ORIGINAL PAPER

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USING CO-CULTURE SYSTEM TO DIFFERENTIATE PERIODONTAL LIGAMENT-DERIVED STEM CELL TO DUCTAL SALIVARY GLAND CELLS

Maryam Koopaie, Sara Biglar, Nafiseh Sheykhbahaei

Oral and Maxillofacial Medicine, Tehran University of Medical Sciences, School of Dentistry, Iran

ABSTRACT

Introduction: Irreversible harm to salivary glands is a complication that is treated using symptomatic treatment only. Stem cell therapy provides a new approach in treating irreversible harm to the salivary glands. Salivary gland's ductal cells transport saliva and change its' composition. Co-culture is a regulatory cell culture technique, in which two or more different cell populations grow with various degrees of interaction.

Objectives: Given the role of salivary glands ductal cells in their final saliva production, this study aimed to examine the differentiation of periodontal ligament stem cells (PDL-SCs) in salivary glands ductal cells using the co-culture technique.

Material and methods: PDL-SCs were isolated from adult human PDL tissue and co-cultured with rat parotid ductal cells using an indirect co-culture system. Trans-differentiation of PDL-SCs was evaluated by polymerase chain reaction (PCR) analysis of kallikrein 1 and keratin 19 genes, with unique functional expression in ductal cells.

Results: Expression of CD90, CD73, CD44, and CD105 mesenchymal markers were proved that the obtained PDL-SCs were mesenchymal stromal cells (MSCs). Positive oil red and alizarin red staining showed differentiation of stem cells into fat and bone cells. Expression of kallikrein 1 and keratin 19 demonstrated successful trans-differentiation of PDL-SCs into salivary ductal cells after co-culturing for three weeks.

Conclusions: These findings highlight the potential usefulness of the co-culture system in the differentiation of PDL-SCs-derived mesenchymal stem cells into salivary gland ductal cells. The proximity of mesenchymal stem cells to salivary acinar cells and the expression of acinar cells' specific markers lead to differentiation.

Key words: periodontal ligament, stem cells, mesenchymal stem cells, co-culture techniques, salivary glands.

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INTRODUCTION

Saliva has influential functions in the oral cavity, including cleaning, regulating the oral microbiome, dissolving food, forming bolus, facilitating chewing and swallowing, and thinning the secretion and lubrication of mucus [1-3]. Furthermore, protecting teeth by neutralizing acid through buffering, maintaining unsaturated calcium phosphate (CaP) with hydroxyapatite, and participating in dental pellicle formation are among the functions related to special components of saliva [4-6]. Oral health is disrupted by salivary gland functional disorders caused by genetic or acquired abnormalities, such as injuries from surgery or radiotherapy of head

Address for correspondence: Dr. Nafiseh Sheykhbahaei, Oral and Maxillofacial Medicine, Tehran University of Medical Sciences, School of Dentistry, Iran, e-mail: dsheykhbahaei@gmail.com

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and neck cancer treatment [7, 8]. These lead to more symptoms, including chewing and swallowing problems, speech disorders, dysgeusia, oral fungal infection, and acceleration of dental caries. Ultimately, these abnormalities severely decrease patients' quality of life [9].

Current preventive and symptomatic treatments are not always effective in controlling irreversible harm caused by radiotherapy [10]. In recent years, damaged tissue repair and new tissue growth approaches have gained much attention using cellular-molecular methods. Salivary stimulants could be used in managing the decreased function of salivary glands and xerostomia; however, the salivary gland needs to be functional [11]. Regarding radiation-induced xerostomia, current preventive treatments, such as salivary glands transfer to outside radiotherapy field, are possible if there is no neck lymph node involvement on the other side (N0), and cannot be carried out in case of any lymph node involvement on the transfer site [12]. Moreover, there is a risk of relapse caused by salivary glands transfer with the presence of concealed cancer [13]. Today, different gene therapy approaches and the use of stem cells in treating many incurable diseases have gained much importance in the medical world, of which final objective is curative rather than palliative (symptomatic) care. Stem cell therapy has provided a new approach for treating irreversible harm to the salivary glands [14].

The unique properties of stem cells, such as self-renewal and the potency required to differentiate into specialized cell types, have made these cells a source for treatments in regenerative medicine [15]. Periodontal ligament (PDL) stem cells are renewable progenitor cells that produce cementoblasts, osteoblasts, and fibroblasts. These cells have shown the properties of mesenchymal stem cells (MSCs) and tissue regeneration capacity [16].

Several techniques, including co-culture, have been suggested to differentiate stem cells from target mature cells. Co-culture is a regulatory cell culture technique, in which two or more different cell populations grow with various degrees of interaction [17]. Some cells cannot quickly become monocultures *in-vitro*, or may not show the intended physiological behavior. However, the presence of another cell population can improve the success of culture or cell behavior. Co-culture systems potentially present a wide range of desired properties compared with mono-culture systems, including modularity, potency, predictability, scalability, and stability [17]. In co-culture, using the paracrine effects of the growth factors secreted from mature cells of the salivary glands for stem cell differentiation, could relieve us from the challenges of accurately identifying extra-cellular factors. In addition, the differentiation of stem cells into target cells in non-*in-vivo* conditions reduces the chance of tumorigenesis and carcinogenesis [18].

OBJECTIVES

Given the role of salivary glands ductal cells in changing saliva composition and modifying primary saliva, this study aimed to examine the differentiation of PDL-SCs into salivary glands' ductal cells using the co-culture technique.

MATERIAL AND METHODS

Normal impacted third molar teeth used in this study were obtained from three patients through surgery at the School of Dentistry of Tehran University of Medical Sciences after the Ethics Committee of Tehran University of Medical Sciences approved the study. Patients varied in age between 15 and 30 years, had no infectious systemic diseases, nor used medications, or consumed tobacco or alcohol. PDL tissue on the surface of the root was isolated by a hoe scaler, and was transferred to the laboratory quickly in a phosphate-buffered saline (PBS) container with 3% PenStrep placed near 4°C ice pack. The sample was washed several times with Hank's buffer containing antibiotics, in the lab in sterile conditions, and under the fume hood. Hank's buffer or salts are a group of salts rich in bicarbonate ions used as a buffer in a cell culture environment to obtain the optimum physiological pH (range, 7.0-7.4). Next, the tissue was placed in Dulbecco's modified eagle medium (DMEM) and type I collagenase enzyme (250 µ/ml) in a shaking incubator at 37°C and 5% CO_2 for 1 to 2 hours. After the tissue digestion, 70 µm and 40 µm filters were used to filter undigested tissue pieces and impurities. Ficoll was used to eliminate mono-nuclear cells from the sample. Afterwards, the sample solution was centrifuged in Eppendorf centrifuge 5810R at 1,500 rpm for 5 minutes, until the cell plate was formed. Then, the cells were cultured in T75 flask in DMEM and 10% fetal calf serum (FCS). The culture environment was changed after 48 hours and then every 72 hours. When 80% of the flask was covered by the cells, they were sub-cultured (passaged) by 1 : 3 ratio using 0.2% trypsin and EDTA. In order to prove they were stem cells, their differentiation into osteoblasts and adipocytes was used after third sub-culture. The environment of differentiation into fat included DMEM/F12 containing 50 µg/ mL ascorbic acid 3-phosphate, 100 ng dexamethasone, and 50 µg/ml indomycin, and the environment of differentiation into bone included DMEM/F12 containing 50 µg/ml ascorbic acid 3-phosphate, 10 ng dexamethasone, and 10 millimolar β-glycerol phosphate. The cells were kept in these induced environments for 14 days, and the environment was changed thrice weekly. To show induction of adipogenesis, oil red staining was applied. Alizarin red staining was used to prove calcium accumulation. Moreover, to prove that the accumulated cells were MSCs, CD90, CD44, CD73, and CD105 mesenchymal markers, CD34 and CD45 hematopoietic markers were used in flow cytometry. Furthermore, immuno-his-

tochemical evaluations of CD44 marker were applied to confirm the presence of mesenchymal stem cells, and were examined by two blinded investigators.

ISOLATION OF RAT SALIVARY GLANDS CELLS

Ductal cells of salivary glands were obtained from submandibular salivary glands of 11 female Wistar rats killed under anesthetic. Next, the submandibular salivary gland tissue was isolated using a scalpel. The tissue was placed in 1% trypsin at 37°C for 2-3 hours for enzymatic isolation of the epithelial part from the mesenchymal. Then, the isolated tissue was washed with PBS and exposed to 1% collagenase at 37°C for 3 hours. As a result, the cells were isolated.

In the next step, it was filtered by 70 mm nylon mesh. Afterwards, it was centrifuged at 2,000 rpm for 15 minutes, and re-washed using PBS. The obtained cells were transferred to a 24-well plate containing 0.5 mm DMEM with 1% antibacterial X100 (containing penicillin-streptomycin), 10% FCS, and 1% glutamine. They were incubated at 37°C, with 5% CO_2 and 95% humidity. The cells were kept for a week to achieve the desired accumulation, with culture medium replaced every 72 hours.

PREPARING CO-CULTURE ENVIRONMENT

After third sub-culture (passage), the stem cells were transferred to a 6-well insert culture plate at 1×10^6 cell/ml density containing 2 ml of DMEM with 10% FBS. Then, salivary gland tissue cells were placed in insert culture plates at 1×10^5 density, in the second sub-culture. After 24 hours of contiguity, keratinocyte growth factor (KGF) was added to the salivary gland cells' environment. The environment was changed every 72 hours. The cells were counted using neobar lam (HBG, Germany), and the stem cells were treated with a differentiation environment for 21 days. The environment was changed every 72 hours.

ANALYSIS OF DIFFERENTIATED STEM CELLS

TOTAL RNA EXTRACTION

RNeasy Plus mini kit by Kiagene Fanavar was applied based on the manufacturer's instruction to extract RNA. PLUS-RNX contained tyrosol, phenol, and a strong detergent to digest tissue and break cellular connections. It should be noted that as RNA is very sensitive to activity of RNAs, the following actions were observed in order to preserve RNA. The whole surface of the hood and surfaces involved in the study, such as centrifuge and racks, were washed with RNaseZAP. RNase-free dishes, microtubes, tubes, and tips, prepared exclusively for working with RNA, were used. Disposable latex powder-free gloves were utilized, and contact with the hand was avoided as the hand skin has an RNase enzyme. The following protocol was used to extract RNA:

- 1 ml RNeasy Plus mini kits were added to the samples, and then they were pipetted by an insulin syringe to be homogenized.
- 200 µl chloroform was added to all samples. Note: chloroform was used to make a phase.
- Vials were incubated at 4°C (inside ice in the fridge).
- Samples were centrifuged at 12,000 rpm at 4°C for 15 minutes.
- Supernatant was removed carefully with a sampler and transferred to a 1.5 ml tube.
- Cold isopropanol was taken at the same volume as supernatant, and then it was added.
- It was mixed and refrigerated for 15 minutes.
- It was centrifuged again at 12,000 rpm at 4°C for 15 minutes.
- Supernatant was discharged, and 1 ml ethanol was added to the resulting sediment. It was mixed and centrifuged at 7,500 rpm at 4°C for 8 minutes.
- Supernatant was discharged and kept under the hood for 10 minutes to dry. The resulting sediment was total RNA, and 35 µl DEPC water was added and transferred to –70°C.

DESIGNING PRIMER

Kallikrein 1 (KLK1), keratin 19 (KRT19), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene sequence was obtained from https://www.ncbi.nlm.nih.gov. Forward primers and reverse primers were designed using Gen Runner (version 3) and Primer Express (version 3.05). Primer sequences of KLK1, KRT19, and GAPDH genes are shown in Table 1.

RESULTS

This experimental study aimed to turn stem cells into salivary glands' ductal cells. The expression results

TABLE 1. Primer sequences (forward and backward) of KLK1, KRT19, and GAPDH

Gene	Primer sequence (forward)	Primer sequence (backward)
KLK1	GACACCTGGAAGGTGGCAAAGA	CATAAGACAGCACTCTGACGGC
KRT19	AGCTAGAGGTGAAGATCCGCGA	GCAGGACAATCCTGGAGTTCTC
GAPDH	GGCAAATTCCATGGCACCGTCAAGGC	TGGTGGTGAAGACGCCAGTGGACTCC

of CD44 marker were examined by immuno-histochemical staining to confirm the presence of mesenchymal stem cells, and are shown in Figure 1. CD90, CD73, CD44, and CD105 mesenchymal markers were examined through flow cytometry to prove that the obtained cells were MSCs (Figure 2).

THE SURVIVAL RATE OF CELLS ON ISOLATION DAY

The assessment was based on cell analysis using staining with propidium iodide (PI). At this stage, the obtained cell plate went under PI staining. Next, the cells went under flow cytometry (Figure 3).

The cells were differentiated into fat and bone using differentiation culture environments in order to prove they were stem cells. Oil red staining was applied to show areas differentiated into fat cells, which were stained red (Figure 4). Alizarin red staining was used to differentiate stem cells into bone cells. The cells differentiated into bone were stained red (Figure 5).

The obtained stem cells were placed in co-culture. The stem cells were transferred to 6-well insert culture

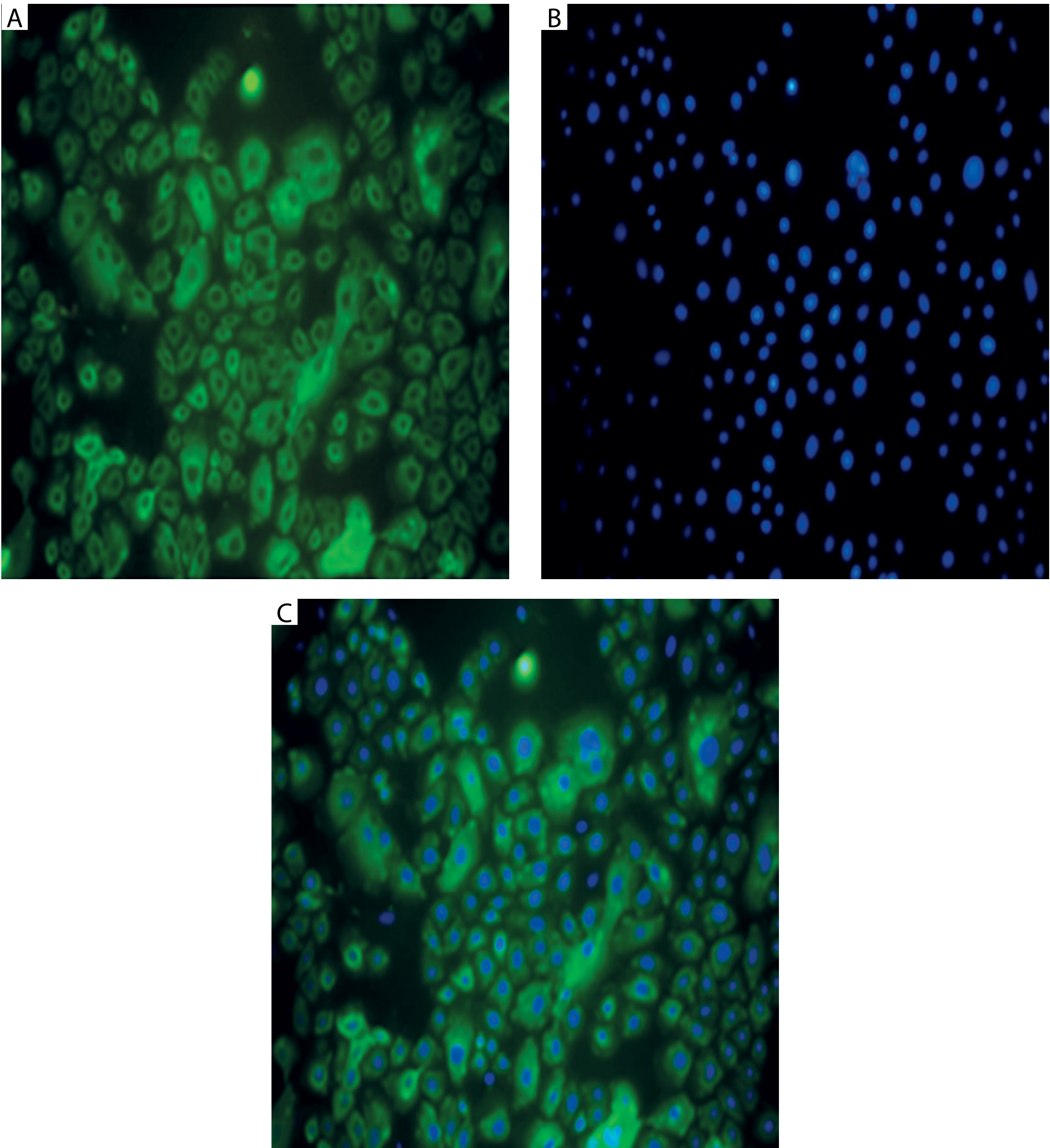

FIGURE 1. Expression of CD44 marker by immuno-histochemical staining. (**A**) ICC, (**B**) DAPI (4′,6-diamidino-2-phenylindole), and (**C**) merge of (**A**) and (**B**)

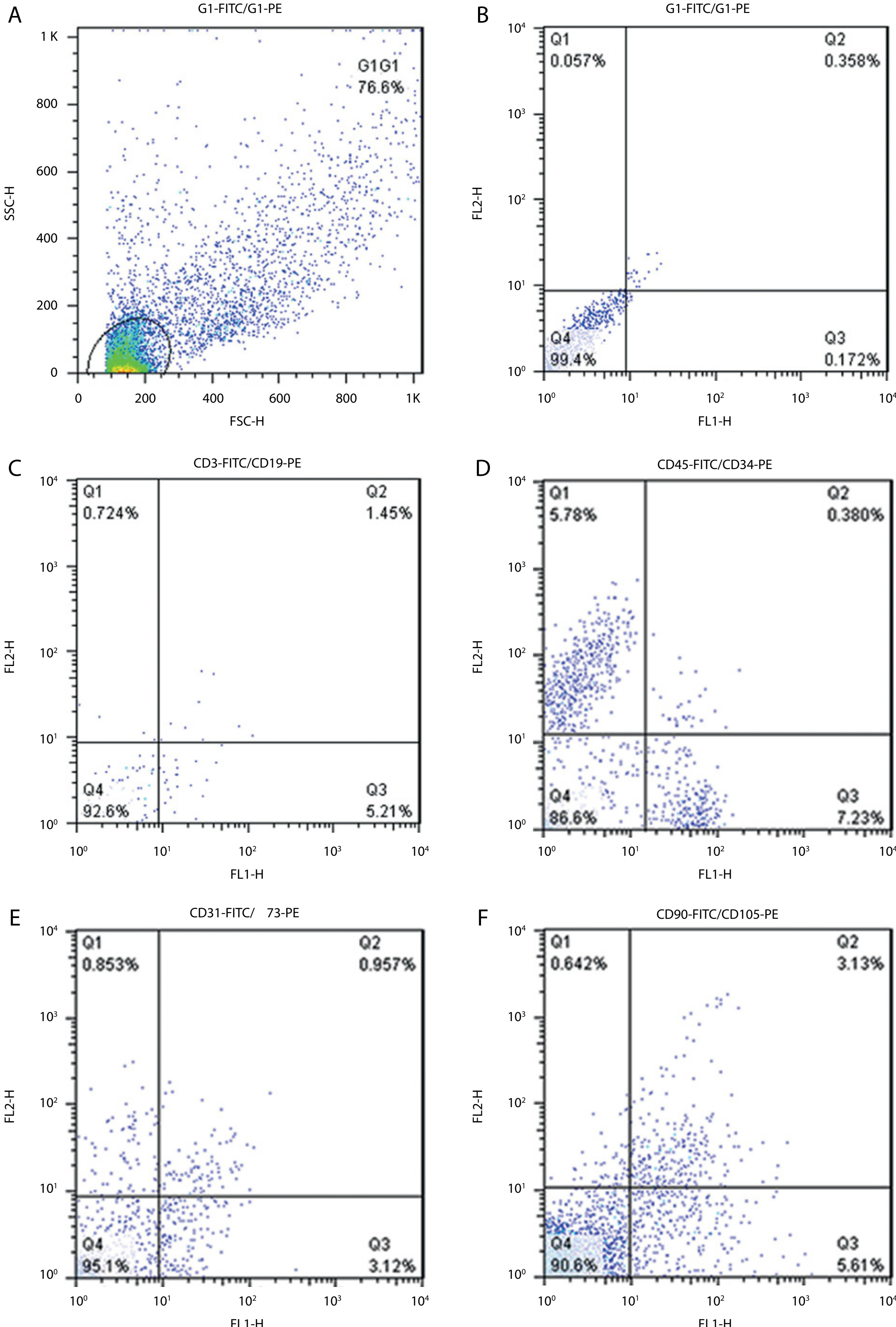

FIGURE 2. Surface markers of stem cells on isolation day

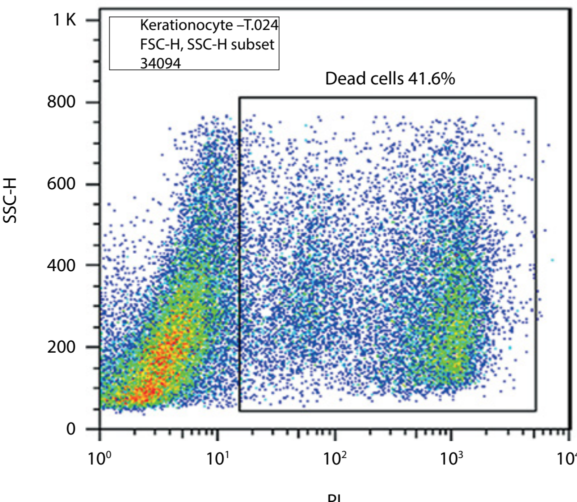

FIGURE 3. Cell viability on isolation day

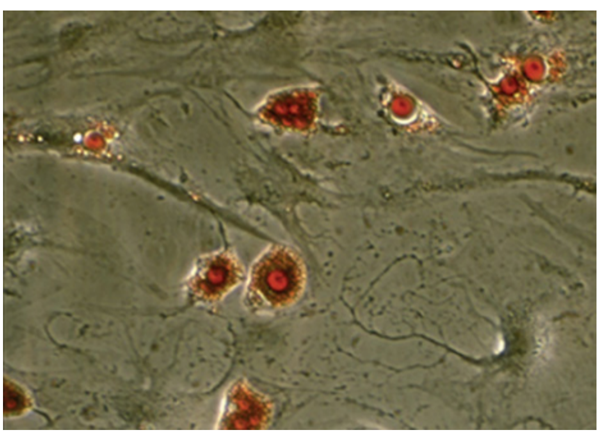

FIGURE 4. Oil red staining (differentiated into fat cells)

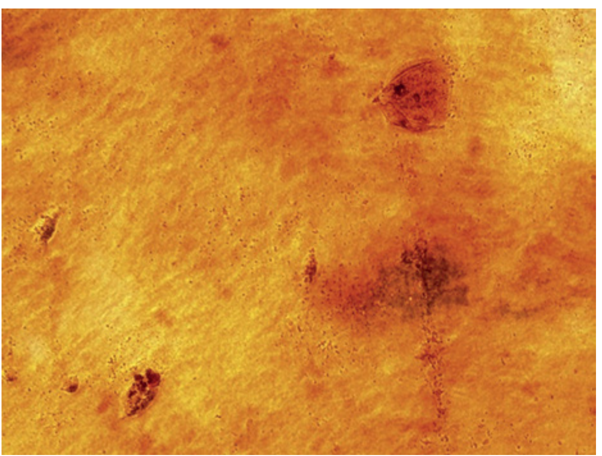

FIGURE 5. Alizarin red staining (differentiation of stem cells into bone cells)

plate after the third sub-culture at 1×10^6 cell/ml density. Salivary glands' tissue cells were placed in insert culture plates. In order to analyze the differentiation of stem cells into salivary glands' ductal cells, *KLK1* (kallikrein 1) gene expression was examined using PCR. Moreover, immuno-histochemistry analysis was carried out to analyze KRT19 (keratin19) marker, which is exclusive for salivary glands' ductal cells [19].

Salivary glands' cells were isolated through cell sorting, total RNA was extracted by TRZol reagent based on the manufacturer's instructions, and was treated with DNase I to prevent genomic DNA contamination. SMObio cDNA synthesis kit was used for cDNA synthesis, and ICycler iQ real-time PCR detection system was utilized for real-time PCR. GAPDH was used as an internal control gene. Melting curve for the exclusive surface marker in differentiated cells is presented in Figure 6.

DISCUSSION

Until now, no effective treatment has been suggested for irreversible harm to salivary glands. Xerostomia management commonly includes symptomatic treatments to relieve xerostomia symptoms [20]. In recent decades, many studies have pointed out the advantages of using stem cells for the regeneration of different tissues and organs [21]. The present study showed the potential of PDL-SCs in differentiation into salivary glands' ductal cells. The advantages of using PDL-SCs include easy culture and the possibility of cell-replication for a long time, without the risk of malignant transformation. PDL-SCs have higher cell reproduction and clonogenic ability than bone-derived stem cells [22]. In addition, obtaining stem cells from bone marrow is associated with pain and discomfort, and fat-derived stem cells are usually limited in number, and in some cases, differentiation into salivary gland cells has not been reported [23]. This points to obtaining stem cells from accessible cells, such as PDL cells. Several studies have shown the differentiation of PDL-derived stem cells into cementoblasts, adipocytes, fibroblasts, osteoblasts, and chondrocytes [24]. Given the potential of PDL-derived stem cells, this study was designed as the first study to differentiate PDL-SCs into salivary glands' ductal cells.

In this study, indirect co-culture was used to differentiate stem cells into ductal cells. The advantage of differentiating stem cells in-vitro is the reduced risk of malignant transformation, as there is evidence of a probable relation between malignant transformation MSCs and undifferentiated sarcoma [25]. Co-culture systems have the advantage of stem cell proliferation in-vitro and reduce the risk of malignant transformation, as stem cells are placed in contiguity with mature cells and the signals from mature cells direct stem cells to differentiate into them in these systems [26]. In this study, the only added factor to the culture environment was KGF. Therefore, reasons that caused the successful differentiation of stem cells were the signals from mature cells present in the co-culture environment. Previous studies have

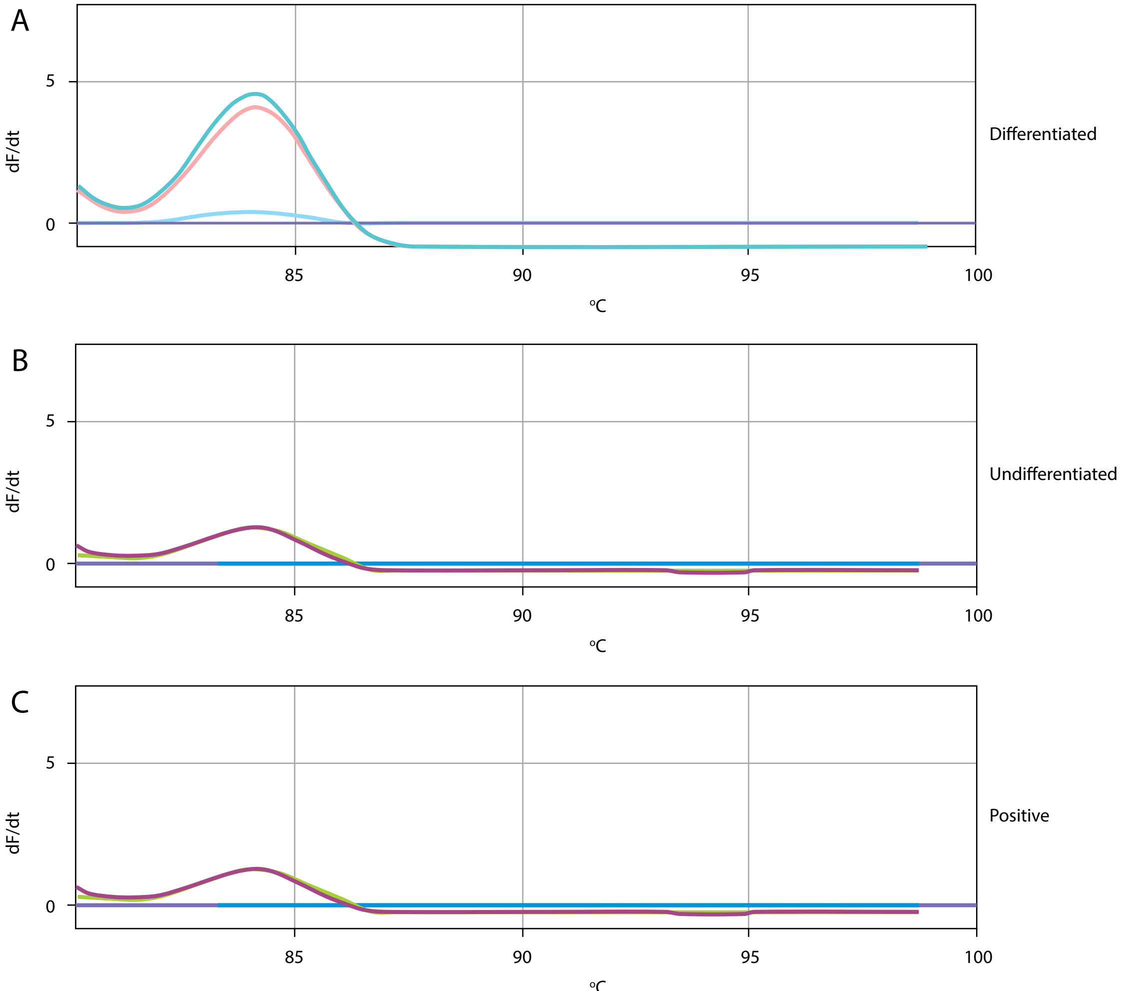

FIGURE 6. Melting curve of exclusive surface marker in differentiated cells.

examined the differentiation of MSCs into salivary glands' cells. Maria *et al.* [27] analyzed the differentiation of MSCs into acinar cells of salivary glands in a co-culture system. In a co-culture system, the assumption is that the factors released from salivary glands' epithelial cells pass through the membrane of PDL-SCs, and signal the differentiation into salivary glands' cells [28].

An important factor in a co-culture system is the amount of released factors in the environment for stem cell differentiation [29]. Several studies have reported different ratios of the release of soluble factors in co-culture environment. The ratio was between 13% to 40% in previous studies, and it has to be sufficient to lead to the differentiation of stem cells [30]. In co-culture systems, stem cells cannot only produce a source of high differentiation potency cells. Also, act as cells that help hemostasis, grow metabolism, and repair. Co-culture systems control the behavior and function of cells through the interaction of several types of cells. These systems can control target cells through feedback from assisting cells [18]. The purpose of tissue engineering is the generation of functional tissues. Cells in a co-culture system can connect, develop, and produce normal physiological structures [31].

The crucial advantage of co-culture systems is feedback. Furthermore, they reduce the limitation of other stem cells with the chance of malignant transformation [18]. Considering the advantages of co-culture systems, this study used a co-culture system for the differentiation of stem cells into salivary glands' ductal cells. In order to prove the stemness of PDL-derived cells, their differentiation to adipocytes and bone was examined. Oil red and aliasing red staining indicated that stem cells were differentiated into adipocytes and bone cells, respectively. In addition, the cells were examined with CD45, CD31, and CD90 markers.

Surface markers of mesenchymal cells include CD90, CD44, CD166, CD106, and CD105. Expression of CD90 in MSCs surface is associated with growth potential and high differentiation of these cells. The cells derived from rat salivary glands tissue were placed in contiguity with PDL-derived stem cells as assisting cells. In this study, indirect technique was used for culture. Soluble factors were transferred from rat salivary glands cells to stem cells and led to the differentiation of these cells into salivary glands' ductal cells.

KLK1 protein is produced in salivary glands ductal cells and is a sub-category of serine proteases [32]. The re-

sults of this study showed that KLK1 expression increased in co-culture environment. The PCR analysis also showed KLK1 expression in differentiated cells. KRT19 gene is expressed in salivary glands' ductal cells and liver cells. The protein encoded by gene KRT19 is a member of the keratin family, and is involved in the organization of myofibers [33]. This study showed that KRT19 gene expression increased in co-culture environments. The findings indicate that PDL-SCs have the potential to turn and differentiate into salivary glands' ductal cells.

An important point is that different approaches have been applied to make complex organs, such as salivary glands, kidneys, and lungs. Thus far, there has not been an approach that covers the complete regeneration of these organs. Future studies on the mechanisms that lead to the regeneration of salivary glands by co-culture systems could hold a bright future for researchers in this field. This study introduced a new view for future studies on salivary glands' regeneration.

CONCLUSIONS

PDL-SCs have the potential for differentiation into salivary gland cells. This study showed the potential of these cells and co-culture environment for differentiation into salivary glands' ductal cells, promising a new treatment approach in patients with irreversible salivary glands' harm. Nonetheless, future animal and laboratory studies are needed to investigate the efficacy of ductal cells obtained from this study for the regeneration of salivary glands.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Tehran University of Medical Sciences Ethical Committee (ethical code: IR.TUMS.VCR.REC.1398.508).

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Pedersen AML, Sørensen CE, Proctor GB, Carpenter GH. Salivary functions in mastication, taste and textural perception, swallowing and initial digestion. Oral Dis 2018; 24: 1399-1416. doi: 10.1111/odi.12867.
2. Uchida H, Ovitt CE. Novel impacts of saliva with regard to oral health. J Prosthet Dent 2021; 127: 383-391. doi: 10.1016/j.prosdent.2021.05.009.
3. Pedersen AML, Belstrøm D. The role of natural salivary defences in maintaining a healthy oral microbiota. J Dent 2019; 80: S3-S12. doi: 10.1111/odi.12867.
4. Farooq I, Bugshan A. The role of salivary contents and modern technologies in the remineralization of dental enamel: a narrative review. F1000Res 2020; 9: 1-14. doi: 10.12688/f1000research.22499.3.
5. Heller D, Helmerhorst E, Oppenheim F. Saliva and serum protein exchange at the tooth enamel surface. J Dent Res 2017; 96: 437-443. doi: 10.1177/0022034516680771.
6. Odanaka H, Obama T, Sawada N, Sugano M, Itabe H, Yamamoto M. Comparison of protein profiles of the pellicle, gingival crevicular fluid, and saliva: possible origin of pellicle proteins. Biol Res 2020; 53. doi: 10.1186/s40659-020-0271-2.
7. Pedersen AML, Sørensen CE, Proctor GB, Carpenter GH, Ekström J. Salivary secretion in health and disease. J Oral Rehabil 2018; 45: 730-746. doi: 10.1111/joor.12664.
8. Mays JW, Sarmadi M, Moutsopoulos NM. Oral manifestations of systemic autoimmune and inflammatory diseases: diagnosis and clinical management. J Evid Based Dent Pract 2012; 12: 265-282. doi: 10.1016/S1532-3382(12)70051-9.
9. de Almeida PDV, Gregio A, Machado M, De Lima A, Azevedo LR. Saliva composition and functions: a comprehensive review. J Contemp Dent Pract 2008; 9: 72-80.
10. Barbieri T, da Costa KC, de Freitas Cuba Guerra L. Current alternatives in the prevention and treatment of xerostomia in cancer therapy. Rev Gaucha Odontol 2020; 68. doi: 10.1590/1981-86372020000163546.
11. Yoo C, Vines JB, Alexander G, Murdock K, Hwang P, Jun HW. Adult stem cells and tissue engineering strategies for salivary gland regeneration: a review. Biomater Res 2014; 18: 1-12. doi: 10.1186/2055-7124-18-9.
12. Zhang Y, Guo CB, Zhang L, et al. Prevention of radiation-induced xerostomia by submandibular gland transfer. Head Neck 2012; 34: 937-942. doi: 10.1002/hed.21859.
13. Collan J, Kapanen M, Mäkitie A, et al. Submandibular gland-sparing intensity modulated radiotherapy in the treatment of head and neck cancer: sites of locoregional relapse and survival. Acta Oncol 2012; 51: 735-742. doi: 10.3109/0284186X.2011.640348.
14. Benderitter M, Caviggioli F, Chapel A, et al. Stem cell therapies for the treatment of radiation-induced normal tissue side effects. Antioxid Redox Signal 2014; 21: 338-355. doi: 10.1089/ars.2013.5652.
15. Rajabzadeh N, Fathi E, Farahzadi R. Stem cell-based regenerative medicine. Stem Cell Investig 2019; 6. doi: 10.21037/sci.2019.06.04.
16. Mrozik K, Gronthos S, Shi S, Bartold PM. A method to isolate, purify, and characterize human periodontal ligament stem cells. Methods Mol Biol 2017; 1537: 413-427.
17. Goers L, Freemont P, Polizzi KM. Co-culture systems and technologies: taking synthetic biology to the next level. J R Soc Interface 2014; 11: 20140065. doi: 10.1098/rsif.2014.0065.
18. Paschos NK, Brown WE, Eswaramoorthy R, Hu JC, Athanasiou KA. Advances in tissue engineering through stem cell-based co-culture. J Tissue Eng Regen Med 2015; 9: 488-503. doi: 10.1002/term.1870.
19. Jang S, Ong H, Gallo A, Liu X, Illei G, Alevizos I. Establishment of functional acinar-like cultures from human salivary glands. J Dent Res 2015; 94: 304-311.
20. Cifuentes M, Del Barrio-Díaz P, Vera-Kellet C. Pilocarpine and artificial saliva for the treatment of xerostomia and xerophthalmia in sjögren syndrome: a double-blind randomized controlled trial. Br J Dermatol 2018; 179: 1056-1061. doi: 10.1111/bjd.16442.
21. Vasanthan J, Gurusamy N, Rajasingh S, et al. Role of human mesenchymal stem cells in regenerative therapy. Cells 2020; 10: 54.
22. Zhu W, Liang M. Periodontal ligament stem cells: current status, concerns, and future prospects. Stem Cells Int 2015; 2015. doi: 10.1155/2015/972313.
23. Nishiwaki S, Nakayama T, Saito S, et al. Efficacy and safety of human adipose tissue-derived mesenchymal stem cells for supporting hematopoiesis. Int J Hematol 2012; 96: 295-300. doi: 10.1007/s12185-012-1140-8.
24. Seo B-M, Miura M, Gronthos S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004; 364: 149-155. doi: 10.1016/S0140-6736(04)16627-0.

25. Kim HJ, Park JS. Usage of human mesenchymal stem cells in cell-based therapy: advantages and disadvantages. Dev Reprod 2017; 21: 1. doi: 10.12717/DR.2017.21.1.001.
26. Chang YH, Chu TY, Ding DC. WNT/β-Catenin signaling pathway regulates non-tumorigenesis of human embryonic stem cells co-cultured with human umbilical cord mesenchymal stem cells. Sci Rep 2017; 7: 1-10. doi: 10.1038/srep41913.
27. Maria OM, Tran SD. Human mesenchymal stem cells cultured with salivary gland biopsies adopt an epithelial phenotype. Stem Cells Dev 2011; 20: 959-967. doi: 10.1089/scd.2010.0214.
28. Lee J, Park S, Roh S. Transdifferentiation of mouse adipose-derived stromal cells into acinar cells of the submandibular gland using a co-culture system. Exp Cell Res 2015; 334: 160-172. doi: 10.1016/j.yexcr.2015.03.006.
29. Lee IC, Wang JH, Lee YT, Young TH. The differentiation of mesenchymal stem cells by mechanical stress or/and co-culture system. Biochem Biophys Res Commun 2007; 352: 147-152. doi: 10.1016/j.bbrc.2006.10.170.
30. Fouad SAA. Dental stem cells: a perspective area in dentistry. Int J Dent Sci Res 2015; 3(2A): 15-25. doi: 10.12691/ijdsr-3-2A-4.
31. Noel G, Baetz NW, Staab JF, et al. A primary human macrophage-enteroid co-culture model to investigate mucosal gut physiology and host-pathogen interactions. Sci Rep 2017; 7: 1-14. doi: 10.1038/srep45270.
32. Zheng C, Hoque AS, Braddon VR, Baum BJ, O'Connell BC. Evaluation of salivary gland acinar and ductal cell-specific promoters in vivo with recombinant adenoviral vectors. Hum Gene Ther 2001; 12: 2215-2223. doi: 10.1089/10430340152710559.
33. Hosoya A, Kwak S, Kim EJ, et al. Immunohistochemical localization of cytokeratins in the junctional region of ectoderm and endoderm. Anat Rec (Hoboken) 2010; 293: 1864-1872. doi: 10.1002/ar.21233.