ORIGINAL RESEARCH PAPER
Volume - 9 | Issue - 12 | December - 2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

IDENTIFICATION OF STEM CELLS IN HUMAN PERIODONTAL TISSUE – AN IN VITRO STUDY.

Periodontology

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ABSTRACT

Aim and Objectives: Periodontal ligament regeneration for treatment of periodontal disease is a daunting task. The current field of tissue engineering using stem cells shows promising results, however, these cells have not yet been fully understood. The present study aimed to isolate and identify the PDL stem cells using CD 73 and CD 90 mesenchymal surface markers.
Materials and Methods: In the present study, samples were obtained from the periodontal ligament of 10 extracted premolars of five healthy patients aged 15 to 28 years, after obtaining written informed consent from the patient. The teeth were extracted for orthodontic reasons. These were then processed and then stained immunohistochemically for detection of cell surface markers, namely CD 73 and CD 90.
Results: The cells so obtained after processing appeared to have a fibroblast-like morphology. Immunohistochemistry revealed the expression of mesenchymal surface markers CD73 and CD 90. The cells showed a stronger expression of CD90 than CD73.
Conclusions: PDL stem cells can be identified by the expression of the mesenchymal stem cell (MSC) markers CD 73 and CD 90.

KEYWORDS

Periodontium, Stem Cells, Periodontitis

INTRODUCTION

Periodontitis is a well-known and relatively common inflammatory destructive pathological phenomenon that occurs in the gingiva, periodontal ligaments (PDL), alveolar bone, and cementum. It is one of the oldest and most common oral diseases. The prevalence of periodontal ailment in exceptional nations varies from 50 % to 90% [1,2] and is the main motive of teeth loss in adults. The typical feature of the stationary segment of periodontitis is defects of the gingiva and other periodontal tissues. The occurrence of gingival recession will increase with age, with 58% of people over age 30 having at least 1 mm of gingival recession and nearly every person over age 50 having varying tiers of gingival recession [2]. In a few elderly sufferers with moderate or severe periodontitis, a wide range of intense gingival defects and alveolar bone loss exists. Severe gingival tissue defects affect most of the patients' appearance, and their phonation along with eating functions. Basic periodontal remedies which are commonly used today can only take away plaque and calculus to control inflammation, whilst the final aim of periodontal remedy is to repair and reconstruct the periodontal tissue. Stem cells represent rather new techniques for periodontal regeneration. Stem cells can proliferate in vitro, and after transplantation can differentiate into desired tissues to restore defects. A critical difficulty is to separate exquisite stem cells from various sources for tissue regeneration.

Mesenchymal stem cells (MSCs) are perfect candidates for regenerative medication. MSCs were, to begin with, distinguished from bone marrow mononuclear cells (BM– MNCs) as fibroblastic colony– forming units (CFU– Fs). This was probably attributable to multipotency and paracrine impact of these cells [3,4]. At present the minimum criteria of MSCs 5 include: (a) remain plastic– adherent under standard culture conditions; (b) express CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA– DR; (c) differentiate into osteoblasts, adipocytes, and chondrocytes in vitro. Currently, there is no consensus on a single surface molecule to identify MSCs from various sources. CD 10, CD13, CD29, and CD4 are some other surface antigens also expressed. Bone marrow (BM) has traditionally been the most widely utilized source of MSCs. Current research, however, has revealed other sources of MSC– like cells, including adipose tissue, placenta, dental pulp, synovial membrane, peripheral blood, periodontal ligament, endometrium, umbilical cord, and umbilical cord blood. In fact, evidence has suggested that MSCs may be present virtually in any vascularized tissues throughout the whole body. [5,6]

It has been shown that mesenchymal periodontal ligament stem cells (PDLSCs) are similar to other mesenchymal stem cells in the expression of the following markers: STRO-1, CD146, CD90, CD29, CD44, CD13, CD105 and CD166, as well as their self-regenerating capacity and their multi-potentiality. Several authors, after isolating PDLSCs showed their ability to differentiate to mesodermal type lineages, differentiating in cementoblast-like cells, osteoblasts, chondrocytes, adipocytes, myofibroblasts, and collagen-forming fibers. Potential of PDLSCs has even been researched to give rise to nervous system-like cells and cementoblasts. [7,8,9]

The purpose of this study was to isolate and characterize human PDLSCs derived from the root, and to investigate their microscopic phenotype, and surface marker expression.

MATERIALS AND METHODS

Tissue procurement

A total of 10 extracted premolar teeth of 5 healthy male patients, who underwent the extraction for orthodontic purposes were included in the study based on a protocol approved by the Institutional Review Board and Ethics Committee. Informed consent was obtained from the patients to use the extracted the teeth. The PDL tissue was obtained from patients, who had no chronic diseases or history of smoking, alcohol consumption or medication use. Included teeth had no endodontic infections and no lucent or opaque lesions observed on radiographs, and fully erupted teeth from patients who exhibited no periodontal infection and had no history of previous treatment.

The following inclusion criteria were used for tooth selection: (1) only patients without systemic alterations, (2) suitable periodontal health, (3) first and second premolar teeth from right and left upper and lower jaws. Exclusion criteria were: (1) patients afflicted with systemic diseases, (2) patients with gingivitis and/or periodontal disease, (3) premolars with presence of dental plaque and/or presence of caries and/or periapical reaction.

Patients rinsed their mouth with 0.2% chlorhexidine, after which their lips and skin were prepped with diluted Betadine. This took control of any infection. After induction of anesthesia, atraumatic extraction was

performed. Care was taken to preserve the periodontium. Root surfaces were scraped with a back-action chisel and isolated tissue samples were placed in a nutrient solution for a minimum of 20 minutes at 4°C, avoiding contact with the walls of the dish. The solution, which contained 15mL of Roswell Park Memorial Institute medium combined with penicillin, streptomycin, gentamicin and amphotericin B, was then transferred to the research laboratory within three hours.

Immunohistochemistry staining

Serial sections, 5 µm in thickness, prepared from archived formalin-fixed, paraffin-embedded (FFPE) tissue blocks, were used for immunohistochemical analysis. All stages of the immunostaining process were carried out at room temperature of 23°C unless otherwise stated. To identify mesenchymal stem cells in the periodontal ligament, mouse monoclonal antibodies (mAbs) anti-CD73, and anti-CD90 were used. The demonstration of cell-surface antigens was significantly improved by pretreatment with antigen-retrieval reagents that broke the protein crosslinks formed during formalin fixation. For antigen retrieval of CD73 surface antigens, the sections were placed in preheated citrate buffer at pH 6 and heated at 80°C in a water bath for 20 min. The slides were allowed to cool to room temperature for 30 min before washing in PBS. No antigen retrieval procedures were necessary for the CD44 surface antigens.

Endogenous peroxidase activity was blocked by incubation, for 20 min, in PBS containing 1% hydrogen peroxide and 0.1% sodium azide. The sections were then rinsed in PBS. The primary antibodies, at concentrations previously determined to give optimal staining (CD73, 4 lg/ml; CD90, 4 lg/ml) were applied to the sections and allowed to incubate at room temperature overnight. The sections were counterstained with haematoxylin and then mounted in aqueous media before light microscopic analysis.

The expression of CD73 and CD90 on the tissue samples were analyzed. The immunoreactivity of the specimens was interpreted based on the intensity of the staining. Score ranks usually lie in a range from 'negative' (mostly marked as '-') to 'positive', which was signed with different amount of '+' depending upon the intensity of the stain. The samples were examined with conventional light microscope. As previously described by Shetty et.al [10], the membranous immunohistochemical staining was considered positive for CD73 and CD90. The staining intensity was graded as (+) weak, (++) moderate and (+++) intense according to the overall appearance at different powers of magnification, in other words, 4×, 10× and 40×, respectively and (-) for no staining, as described by Shetty et. al.[10]

RESULTS:

The first adherent cells appeared 2 h after the primary culture and reached 80% of confluence at about day 10. Under optical microscopy, all the primary cells appeared spindle-shaped fibroblast-like morphology, as seen in Figure-1. After subculture, cells grew rapidly and appeared radial or whorled arrangement. Immunocytochemistry staining showed that the cultured cells were detected positive for CD73 in 8 out of 10 samples and all the cells from the samples were strongly positive for the marker CD90. Immunohistochemical examination, as shown in Table-1, demonstrated that both CD73 and CD90 were expressed. However, expression of CD90 markers was stronger than the Cd73.

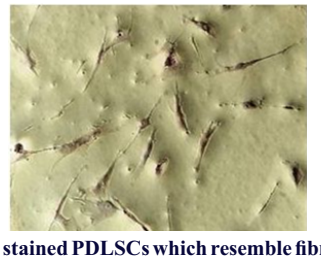

Figure 1: CD90 stained PDLSCs which resemble fibroblasts.

Table – 1 Immunohistochemistry Examiation

MEMBRANOUS IMMUNO HISTOCHEMICAL MARKER	DEGREE OF STAINING		
	Weak (+)	Moderate (++)	Intense (+++)
CD 73	2	5	3
CD 90	0	4	6

The above table shows the degree of staining in the 10 sample teeth. CD90 was more intensely stained than CD70.

DISCUSSION:

The PDL is a specialized, highly vascularized, connective tissue encompassing the root of the tooth. Beside the role in maintaining tooth attachment to the surrounding alveolar bone, PDL possess a regenerative capacity. Potential use of these cells in cell-based therapy for the treatment of damaged periodontium has encouraged researchers to identify PDLSCs and explore their characteristics. PDLSCs are an excellent source of generation of a huge number of mature cells through a sequential procedure of proliferation and differentiation, retaining their ability to self-renew to maintain the stem cell pool. [10] Stem cell markers are the protein products that identify a multipotent single cell capable of recapitulating. There are four types of cell: embryonic stem cell (ESC), hematopoietic stem cell (HSC), mesenchymal/stromal stem cell (MSC/SSC) and neural stem cell (NSC). The purpose of this study was to characterize the stem cellness of the periodontal tissue obtained from extracted teeth, which are in general considered waste teeth, using histological and immunohistochemical methods. The PDLSCs isolated in this study showed the property to adhere as spindle-shape fibroblast-like cells when maintained in standard culture conditions. In addition, obtained PDLSCs exhibited the ability to proliferate and to produce colonies from a single cell, this being in agreement with previous observations made by other authors. [8,10,11]

Prior to investigating the PDLSCs, an overview of marker expression in basic pulp tissue is crucial. In the present study, we demonstrate the immunohistochemical characterization of stem cell and differentiation markers in PDL tissue sections of premolar teeth. CD73 and CD90 serve as reliable markers for identifying the PDLSCs population. CD73, originally defined as a lymphocyte differentiation antigen, functions as a cosignalling molecule on T lymphocytes and is required for lymphocyte binding to endothelium [12,13]. Expression of CD73 has been demonstrated on various cell types including lymphocytes, endothelial cells, and MSC. [14,15,16] It is thought to play physiological roles in epithelial ion and fluid transport, maintaining barrier functions, mediating endothelial permeability, adapting to hypoxia, and contributing to microbial responses [17,18]. CD73 is an extracellular enzyme that catalyzes the formation of immunosuppressive adenosine by converting adenosine 5′-monophosphate (AMP) to its bioactive intermediate, adenosine, which in turn activates adenosine receptors, when released into the extracellular space, and regulates various physiological functions [19,20]. Adenosine signalling, modulated by CD39 and CD73 expression, has been highlighted as a novel modulator in the immunosuppression of T-cell proliferation by MSC [21,22]. As such, this may be contributory to immunomodulatory properties of PDLSC [8] and may highlight an avenue for anti-inflammatory therapy in periodontal disease [23].

The expression of CD90 on PDLSC has been well documented [23,24,25]; however, its role in PDLSC function remains largely unknown. CD90, also known as Thy-1 (thymocyte differentiation antigen-1), is found to be expressed in various cell types such as hematopoietic stem/progenitor cells [26], hepatic stem cells in human fetal liver [27], liver cancer stem cells [28], neurons, fibroblasts, vascular pericytes, and MSC [29]. Its expression is developmentally regulated [30] and remains one of the minimal criteria for defining human MSC, proposed by the committee for the International Society for Cellular Therapy (ISCT) [29,30]. While the biological role of CD90 is unclear, a number of associated immunological and nonimmunological functions have been previously addressed [30]. In addition to its involvement in T-cell activation [30], it is believed to be associated with many cellular processes and pathological conditions in a context-dependent manner [29], including cell-cell and cell-matrix interactions, cell motility, and thymocyte adhesion to epithelium [30]. Moreover, CD90 expression is also associated with fibroblast phenotypes relevant to wound healing and fibrosis. The differential CD90 expression is associated with cell-extracellular matrix interactions and cell migration and, as such, is correlated with distinct cellular morphology [30].

Certain studies have detected the expression of CD44, CD73, CD90, CD105, CD166, STRO– 1, CD14, CD34 and CD45 in PDLSCs by flow cytometry. [31] Several studies have reported that PDLCs express MSC markers such as CD10, CD13, CD29, CD44, CD59, CD73,

CD90, and CD105 and do not express CD14, CD34, CD45, and HLA-DR as stated by Lindroos et al. 2008; [32] Xu et al. 2009; [33] Park et al. 2011 [5]. Our study data showed that the PDLSCs were positive for MSCs markers CD73 and CD90 in accord with the marker expression profile defined for multipotent stromal cells in the International Society for Cellular Therapy position statement.[34]

CONCLUSION:

The results of this study show that CD73 and CD90 are reliable markers of PDLSCs. This may support the use of periodontal ligament as a potential source of pluripotent PDLSCs for future cell-based therapies. They will also be useful in periodontal tissue engineering. However, the stem cells of periodontal origin have been heretofore underestimated due to the sparse available literature and hence future research in this direction is warranted to use them to their maximal potential.

REFERENCES:

[1] Nazir M. A. (2017). Prevalence of periodontal disease, its association with systemic diseases and prevention. International Journal of Health Sciences, 11(2), 72–80.
[2] Kassab M. M. and Cohen R. E. (2003). The etiology and prevalence of gingival recession. Journal of American Dental Association, 134(2), 220-5.
[3] Lv F. J., Tuan R. S., Cheung K. M., and Leung V. Y. (2014). Concise review: the surface markers and identity of human mesenchymal stem cells. Stem Cells, 32(6), 1408-19.
[4] Huang G.T., Gronthos S., and Shi S. (2009). Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in regenerative medicine. Journal of Dental Research, 88, 792– 806.
[5] Park J. C., Kim J. M., and Jung I. H. et al (2011). Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: In vitro and in vivo evaluations. Journal of Clinical Periodontology, 38, 721– 731.
[6] Iriarte C. G. T., Ramírez O. R., García A. M., Terán S. L. V and Clavel J. F. G (2017). Isolation of periodontal ligament stem cells from extracted premolars. Simplified method. Revista Odontológica Mexicana, 21, e12-e20.
[7] Saito M. T., Silverio K. G., Casati M. Z., Sallum E. A., and Nociti Jr. F. H. (2015). Tooth-derived stem cells: update and perspectives. World Journal of Stem Cells, 7, 399-407
[8] Seo B. M., Miura M., Sonoyama W., Coppe C., Stanyon R., and Shi S. (2005). Recovery of stem cells from cryopreserved periodontal ligament. Journal of Dental Research, 84, 907-912
[9] Guo S., Guo W., Ding Y., Gong J., Zou Q., and Xie D., et al. (2013). Comparative study of human dental follicle cell sheets and periodontal ligament cell sheets for periodontal tissue regeneration. Cell Transplant., 22, 1061-1073
[10] Shetty H., Kakade A., Shetty S., Neelakantan P., Nagar S., Desai R. S., and Beri K. (2018). Immunohistochemical characterization of stem cell and differentiation markers of the dental pulp of human natal teeth. Future science OA, 4(10), FSO342.
[11] Miletić M, Mojsilović S, Đorđević IO, Kukolj T, Jauković A, Santibañez J. F., Jovčić G, and Diana Bugarski D (2014). Mesenchymal stem cells isolated from human periodontal ligament. Archives of Biology and Science, Belgrade, 66, 261-271
[12] Seo, B.M., Miura, M., Gronthos, S., Bartold, P.M., Batouli, S., Brahim, J., Young, M., Robey, P.G., Wang, C.Y. and S. Shi (2004). Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364, 149-155
[13] Scholar H., Rupert S., Suzuki N., Chowdary K. and Gruss P (1990). New type of POU domain in germ line-specific protein in Oct-4. Nature, 344, 435–439.
[14] Liu P., Cai J., and Dong D., et al (2015). Effects of SOX2 on proliferation, migration and adhesion of human dental pulp stem cells. PLoS ONE. 2015;10(10):e0141346.
[15] Gronthos S., Brahim J., and Li W., et al. (2002). Stem cell properties of human dental pulp stem cells. Journal of dental research, 81(8), 531–535.
[16] Waddington R. J., Youde S. J., Lee C. P., and Sloan A. J. (2009). Isolation of distinct progenitor stem cell populations from dental pulp. Cells Tissues Organs, 189, 268–274.
[17] Xiong, J., Menicanin, D., Zilm, P. S., Marino, V., Bartold, P. M., and Gronthos, S. (2016). Investigation of the Cell Surface Proteome of Human Periodontal Ligament Stem Cells. Stem cells International, 2016, 1947157.
[18] Colgan S. P., Eltzschig H. K., Eckle T., and Thompson L. F. (2006). Physiological roles for ecto-5′-nucleotidase (CD73). Purinergic Signalling, 2, 351–360.
[19] Beavis P. A., Stagg J., Darcy P. K., and Smyth M. J. (2012). CD73: a potent suppressor of antitumor immune responses. Trends in Immunology, 33, 231–237.
[20] Sattler C., Steinsdoerfer M., and Offers M., et al (2011). Inhibition of T-cell proliferation by murine multipotent mesenchymal stromal cells is mediated by CD39 expression and adenosine generation. Cell Transplantation, 20, 1221–1230.
[21] Saldanha-Araujo F., Ferreira F. I. S., and Palma P. V., et al (2011). Mesenchymal stromal cells up-regulate CD39 and increase adenosine production to suppress activated T-lymphocytes. Stem Cell Research, 7, 66–74.
[22] Wada N., Menicanin D., Shi S., Bartold P. M., and Gronthos S. (2009). Immunomodulatory properties of human periodontal ligament stem cells. Journal of Cellular Physiology, 219(3), 667–676.
[23] Kato T., Hattori K., and Deguchi T., et al (2011). Osteogenic potential of rat stromal cells derived from periodontal ligament. Journal of Tissue Engineering and Regenerative Medicine, 5(10), 798–805.
[24] Tomokiyo A., Maeda H., and Fujii S., et al (2012). A multipotent clonal human periodontal ligament cell line with neural crest cell phenotypes promotes neurocytic differentiation, migration, and survival. Journal of Cellular Physiology, 227(5), 2040–2050.
[25] Trubiani O., Zalzal S. F., and Paganelli R., et al. (2010). Expression profile of the embryonic markers nanog, OCT-4, SSEA-1, SSEA-4, and frizzled-9 receptor in human periodontal ligament mesenchymal stem cells. Journal of Cellular Physiology, 225(1), 123–131.
[26] Baum C. M., Weissman I. L., Tsukamoto A. S., Buckle A. M., and Peault B. (1992). Isolation of a candidate human hematopoietic stem-cell population. Proceedings of the National Academy of Sciences of the United States of America, 89(7), 2804–2808.
[27] Masson N. M., Currie I. S., Terrace J. D., Garden O. J., Parks R. W., and Ross J. A. (2006). Hepatic progenitor cells in human fetal liver express the oval cell marker Thy-1. American Journal of Physiology—Gastrointestinal and Liver Physiology, 291(1), G45–G54.
[28] Yang Z. F., Ho D. W., and Ng M. N., et al. (2008). Significance of CD90+ cancer stem cells in human liver cancer. Cancer Cell, 13(2), 153–166.
[29] Bradley J. E., Ramirez G., and Hagood J. S. (2009). Roles and regulation of Thy-1, a context-dependent modulator of cell phenotype. BioFactors, 35(3), 258–265.
[30] Haeryfar S. M. M., and Hoskin D. W. (2004). Thy-1: more than a mouse pan-T cell marker. Journal of Immunology, 173(6), 3581–3588.
[31] Tran H. L. B., Doan V. N., Le H. T. N., and Ngo L. T. Q. (2014). Various methods for isolation of multipotent human periodontal ligament cells for regenerative medicine. In Vitro Cellular & Developmental Biology Animal, 50(7), 597-602.
[32] Lindroos B., Mäenpää K., Ylikomi T., Oja H., Suuronen R. and Miettinen S. (2008). Characterization of human dental stem cells and buccal mucosa fibroblasts. Biochemical and Biophysical Research Communications, 368, 329–335.
[33] Xu J., Wang W., Kapila Y., Lotz J., and Kapila S. (2009). Multiple differentiation capacity of STRO-1 + /CD146+ PDL mesenchymal progenitor cells. Stem Cells and Development, 18, 487–496.
[34] Dominici M., Le Blanc K., Mueller I., Slaper-Cortenbach I., Marini F., Krause D., Deans R., Keating A., Prockop Dj., and Horwitz E. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, 8(4), 315-317.