
Research Article

Periodontal ligament stem cells-current review of the literature

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Abstract: The presence of mesenchymal stem cells (MSCs) in human periodontal ligament (PDL) were first described by Seo et al. in 2004. These cells are capable of plastic adherence, colony forming and differentiation into various cell types when cultivated in appropriate cell culture conditions. PDL stem cells are investigated by high number of scientists nowadays because of their high potency for tissue regeneration. However, little is known about the effects of various active molecules and growth factors on their stem cell properties. There is limited number of reports revealing the activity of these cells when applied in clinical studies. The purpose of the current review is to highlight the stem cell properties of human PDL MSCs and to briefly systematize the data from various sources.

Keywords:

I. Introduction:

Periodontal ligament (PDL) mesenchymal stem cells (MSCs) were firstly isolated and described by Seo et al. [1] in 2004. The authors reveal the stem cell phenotype of PDL cell population through positive expression of specific stem cell markers and the cell ability of multilineage differentiation. Periodontal MSCs are capable of unique cementum to PDL complex formation and stimulation of periodontal regeneration when transplanted into immunocompromised animal models. MSCs in human periodontium are mainly localized perivascularly. Their morphology strongly resembles pericyte-like cells as they are able to form capillary-like structures *in vitro* [2].

II. Isolation of Mscs From Human Pdl:

Periodontal MSCs are isolated from the middle third of the root of permanent teeth and also from the walls of the dental socket after tooth extraction [3]. After scraping with sterile scalpel blades, the tissue explants are incubated in enzymatic solutions to obtain a single-cell suspension [4,5,6]. The resulting suspension is seeded in plastic cell culture dishes. According to some authors, the cells isolated from the tooth root and from the alveolar wall have different properties according to their proliferation and differentiation ability [7]. Periodontal MSCs can also be isolated from deciduous teeth [8]. Their properties when compared to MSCs isolated from permanent teeth are still debated. It has been postulated that deciduous teeth PDL stem cells have better ability of proliferation and differentiation into osteoblasts and adipocytes than cells isolated from permanent teeth [8]. According to other authors, there is no significant difference in the properties of both stem cell types [9]. With regard to periodontal MSCs from deciduous teeth, a question arises

about their potential to induce bone resorption when implanted into vital tissues.

PDL stem cells have also been obtained from permanent teeth with periodontal inflammation [10]. They appear to have high proliferation and migration ability, but exhibit limited differentiation potential when compared to cells isolated from teeth with healthy periodontium [10]. Limited number of vital MSCs can be isolated from inflamed periodontal tissue [7].

III. Cultivation of Human Pdl Stem Cells:

The colony forming ability of the MSCs and their plastic adherence to cell culture dishes, allows for thorough examination of the cell morphology and stem cell properties [11]. Their adhesion ability provides the mechanical separation from non-adherent hematopoietic cells. Initially some of the hematopoietic stem cells adhere to the cell culture dishes, but after a couple of days cultivation they have been "washed away" with only fibroblast-like cells remain adherent [12,13]. Usually the isolated MSCs cultures are heterogeneous, with multiple endothelial cells, macrophages, osteoblasts, etc., also found in the cell culture flasks. Various procedures, i.e. Fluorescent-Activated Cell Sorting (FACS) or Magnetic-Activated Cell Sorting (MACS), have been applied to provide proper separation of different cell types and to maintain homogeneous cell cultures.

MSCs are cultivated at standard cell culture conditions: 37°C, 5% CO₂ and 50% humidity in cell culture medium, supplemented with glucose, salts, essential amino acids, vitamins and microelements [14,15]. Various media for MSCs *in vitro* culturing, capable of inducing a specific type of cell differentiation (i.e. into osteoblasts, chondroblasts, adipocytes, hepatocytes, etc), are currently well known [16].

Fetal bovine serum (FBS) is usually added to the cell culture

media. It significantly enhances cell proliferation and stimulates various biological processes in the cells [17]. However, it is a product of animal origin and long-term cell cultivation in a FBS media is associated with a potential risk of immune reactions and infections [17]. The use of FBS should be avoided when MSCs are cultured and prepared for clinical application [18]. Thus a significant number of scientists conduct their MSCs studies in serum-free media, including various FBS substitutes such as insulin-transferrin-selenium-ITS [16,18] or platelet concentrates (PC) [17,19]. Wide range of studies have concluded that stem cells can implement their properties quite well in a serum-free culture media.

IV. Characterization of Human Pdl Stem Cells

Mesenchymal cells isolated from PDL represent a heterogeneous population where the stem cell number predominates. Characterization of the stem cells and their stem cell properties investigation are performed using specific markers via well-known methods, including immunofluorescence, immunohistochemical staining [1,20], magnetic cell sorting [21] and flow-cytometry [2]. Seo et al. [1] reveal that periodontal MSCs are able to express two specific mesenchymal stem cell surface markers: CD146 and STRO-1. CD146 is one of the main markers for perivascular and multipotent progenitor cells in human connective tissue [22]. It is also expressed by endothelial cells, smooth muscle cells, Schwann cells, some neoplastic cell types [23]. The exact function of this protein is not fully elucidated, but it is known to have relevance to cell morphology, adhesion, cytoskeleton reorganization, migration, transmembrane signaling, etc. [24]. A number of studies have shown that genuinely CD146-positive cell populations have all the characteristics of MSCs [25]. STRO-1 is one of the most commonly applied cell surface markers in dental MSCs characterization [26]. Iwasaki et al [2] detected the presence of PDL stem cell niches by positive expression of CD44, CD73, CD90, CD146 and CD166. Chen et al. [7] characterize the stem cells via CD44, CD146 and STRO-1 antibodies. Silverio et al. [27] separate cells into two groups based on their CD105 expression (CD105-positive and CD105-negative cells). Positive cells are additionally labeled with CD166 as cells dually-positive for stem cell markers were established. Since it is known that the periodontal stem cells have mesenchymal origin, expression of hematopoietic markers like CD14, CD34, CD35, etc., is not expected [27,28]. The PDL stem cell properties are further demonstrated by their ability to form colonies and differentiate into various cell types in vitro [1,7,14]. Many authors investigate the proliferation rate of periodontal MSCs and their ability to form colonies for a particular period of time (2-3 days to 3 weeks) [9,27,29]. The differentiation potential of periodontal MSCs is often revealed after cultivation with active substances (i.e. cytokines, growth factors), initiating processes of tissue osteogenesis, chondrogenesis, adipogenesis, etc. Osteogenic differentiation of MSC occurs after nearly 3 weeks cultivation with cell culture media supplemented with ascorbic acid,

dexamethasone and beta-glycerophosphate. According to reports in the literature, various active molecules and growth factors are able to influence cell culture mineralization by stimulation or inhibition effect [30]. It has been shown that MSCs can also differentiate into chondroblasts and adipocytes, which confirms their multi-linear differentiation potency [27,31].

The properties of the MSCs are directly dependent on the age of the donor of tissue explants. Cells obtained from older patients have minimal stem cell expression potential [32,33]. Expression of stem cell markers such as STRO-1 and CD146 as well as the ability of cells to form cementum/PDL-like structures in vivo is significantly diminished with the donor aging [34,35]. Disturbance in the stem cell properties of human PDL cells in elderly patients is probably related to the fact that people over 41-years old are at higher risk of severe periodontitis.

Periodontal MSC also possess high immunomodulatory properties. According to the literature, they have the potential to inhibit proliferation of allogeneic T-cells by increasing the levels of cyclooxygenase-2 and prostaglandin-E2 [36]. These MSCs suppress B-cell proliferation and migration by means of intercellular signaling and specific cellular protein synthesis mechanisms for programmed cell death [37]. It has been reported that MSCs do not induce immune responses in recipients because they do not express HLA-II (human leukocyte antigen) as well as T-cell co-stimulatory molecules, such as CD80 and CD86 [36].

V. Effects of Growth Factors On Human Pdl Stem Cells

The effects of various growth factors on the periodontal MSCs are discussed in many scientific reports [38,39,40]. It has been stated that some active molecules stimulate their differentiation into osteo- or fibroblast-like cells, while others are involved in maintaining they non-differentiated phenotype [41]. Long-term cultivation of MSCs in an autocrine media is associated with a high risk of auto-differentiation, especially in cells of later passages [42]. Recently, scientists have been interested in the possibility of maintain early cell passages undifferentiated through various active molecules [43]. The success of this kind of experiments will lead to diminished cell aging process and lower risk of stem cell auto-differentiation during in vitro culturing. Auto-differentiation and cell aging are accompanied by changes in the expression of specific cell markers. The expression of Sox-2 and Oct-4 (MSC nuclear markers) in PDL stem cells remains optimal up to the 3rd passage of culturing, and then begins to decrease [44]. Reduced expression of stem cell markers is a sign of cell aging and reduced cell proliferation and differentiation ability.

Each one of the growth factors can initiate a particular type of cell differentiation. BMP-2, BMP-7 and VEGF potentiate osteogenic differentiation and their activity is directed to regenerative processes in bone defects [30,45]. TGF-beta 1 (transforming growth factor beta 1) is able to induce increased expression of proteins specific for fibroblast-like cells - collagen type I, alpha-smooth muscle actin, peristin [39].

An accurate evaluation of the effects of each growth factor on

undifferentiated stem cells, could be made after comparison between cells cultivated in highly specific cell media supplemented with active molecules and control cells cultivated in autocrine medium. The effects of every single growth factors is clarified when it has been added to the cell culture media as a single active molecule or in a combination with other growth factors. Culture media containing two or more growth factors have been developed in an attempt to assess the extent to which the active molecules mutually potentiate or inhibit their activity [45].

According to reports from in vitro studies, a major role in the growth factors` activity plays their concentration as well as additional factors of the microenvironment (temperature, humidity, presence of other active agents whose concentration is not specified) [30,39,44].

VI. Pdl Stem Cells in Preclinical Studies

Current in vitro MSC studies have been conducted in order to develop more predictable and minimally invasive therapeutic approaches in the clinical practice. Therefore, an increasing number of reports from preclinical and animal studies are currently known [46]. In many in vivo animal models have been found that transplantation of PDL MSCs into periodontal defects leads to an excellent outcomes and lack of local complications [36, 47].

It has been found that application of PDL MSCs in replanted tooth after tooth avulsion could establish optimal local environment for tissue regeneration [48]. The best clinical results are found in a tooth replantation back in the alveolar socket within 5 minutes of the expulsion [48]. In most cases, the teeth are replanted after several hours or even days, depending on the time the patient seeks medical assistance. By this time, more cells in the PDL lose their vitality, resulting in compromised treatment outcomes. Data in the literature suggests that in 73% to 96% of the cases, the avulsed teeth do not integrate after replantation into the alveolar socket [49]. Zhou et al. [47] stimulate isolated autologous PDL MSCs in vitro to synthesize their own extracellular matrix and then applied them to the root surface of avulsed teeth. The results demonstrate very good regeneration of PDL and cementum as well as high levels of expression of collagen type I, type III and fibronectin in the regenerated tissue.

A large number of preclinical animal studies have shown that the open curettage of furcation and interdental peroidontal defects in combination with the local application of autologous stem cells from the PDL leads to the formation of new bone, cementum, and ligaments [50,51]. In addition to the autologous cells, allogeneic periodontal MSCs can also be applied locally. In an experiment conducted with pigs it was found that they stimulate regenerative processes in periodontal tissues in a way quite similar to the autologous stem cells [36]. The authors do not report any immune response in the recipients.

Sonoyama et al. [52] conducted an experiment where they applied either PDL MSCs or MSCs from apical papilla in a combination with Gelfoam® gelatin sponge around dental implants in pigs. At first, immediatelly after the tooth

extraction the PDL has been thoroughly removed from the alveolar socket. Three months after the implant placement, a ceramic restoration was added. Results from the computed tomography and histological examination demonstrate the regeneration of the PDL-like structure and greater stability in the areas treated simultaneously with both stem cells types and with Gelfoam®.

In a study conducted by Zhao et al. [53] autologous PDL MSCs in combination with autologous blood-derived products (platelet-rich plasma) were used for the regeneration of the tooth-supporting tissues in avulsed teeth. The results were reported eight weeks postoperatively. There is an excellent regeneration PDL structures, lack of inflammatory complications and lack of ankylosis. The effect of these autologous products is due to the high amount of growth factors and other active substances they contain.

VII. Pdl Stem Cells in Clinical Studies

There is a very limited data in the literature about the application of PDL MSCs in clinical studies with patients. Feng et al. [54] determined the effect of autologous cells isolated from PDL when implanted into periodontal bone deffects. The teeth treated were 16 in total. Patients were followed for a period of 32 to 72 months. In the clinical experiment, a positive effect of the PDL MSCs on the healing process and on the tooth stability has been identified. No complications were observed during and after the treatment.

In a study of Gault et al. [55] the effect of human autologous PDL MSCs covering the dental implant surface immediately prior their placement in the dental socked was observed. The cells were previously isolated and cultured in vitro. In the implants whose surface was covered by stem cells, a proper osteointegration has been established, with no further complications.

All currently known in vitro and in vivo reports on human MSCs are not sufficient for their introduction into routine clinical practice. Periodontal MSCs should be first isolated, cultured and multiplied in vitro, as their clinical application requires a very large number of vital cells with a stable phenotype [56]. An important fact is that prolonged cell growth in in vitro conditions leads to aging, gradual loss of stem cell properties and limits their proliferative potential.

VIII. Conclusion:

The presence of MSCs in human PDL is well known nowadays. However, further research is needed in order to clarify their properties and the effects of various active molecules on the stem cell phenotype. Wide range of preclinical studies should be conducted before the routine application of PDL stem cells in dental practice.

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