CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.4007767

Available online at: http://www.iajps.com Research Article

STEM CELLS OF THE PERIODONTAL LIGAMENT: USE IN CLINICAL DENTISTRY

[1]Shpitser Ivan Michailovich, [2]Gazarian Aram Valerievich,[3]Balobanova Natalya Petrovna, [4]Chizhova Darya Sergeevna, [5]Frolova Margarita Yurievna, [6]Ilia Igorevich Dementev, [7]Dereviankin Aleksandr Aleksandrovich, [8]Rasamatov Bekhruz

[1]Student of Institute of Dentistry, Sechenov University; (https://orcid.org/0000-0003-4621-5739), schpiczeriwan@yandex.ru, +79999652130

[2] Cand. Sci. (Biology), Assoc. Prof., the Department of Pharmaceutical, PhD in Biology, Sechenov First MSMU, Sechenov University (8-2 Trubetskayast., Moscow 119991, Russia, м.т.(+79263789419); e-mail: Balobanova.NP@yandex.ru . ORCID: 0000-0003-1946-137

[3]Student of Institute of Dentistry, Sechenov University +7 916 925 94 92, aramgazaryan03@gmail.com

[4]Student of Institute of Dentistry, Sechenov University, dariya.chizhova@mail.ru, +7 910 947 47 46

[5]Student of Institute of Dentistry, Sechenov University, +79191055994 (https://orcid.org/0000-0002-7120-3206) m.frolova97@yandex.ru

[6]Student of Institute of Dentistry, Sechenov University,+79114119450, Ilya.98.dementev@gmail.com

[7]Student of Institute of Dentistry, Sechenov University, +79168006461, derevyankinwf@mail.ru

[8]Student of Institute of Dentistry, Sechenov University. +79019069405 behruzrasamatovmoskva@gmail.com,

Article Received: December 2019 **Accepted:** January 2020 **Published:** February 2020

Abstract:

This literature review contains information about discovery, markers and substances expressed by periodontal ligament stem cells (PDLSCs), about clinical experiments in vivo and in vitro studies, as well as on scaffolds used for cell transplantation, and the application of mesenchymal stem cell (MSC) data in general medicine. In recent years, tissue engineering has become increasingly popular in medicine and dentistry is not an exception. Many discoveries have already been made at the PDLSC gene level, which provide comprehension of certain differentiation processes, both in vivo and in vitro, and in ex vivo culture conditions. The niche of MSC data is easily available, therefore, for a number of other reasons, PDLSCs attract attention for use in regenerative medicine. Numerous clinical studies have shown the promise of use these cells for regenerative purposes.

Key words: PDLSC, SC, regenerative medicine, Stem cells niche.

Corresponding author:

Shpitser Ivan Michailovich,

Student of Institute of Dentistry,

Sechenov University; (https://orcid.org/0000-0003-4621-5739),

schpiczeriwan@yandex.ru, +79999652130

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Please cite this article in press Shpitser Ivan Michailovich et al., ***Stem Cells Of The Periodontal Ligament: Use In Clinical Dentistry.,*** *Indo Am. J. P. Sci, 2020; 07(02).*

INTRODUCTION:

One of the fastest growing and perspective areas of medicine is regenerative medicine. Regenerative medicine is usually associated with stem cells (SC). All stem cells are classified by origin and by their ability to differentiate. According to the source of origin, there are: embryonic stem cells, postnatal stem cells, reprogrammed stem cells [1]. By the ability to differentiate: totipotent stem cells, forming cells of any type; pluripotent, forming cells of many types, but not all; multipotent, forming cells of several types, and unipotent, forming only one type of cells [2, 3]. Stem cells are cells that have the following capabilities: first, they are able to permanently produce daughter cells that have the same characteristics as self-renewal; secondly, they can generate daughter cells that have different, more limited properties, and, finally, they can re-populate the body's cells in vivo [4].

The first scientist who introduced the SC to scientific world was A.A. Maximov (1908). He called them so because they are in the "trunk" of the blood-forming organ. It was he who suggested the differentiation of precursor cells into different types of blood cells in response to external signals. Thanks to this domestic scientist, impetuous stem cell research began in the 20th century. [5, 6] So already in 1970 A.Ya. Friedenstein described mesenchymal stem cells (MSCs), which were clonogenic, plastic, stuck with each other, obtained from bone marrow, they were called fibroblasts, which can form a colony [7, 8]. Part of regenerative medicine is tissue engineering, which includes the study of: bioactive growth factors, cell populations and scaffolds, as cell scaffolds [9]. The term “tissue engineering” was first used by Langer and Vacanti in 1993 and described tissue regeneration by transplanting cells with or without a scaffold for cell growth [10]. Since their discovery, these cells have been called bone marrow mesenchymal stem cells (BMMSCs) and stromal progenitor cells, and more recently, MSC [11]. The Committee of Mesenchymal and Tissue Stem Cells of the International Cell Therapy Community has proposed criteria for determining multipotent mesenchymal stem cells. Their definition includes three criteria: adhesion to plastic; specific patterns of expression of surface antigens; multipotent potential of differentiation [12]. In particular, MSCs should be adhesive to plastics when cultured under standard conditions in standard culture flasks. At least 95% of the mesenchymal stem cell population must also express CD105, CD73 and CD90. In addition, <2% of the population must express markers of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II. Finally, the population of mesenchymal stem cells should be able to undergo trilinear mesenchymal differentiation with the formation of osteoblasts, adipocytes and chondroblasts under standard in vitro differentiation conditions [12]. Only if all the above conditions are met, the cells can be called MSCs.

MSCs isolated from the tissues of the maxillofacial region include dental pulp stem cells (DPSCs), periodontal ligament stem cells, (PDLSCs), stem cells from human exfoliated deciduous teeth (SHED), stem cells from apical papilla (SCAP), dental follicle progenitor cells (DFSCs), gingival mesenchymal stem cells (GMSCs), oral mucosa stem cells (OMSCs), bone marrow mesenchymal stem cells (BMMSCs), periosteum stem cells (PSCs), salivary gland stem cells (SGSCs) [13, 14].

PDLSC features.

The periodontal ligament (PDL) is a connective tissue located between the cementum (a thin layer of mineralised tissue covering the roots of the teeth) and the inner wall of the tooth alveolus. Its main function is the creation of the mechanical stability of the tooth. PDL not only plays an important role in supporting the teeth, but also contributes to the nutrition of the teeth and surrounding tissues, homeostasis and the regeneration of damaged tissue [15, 16]. Byoung-Moo Seo et al. first identified postnatal PDLSCs as stem cells in 2004. Their results showed that postnatal PDLSCs were clonogenic, with high proliferative potential and able to regenerate cementum, periodontal fibers and alveolar bone [17]. PDLSC primary cultures are colonies of bipolar fibroblast-like cells with 2–3 oval-shaped nuclei. In nuclei, euchromatin indicates the active status of gene transcription. Flow cytometry analysis put in evidence the homogeneous expression of the mesenchymal-related antigens CD13, CD29, CD44, CD73, CD90, CD105, CD146, CD166, other than OCT3/4, Sox2, and SSEA4 intracellular antigens, p75, Nestin, CD49, SLACS, SOX10 neural crest related markers [18, 19] . Stem cells of the periodontal ligament also express a variety of osteogenic markers, such as alkaline phosphatase (ALP), matrix extracellular phosphoglycoprotein (MEPE), bone sialoprotein (BSP), and osteocalcin; STRO-1 mesenchymal stem cell marker; and the tendon marker scleraxis [20]. The viability and the ability to divide (population doubling time) stem cells of the periodontal ligament, isolated from the periodontal teeth of healthy teeth, is approximately 4 hours [21]. At the same time, in vitro, the proliferative potential of MSCs begins to decrease markedly after 120 days of expansion [22].

Stem cells niches and their selection.

Stem cells are known to be responsible for the growth, homeostasis and repair of many tissues. The viability and division of stem cells is regulated by the input data of their local microenvironment, often called the "stem cell niche". The stem cell niche hypothesis was developed in 1978 by Schofield [23].

A stem cell niche is an in vivo microenvironment in which stem cells live and receive stimuli that determine their fate. Therefore, a niche should not be viewed only as the physical location of stem cells, but rather as a place where external signals interact and integrate, affecting the behavior of stem cells. Cross-talk between different cell types in the stem cell niche makes it possible to cooperate with these cell communication networks and adapt the dynamics of normal stem cells to increase their ability to respond to therapeutic needs [24]. Stem cells are fundamental for the maintenance and regeneration of tissues; the signals they receive from the extracellular matrix and the external environment play a critical role in tissue homeostasis. Non-cellular matrix proteins act as an anchor of stem cells and constitute a mechanical unit of scaffolds for transmitting stem cell signals [25].

In vivo, the stem cell situation is completely different compared to in vitro studies. The limited proliferative potential observed in vitro may not reflect the ability to proliferate in 3 directions, which is most likely due to the lack of a physiological niche that provides self-maintenance [26]. Criticism for experiments based on cultured ex vivo SCs conducted in vivo is the gap between the physiological niches in which stem cells are located and the cultivation dishes.

PDLSC niches with an extracellular matrix, which contains endogenous growth factors to stimulate dormant SCs for differentiation into other cell types and help in regeneration, are soft tissues located on the extracted root surface [25]. It is noted that PDLSCs are located in the region of 0–20 μm from the perimeter of blood vessels [27]. These stem cells are obtained by separating PDL tissues from the middle third of the root surface. [28, 29].

Clinical and laboratory studies.

Inflammation in the periodontal tissues destroys its tissues. Increasing evidence suggests that inflammation also reduces the regenerative capacity of PDLSCs. PDLSCs derived from the inflamed periodontal ligament are inflamed periodontal ligament stem cells, which have a higher proliferation rate and ability to migrate, but have a lower osteogenic ability [30] and a lower potential for cementogenesis compared to PDLSC obtained from healthy tissues.

PDLSCs promote self-renewal and regeneration of periodontal tissues, especially with regard to bone formation [31, 32]. Therefore, PDLSCs can be an important source of cells for the treatment of periodontal disease. But it is known that periodontitis can reduce the expression of the KAT2A gene in PDLSCs, activating the classical Wnt pathway [33]. Also, the microenvironment for chronic inflammation of the periodontium can reduce the potential for differentiation of PDLSCs [34].

Regarding inflammation, there is growing evidence recently that, as an ATP-gated ion channel expressed in most stem cells, the P2X7 receptor (P2X7R) plays a wide physiological and pathological role in inflammation [35]. It is reported that extracellular ATP is a key modulator of inflammation, and ATP signaling through P2X7R can initiate a proinflammatory cascade with pathological loss of alveolar bone during periodontitis [36], as well as affecting the periodontal function [37, 38]. P2X7R has been shown to have a significant positive effect on osteogenesis [39] and bone formation [40]. There are studies showing that the P2X7R BzATP agonist can induce osteoblast SaOS-2 apoptosis (human osteosarcoma cell line) [41]. The first study was conducted by Xu X. et al., In which the role of P2X7R in PDLSC during inflammation in vitro was studied [29].

The authors showed that P2X7R expression increased in the osteoinductive environment, but was inhibited by inflammatory factors. Based on a number of experiments, the authors concluded that P2X7R expressed on PDLSC plays a positive role in their osteogenic differentiation in inflammatory conditions.

In the study of Zhu-Ling Guo et al. which was conducted in vitro, the function of PDLSC in a simulated medium in diabetes mellitus was evaluated. The authors used different doses of Advanced Glycation Endproduct-BSA (AGE-BSA) - 50, 100 and 200 μg / ml. The percentage of PDLSCs in the experimental group was clearly lower than in the control group. AGE-BSA showed a dose-dependent inhibition of osteogenic differentiation of PDLSC, significantly reduced at 200 μg / ml. An increase in RAGE and intracellular ROS content was also observed [42]. Other studies show that locally deposited AGEs can reduce the production of extracellular matrix, reduce collagen expression, prevent osteoblast differentiation and maturation, mediate apoptosis, and delay the repair of periodontal tissues [43, 44]. The experiments indicate the inclusion of the signaling pathways of MAPK and WNT during the cascade activation of

AGE and RAGE [45, 46]. It was found that the MAPK signaling pathway in PDLSC is key to bone formation and periodontal homeostasis, and its activation is directly related to the release of proinflammatory cytokines [47, 48, 49]. AGEs induce apoptosis, the synthesis of intracellular ROS, which is a critical pathological mechanism in PDLSC in diabetes mellitus [39, 50]. So research conducted by Deng C. et al. showed that a glucose content of 25 mmol / l can inhibit osteogenic differentiation of PDLSC; (2) may contribute to the formation of lipid droplets and enhance adipogenic differentiation of PDLSC; (3) can stimulate the lipidation process by increasing the expression of C / EBP β and PPAR-γ mRNA [51].

In the study of He Wang et al. proteomic profiling of PDLSC and DPSC was performed. It provides insights into the exact differences in the proteome and the corresponding pathways of the osteogenic mechanisms. The study was conducted using the iTRAQ proteome [52]. The authors found a set of more than 3000 proteins in both PDLSC and DPSC, while only less than 5% of them were differentially expressed. This suggests that PDLSC and DPSC have a wide spectrum of their proteome, which may be due to their similar origin. However, it is noteworthy that these differentially expressed proteins may reflect different differentiation potentials and different functions in their respective niches. The results showed that PDLSC have a stronger mineralization capacity than DPSC, which is an indicator of osteo / odontogenic differentiation. Profiled and heat shock proteins. A higher level of HSPB1 was noted in the PDLSC compared to DPSC, which may contribute to a higher mineralization capacity. The S100-S100A4 protein family (S100-A4 protein), S100A10 and S100A11 (S100-A11 protein) were more highly expressed in PDLSC, while S100A9 was more highly expressed in DPSC. The authors note that this may partially contribute to the higher mineralization and migration capacity of the PDLSC. The S100 family of proteins are synthesized when damaged, promote the absorption of Ca2 +.

It is already known that MSCs lose their properties of stem cells due to replicative aging or spontaneous differentiation into different cell lines in vitro. This happens due to various factors, such as doubling of the population, different number of planted cells, a certain coverage of the extracellular matrix of the culture plate, the presence of growth factors in the serum [53; 54; 55; 56]. Therefore, the study of Kengo Iwasaki et al. is important to the scientific world. In the study, the authors described the morphological changes in PDLSC during ex vivo expansion under standard culture conditions. PDLSC changed its shape from spindle-shaped to flat and wide, developing a stress-like structure of the cytoskeleton and a phenotype similar to that of myofibroblasts. The authors note that the cells acquired a myofibroblast-like structure at passage 9, but at the same time, the cells could change the morphology at earlier passages. In PDLSC with altered morphology, there was an increase in contrast when staining with vinculine, an increase in contractile activity and an increase in the synthesis of myofibroblast genes. The expression of CD166 was significantly reduced in PDLSC, similar to myofibroblasts, compared with PDLSC in the form of spindles. Thus, the periodontal ligament stem cells exhibited various properties of myofibroblasts, while at the same time losing the characteristics of MSC. The authors attributed this change in morphology to the fact that PDLSCs are a population of stem cells from the periodontal ligament, the anatomical characterization of which can explain the spontaneous differentiation of PDLSC in this study [57]. In addition to spontaneous differentiation into myofibroblasts, PDLSC can also differentiate into osteoblasts [58, 59].

It should be said about PDLSC temporary teeth. The authors found that PDLSCs derived from resorbable milk teeth expressed increased RUNX2, which activated RANKL and decreased osteoprotegerin (OPG) regulation, both at the mRNA level and at the protein level. These imbalances between RANKL and OPG ultimately led to differentiation into osteoclasts and root resorption. Thus, PDLSC from resorbed milk teeth can cause an unexpected activation of osteoclasts when used in periodontal regeneration [60].

In addition to in vitro studies, many in vivo experiments are being conducted. Thus, it was shown that transplantation of PDLSCs ex vivo (outside the body) to immunocompromised mice confirmed the ability of these cells to form functional cementum-like structures and tissue of the perodontal ligament, including Sharpey's fibre, in vivo [60, 61].

Recent advances in tissue engineering open up the possibility of regenerating lost tissues using stem cells grown ex vivo. Several studies have shown that the transplantation of PDLSC into periodontal bone defects accelerates the regeneration of periodontal tissues and enables new regenerative treatment of periodontal diseases [62, 63, 64, 65]. There are reports on the use of PDLSC in conjunction with the method of guided bone regeneration (GBR) and osteoplastic materials for periodontal regeneration [66, 67, 68].

Today, the stem cell assistance in periodontal regeneration technique (SAI-PRT) method is

promising. This technique involves the regeneration of periodontal tissues using PDLSCs, a niche with growth factors and a gelatin sponge as a scaffold [69, 70, 71]. SAI-PRT was applied in a prospective, randomized single-blinded controlled trial, cited by Shalini H.S. [24]. Patients were divided into 2 groups: scaling and root polishing were performed in the control group, and the same was followed in the test group, but with the subsequent use of PDLSC. PDLSC together with a niche was obtained from the surface of the root of the extracted tooth, which was removed according to orthodontic indications. In this case, the ICs were placed together with the niche in the bone defect without any treatment and ex vivo cultivation. The depth of sounding, the height of the newly formed bone, the clinical level of attachment of the gums at 3, 6, 9 and 12 months showed an improvement in the test group. At the same time, bone density increased significantly in the test group from 6 months. In the current study, improvements in the clinical level of gingival attachment and radiographic changes, such as reducing the distance from the cement-enamel border to the bone and filling the defect area with a bone, were not statistically significant compared to the control group. However, these parameters were higher than in the control group. Shalini H.S. et al. proposed a new term "niche autograft PDLSC". This method using niche autograft PDLSC avoids the disadvantages of culturing stem cell data ex vivo, which are indicated in article [72]. The above method was based on the concept of "best of waste" for periodontal regeneration, it does not have a biohazard, because the patient's own tissue is used. The principle of SAI-PRT was recommended on a regular clinical basis, as complex tissue engineering becomes easy at the chair. The minuses of the technique were indicated: it requires tooth extraction, ex vivo cultivation is required to close large defects [24]. But there is a study proving that PDLSC can be obtained during standard scaling of root surfaces and their planning [73, 74].

Studies have been conducted on PDLSC behavior during orthodontic treatment [75, 76]. The orthodontic tooth movement (OTM) occurs due to mechanical forces and stimulates the remodeling of the PDL and the alveolar process. Under the influence of an appropriate orthodontic force, periodontal tissue is restored at the molecular, cellular, and tissue levels [77]. On the compression side, PDLs become compressed and disorganized, resulting in bone resorption, while stretching of PDL fibers induces bone deposition [78]. Feng and his colleagues demonstrated that the application of orthodontic power affects the amount of collagen in the PDL, as an important component of the extracellular matrix in the PDL. Its quantity decreases on the side of compression and is restored after the elimination of force within 5 days. Accordingly, the expression of type 1 collagen (Col-I) in PDLSC was reduced at the beginning of the application of orthodontic power and was restored after elimination [76]. During OTM, various molecules induce PDLSC, such as interleukin- 11 (IL-11), collagen triple helix containing 1 (CTHRC1) [79], microRNA-21 (miR-21) and hydrogen sulfide (H2S) [80, 81]. IL-11 is known to be associated with bone remodeling [82, 83]. After OTM, it was observed that IL-11 increased in rat PDL. Accordingly, IL-11 can stimulate osteoblastic and cement-blister markers, increase the expression of sialoprotein in bone, and stimulate the proliferation of human PDLSC in osteoblasts and cementoblasts [84]. MicroRNAs, play important role in the osteogenic differentiation of PDLSCs after application of orthodontic force. Thus, microarray data showed that microRNA 53 in PDLSC was differentially expressed after tension [85], including hsa-miR-21, which was found to be involved in stress-induced osteogenesis of human PDLSC in vitro [86]. To study the effect of stress on the function of PDLSCs for the regulation of osteoclastogenesis, J. Liu et al. subjected to PDLSC applied to the article static mechanical strain (SMS) with a range of values from 6% to 14% at 0.1 Hz for 12 hours. When the stress was less than 12%, the genes of osteoclast populations (RANKL) showed no significant differences. However, when the strain was above 12% in PDLSCs, the levels of osteoclastic genes were clearly increased. Moreover, PDLSCs obtained from periodontitis patients and healthy donors respond differently to stress. Under the same conditions, the best SMS values for the balance between osteogenesis and osteoclastogenesis for healthy PDLSC were determined to be 12%, while the optimal effort for PDLSC isolated from patients with periodontitis was 8% [87]. Compression is one of the parameters for orthodontic treatment. Short-term compression (application of force for 1 hour) has been shown to contribute to osteogenic differentiation of PDLSC, at the same time inhibiting osteogenesis and promoting osteoclastogenesis by increasing the RANKL / OPG ratio, which decreases after 12 hours and osteogenesis is activated [75]. Studies were also conducted on the behavior of PDLSC in vibration [88, 89], ultrasound exposure [90, 91].

Scaffolds

In addition to stem cells and growth factors, tissue engineers require the use of scaffolds to support and ensure the organization of tissues [92, 93]. The ideal framework should provide the physiological needs of the cells; it have to remind its own niche SC. In addition to biocompatibility, osteoinduction and structural support, scaffolds also require

predictable and controlled biodegradation with the formation of non-toxic wastes [93, 94].

Recent advances in the field of biomaterials have led to a transition from non-porous, biologically inert materials to more porous, other ongoing biomaterials and, in particular, to the use of cell matrix composites. A number of delivery vehicles have been successfully used in in vivo cell-matrix composites, such as porous ceramics, collagen sponges and β-tricalcium phosphate, filled with autologous MSCs [95-115]. Bioceramics based on biphasic calcium phosphate (BCP) with recommended proportions recommended for use in dentistry due to their excellent biological activity [96]. BCPs made from HA and β-TCP in 40:60 weight mode were obtained using a gel combining technique using the polymer sponge element [97]. It has been proven that it is able to act as a reliable framework for the effective stimulation of periodontal regeneration [28]. In the study, Han Shi improved PPG, which is a type of nanopowder with constituent dimensions of 30-50 nm. It had abundant micropores, which are widely distributed in the walls of macropores, with an average number of portions of 162 microns and porosity of up to 80%. This allows cells to fasten in a scaffold [28].
In the Kengo Iwasaki study, as a scaffold for transplantation, PDLSC used decellularized amnion and implanted them into surgically detected periodontal defects in rats. PDLSC-amnion significantly enhances the formation of periodontal tissues in vivo. These transplants can lead to new regenerative therapy for periodontal disease [70].

CONCLUSION:

On the basis of the analyzed literature, it can be said that regenerative medicine, and in particular, regenerative developments in dentistry, are finding increasing use and will soon become an integral part of treatment. PDLSCs have tremendous differentiation potential, which makes it possible to carry out therapy using SC data not only in the oral cavity, but also in many other areas of the body. The niche of these cells is readily available, thanks to which the prospect of using these cells increases. At the moment, the use of PDLSCs has proven itself in clinical practice as a way to regenerate and treat certain diseases, but not all problems have been solved. For example, in working with SCs during ex vivo cultivation, difficulties lie in the possibility of erroneous cell differentiation, the onset of the oncological process, the complexity of cell delivery, and the immunogenicity of cells. Therefore, when it is possible to implant cells without ex vivo cultivation, it is better to avoid this. Further research is needed to improve cell culture methods, predicted transplantation and solve some problems with the storage of these cells in IC banks. Scaffolds are an integral part of cell transplantation. Like the SC niche, scaffolds are very important and their use is advisable for the success of the treatment result. Thanks to such devices as a disaggregator, it is possible to quickly isolate the desired cells from their niche.

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