol groups in the SPCL provide the scaffold with osteoconductive capabilities by providing an environment on one side of the membrane that creates an ideal environment for bone regeneration and assists periodontal regeneration [49]. The second layer of the scaffold consists of a membrane that acts to prevent any gingival epithelium invasion into the defect [49]. Canine adipose-derived stem cells (cASC) were seeded on these scaffolds and implanted into rats, ultimately determining the promotion of cASC osteoblastic differentiation and formation of periodontal ligament and alveolar bone [50]. However, these adipose-derived stem cell biphasic scaffolds did not have the capability to regenerate the entire periodontium due to the lack of cementum-tissue formation. Therefore, research is currently being conducted on the creation of tri-layered scaffolds to induce cementogenesis in these defects while using computer software to precisely fit the scaffolds into the periodontal defects [51,52]. The triphasic scaffolds are currently being created with gingival, bone, and periodontal compartments using additive manufacturing. Although the scaffold was found to promote neogenesis of the periodontium, the fiber arrangement was not fully functional [53].

Scaffolds with PDLSCs and Dental Pulp Stem Cells

The constructs seeded with bone marrow and adipose-derived stem cells proceeded with little success, leading to the scaffolds being developed with PDLSC and dental pulp stem cells, which both confirmed efficacy and safety for periodontal regeneration in both human and animal studies [54,55]. For instance, a study to treat intrabony defects using autologous PDLSCs mixed with bovine-derived bone minerals in guided tissue regeneration used a sample size of 30 people, aged 18 to 35 and with 20 teeth treated in the cell group and 21 in the control. It was concluded that the use of autologous PDLSCs were safe to use, however, further studies need to be conducted to determine whether the PDLSCs show any statistically significant increase in osteogenesis in the defects by using a larger sample size [56].

The dental-derived postnatal stem cells have just begun to be researched in their abilities to be used in periodontal regeneration and are easily accessible due to them being routinely discarded in dental clinics. This allows for easy access to the stem cells that have been discovered to give rise to each of the four periodontal tissues rather than the post-natal non-dental derived stem cells such as the adipose and bone marrow-derived stem cells. The PDLSCs have the ability to differentiate into multiple cell lineages and undergo self-renewal, expressing mesenchymal stem cell markers (CD44, CD73, CD 90, CD105, CD106 (VCAM-1), CD146 (MUC-18), and Stro-1) [57]. In addition, these stem cells have a higher proliferation rate than bone marrow-derived stem cells and express scleraxis (tendon/ligament transcription factor) at higher levels than BMDSCs and stem cells derived from dental pulp [37]. They are even able to generate a PDL attachment apparatus in vivo by generating collagen-like bundles that attach to cementum-like tissues [37]. The expression of these markers, PDL generation, and high proliferation rate make the PDLSCs an attractive candidate for complete periodontal therapy.

PDLSC Research Barriers

The clinical research on the PDLSCs ability to regenerate the periodontium has many barriers including the tight regulations imposed on stem cell culturing, the possibility of the treatment

not being cost effective, and lack of knowledge of the complete periodontal regenerative process. The FDA has become very strict with the guidelines on stem cell culturing due to the use of fetal bovine serum in many culture mediums, leading to difficulty performing studies without cross-contamination or infection [58,59]. The PDLSC culturing protocol needs to be standardized in terms of isolating and culturing in order to make comparisons between studies [60]. In addition, even if the studies produced significant results, the stem cell therapy methods for periodontal treatment may not be any more cost-effective than the current treatments in use, leading to dental clinics not implementing the procedures or taking the time to learn how to perform the procedure [32]. Lastly, the knowledge is lacking in the understanding of spatial and temporal requirements for regeneration or whether the process can be turned off once initiated, leading to possible tumor growth [32]. In order to fully understand this process, more needs to be researched about the process of cementogenesis and the requirement for reattachment of the gingiva and other periodontal tissues. Although growth factors are proven to positively contribute to the regenerative process, there are a broad range of them, and they lack any tissue specificity. Therefore, in order for the PDLSC therapy to be deemed as a new clinical procedure, stem cell biology and their growth process needs to be more thoroughly understood [17].

Conclusion

Ever since mesenchymal stem cells were officially isolated from the periodontal ligament in 2004, the cellular interactions between the cells populations in the periodontal ligaments have been studied [37]. When compared to the rest of the intraoral stem cells such as DPSCs, DFSCs, and SHEDs, the PDLSCs show the most potential to perform complete regeneration of the periodontal ligament and possibly the entire periodontium [61]. The PDLSCs have confirmed multipotency in humans, mice, sheep, and rats with the ability to differentiate into cementoblasts, osteoblasts, and lipogenic cells [62]. This multipotency provides the PDLSCs with multiple potential clinical applications such as regenerative periodontal therapy, tissue engineering, and gene and cell-based therapies in regenerative dentistry [17]. The goals of this regenerative gene therapy are to allow the damaged periodontal ligament space to regain its regenerative capacity [63]. PDLSCs are being used in conjunction with tissue engineering in current studies, providing the highest capability for regeneration when adapted to be used with fibroblasts found in the periodontal defect by mimicking the normal biological process of periodontal development in early childhood [64,65].

Although PDLSCs show very promising results in current research, there are many biological, technical, and clinical challenges. For instance, the regeneration in animal models cannot be completely translated to the periodontal development in a human and, although there is evidence that regeneration can occur, complete and predictable regeneration is still a research goal due to the high variability and overlapping of growth factors. Due to this high heterogeneity, there is a limited usefulness of prolonged PDLSC cell cultures and their incorporation into 3D scaffolds. In addition, immune rejection and tumor growth are also possible dangerous side effects of the PDLSC proliferation and differentiation [17]. These challenges make it necessary to understand the specific growth factors that are expressed at every stage of periodontal development in order to refine the mechanisms of incorporating the PDLSCs into the 3D matrix scaffolds. Once these scaffolds are refined, they will then need to be tested in sample sizes large enough to determine whether the incorporation of PDLSCs provides a statistically significant growth of the periodontal tissues.

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