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Research article

Assessment of odontogenic potential of mesenchymal stem cells derived from adipose tissue and oral mucosal tissue (comparative study)

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Abstract

Stem cell research has reached new heights in recent times and is implemented in regenerative medicine and dentistry. The oral cavity is the richest stem cell source in the human body. These stem cells are now recognized as being vital to different types of dental and non dental tissue regeneration. The aim of this study was to isolate human mesenchymal stem cells (MSCs) from Adipose tissue (ASCs) and oral mucosal tissue (OMSCs) and confirm their differentiation potentials, including the odontogenic lineage. ASCs and OMSCs cultures were nalyzed for cell shape, cell cycle, proliferation potential (MTT assay) and stem cell markers (CD90, CD105). The odontogenic differentiation potential of ASCs and OMSCs induced with odontogenic induction medium and was assessed by means of Alizarin Red stain and quantitative real time RT-PCR using dentin sialophosphprotein (DSPP). Our data revealed that ASCs and OMSCs showed a significant increase in cell viability from day 14 to day 21, representing high cell proliferation rate(80-90%), where ASCs proliferated faster than OMSCs. Moreover, ASCs and OMSCs can efficiently differentiate into dentin forming cells expressing odontoblastic markers (DSPP), where OMSCs significantly expressed (DSPP) higher than ASCs. This study provides evidence that ASCs and OMSCs can be used in tissue engineering/regeneration protocols as an approachable stem cell source.

Key words: stem cells, differentiation, odontogenic, adipose tissue, oral mucosa.

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1. Introduction

The goal of tissue engineering and regenerative medicine is to improve or restore the functions of diseased tissues and organs. Tissue engineering strategies have need of main fundamentals such as stem cells, scaffold or matrix, and growth factors [19, 36].

Mesenchymal stem cells (MSCs) are multipotent stem cells that can be isolated from many tissues/organs, such as bone marrow and adipose tissue, with the properties of self-renewal for long periods through cell division [16]. In addition, under certain physiological or investigational circumstances, MSCs are promising progenitor cell sources for stem cell transplantation, tissue engineering and regeneration. Exploring suitable sources of stem cells for reparative and regenerative purposes is an important mission in front of researchers [31, 38]. Adipose tissue-derived stem cells (ASCs) were first isolated by Zuk et al. [42] as a rich accessible source of MSCs with minimal patient discomfort, less invasive and low risk of side effects. ASCs were found to be able to keep strong proliferative ability, maintain their phenotypes and have stronger multi-differentiation potential [5]. ASCs have many clinical advantages over bone marrow mesenchymal stem cells, by its unique expression of antigens CD49d and CD106, whereas they did not express the MSCs marker CD106 (38,39) [22]. In addition, the differentiation potential of ASCs can be maintained with aging [15] [27].

Recent attention has been focused on the exploitation and existence of dental tissue-derived stem cells in tissue engineering, providing potential cell sources for regeneration of tooth structures as well as other tissues/organs [30, 24, 8]. The most widely known MSCs of dental origin are dental pulp stem cells (DPSCs) [23, 35] and periodontal ligament stem cells (PDLSCs)[3, 4]. Besides that, several other kinds of MSCs of dental origin have also been gradually secluded by the researchers, such as, exfoliated deciduous teeth (SHED [21], apical papilla (SCAP) dental follicle (DFPCs)[36] as well as oral mucosal tissue [3]. The oral mucosa is the term used to illustrate the soft tissue lining of the oral cavity, including the buccal mucosa and the gingivae [34, 18]. Although the capability of dental mesenchymal stem cells to give rise to dental tissue as well some other tissues has been reported, unfortunately the accessibility and availability of these stem cells are quite limited [31]. Comparatively, gingival MSCs (GMSCs) constitute more tempting alternatives to the other dental originated MSCs in terms of that they are much easier to get as a byproduct from the clinically resected gingival tissues.

Hence, it is of great interest to validate the multiple differentiation potentials of GMSCs for potential tissue engineering applications [17].

Zhang Q et al. [44] first sheltered a population of oral progenitor cells within gingival tissue, termed GMSCs, which formed clonogenic colonies, expressed a typical MSC surface marker profile(CD90, CD105, CD73, CD44 and CD13 positive and CD34, CD38, CD45 and CD54 negative)[40] and gripped the ability to differentiate into multiple mesodermal lineages *in vitro* [39, 11]. Notably, single colony derived GMSCs verified the capacity for self-renewal and formation of connective tissue-like structures *in vivo*. Jin SH et al., [17] and Geetanjali B et al., [13] confirmed that GMSCs are superior to BMSCs for clinical applications.

Dental stem cell-based tissue engineering approaches can diminish the problem of

endodontic treatment by preserving tooth vitality [6, 38]. Thus, the present study aims to investigate odontogenic potential of adipose tissue and oral mucosal tissue when exposed to odontogenic induction medium in vitro.

2. Materials and Methods

Sample Collection

Adipose tissue and oral mucosal samples were collected from the ten white healthy albino rats from the animal house of the National Research Center. Under general anesthesia, adipose tissue from inguinal region and the oral mucosa from cheek were aseptically collected. Incineration of the deceased rats was done at the incinerator of the National Research Center.

Isolation and culture of ASCs and OMSCs

The oral mucosa was scraped with a lancet to peel the epithelium from it and both specimens were minced into small 1mm 3 pieces. The minced pieces were collected in sterile, labeled 1.5 ml Eppendorf tubes to which a digesting solution was added. Enzyme digestion (3 mg/ml collagenase type I and 4 mg/ ml dispase) was carried out according to Gronthos et al [14], for 60 minutes at 37°C. The culture medium (DMEM) with L- glutamine supplemented with 10 % fetal bovine serum (Gibco, Invitrogen Life Technologies, USA), antibiotics and finally antimycotic agent was added. The tubes were centrifuged for 20 minutes at room temperature to obtain a cell pellet of isolated cells.

The supernatant was discarded and then the cells in the pellet were re-suspended in complete culture medium by successive pipetting. Media were changed every 2-3 days.

Passaging was performed when the primary cell culture of adherent cells reached 70% confluence and was named passage zero (P0). Later passages were named accordingly. And cells were propagated and expanded till passage 3 (P3). Cultures were washed twice with (PBS) and trypsinized with 0.25% trypsin in 1m (EDTA) (GIBCO/BRL) for 5 minutes at 37°C. After centrifugation, cell pellets were re-suspended in 1 ml complete medium and divided into two plates (passaging) both followed by immersion in complete culture medium to increase cell numbers. Thus the primary cell culture was propagated and expanded. The cells were counted under inverted microscope.

Characterizations of ASCs and OMSCs by Flow cytometry

Adherent cells (at the end of the 4th passage) were trypsinized and adjusted to 1×106 cells/ml. Then 1x105 cells were incubated with 10µl of monoclonal antibodies: CD45 FITC, CD90 PE and CD105 PE, (Beckman coulter, USA) at 4 ◦C in the dark. Same species iso-types served as a negative control. After 20 minute incubation, 2 ml of PBS containing 2% FCS solution were added to each tube of monoclonal treated cells. The mixtures were then centrifuged for 5 minutes at 2500 rpm followed by discarding the supernatant and re-suspending cells in 500µl PBS containing 2% FCS. Cell analysis was performed using CYTOMICS FC 500 Flow Cytometer (Beckman coulter, FL, USA) and analyzed using CXP Software version 2.2.

Assessing Proliferation Capability:

MTT Assay Protocol

The proliferation capacity was judged by close follow up of confluence rate i.e. culture plates reaching 70 % confluence according to culture days Cultures from ACSs and OMSCs monitored using inverted light microscope (Olympus, USA).Cells were cultured in 100 ml of culture medium in a flat-bottomed 96 well plate. The MTT reagent was added (10 ml per well) and the plate was incubated for 2 to 12 hours.

Detergent reagent was added to each well and the absorbance of each sample in a microplate reader was measured at 550 - 600 nm, depending upon the filters available

Odontogenic differentiation

The 3rd passage culture of the cells was grown to 80%-90% confluence in non-coated 3.5 cm dishes in culture media. Odontogenic differentiation was induced by using odontogenic induction medium containing DMEM, 10% FBS, 5 mM β-glycerol phosphate, 100 µM L- ascorbic acid 2- phosphate, 0.01 µM dexamethasone, 2 mM L-glutamine, 100 units/ml penicillin, 100mg/ml streptomycin and 1.8 mM and Mono-potassium phosphate (#A10072-01 kit) for 21 days. The medium was changed twice a week

Flow cytometric analysis:

Differentiated cells were tested for the expression of stem cell surface markers (CD90, CD105). Cell analysis was performed using CYTOMICS FC 500 Flow Cytometer (Beckman coulter, FL, USA) and analyzed using CXP Software version 2.2[7].

Staining and RT-PCR:

On day 14 and 21, the tissues were stained using Alizarin Red stain to identify mineralization. On day 14 and 21, the total RNA was extracted from all dishes and real time RT-PCR was performed to analyze the mRNA level of the dentin sialophosphprotein (DSPP).

Statistical analysis

Data were coded and entered using the SPSS (version 21). Data was summarized using mean and standard deviation. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables while non-parametrical Kruscal-Wallis test and Mann-Whitney test were used for non-normally distributed quantitative variables by Pearson's correlation. P values < 0.05 were considered as statistically significant.

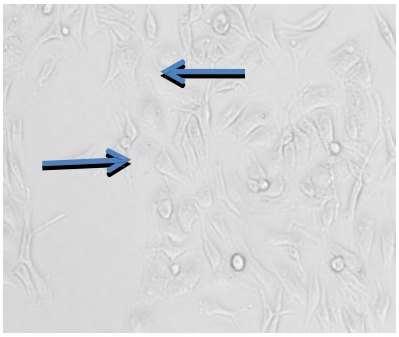
3. Results and discussion

Results

Isolation and Culture

Stem cells were successfully isolated from Adipose tissue. After enzymatic digestion and initial seeding, ASCs conformed an elongated shape and some cells started to be spindle shaped (Figure 1). Adipose tissue continued to proliferate and propagate reaching 80-90% confluence by day 10.

In addition, stem cells were also successfully isolated from oral mucosal tissue. After enzymatic digestion and initial seeding, OMSCs conformed an elongated shape and some cells started to be spindle shaped than ASCs (Figure 2). OMSCs continued to proliferate and propagate reaching 80-90% confluence by the end of the second week.



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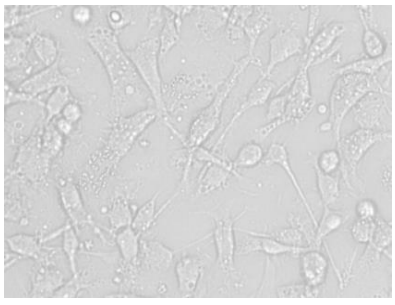

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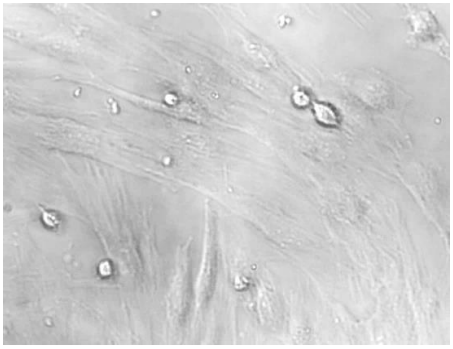
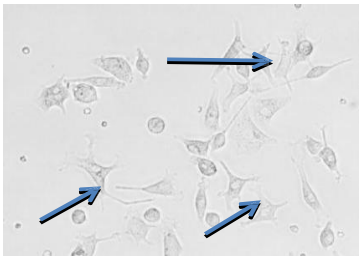


C

Figure 1. Showing ASCs after isolation a) one week; show a stellate cells, b) two weeks: cells increase in number and attain more spindle appearance, c) three weeks: cells increase in number and become compressed.



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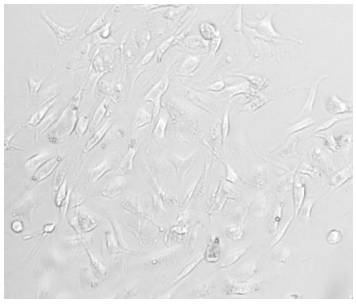

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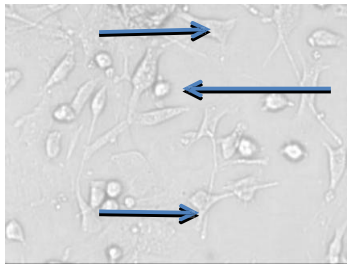
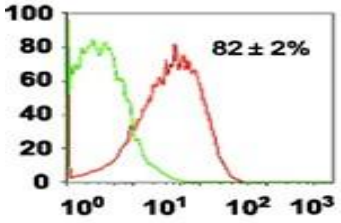


C

Figure 2. Showing OMSCs after isolation a) one week; show morphological diversity, b) two weeks: cells increase in number and attain more spindle appearance, c) three weeks: cells become more compressed and showing confluence 80- 90%.

Characterization of stem cells by flow cytometry

The expression of CD90, CD105 and CD45 were assessed using flow cytometric analysis which revealed that ASCs and OMSCs were positive for CD90 (ASCs: 82 ±2%, OMSCs: 97 ±1%) as well as for CD105 (ASCs: 98.58% and OMSCs: 99.75%). On the other hand, the cells were negative for the leucocyte precursor marker CD45 (ASCs: 0.04 and OMSCs: 0.2%).



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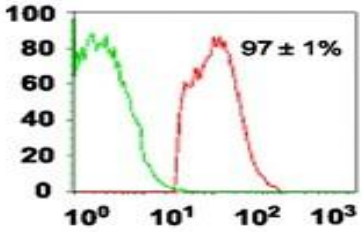
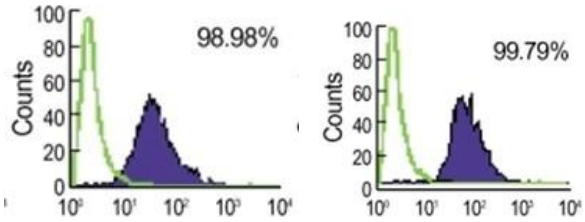


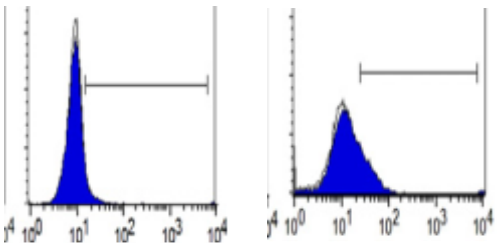
B

Figure. 3. Flow cytometric analysis of ASCs(A) and OMSCs(B) for CD90.



A B

Figure 4. Flow cytometric analysis of ASCs(A) and OMSCs(B) for CD105.



A B

Figure 5. Flow cytometric analysis of ASCs(A) and OMSCs(B) for CD45.

MTT Assay

On day 14, the number of viable cells in ASCs cultures was found to be significantly higher than that of OMSCs and this was also demonstrated on day 21. It was also demonstrated that the proliferation of ASCs and OMSCs increased significantly (P value ≤ 0.05) from day 14 to day 21

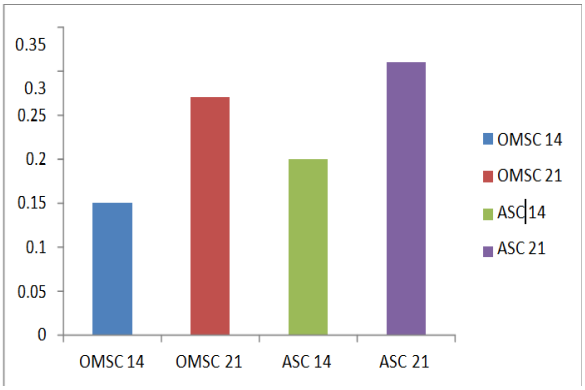


Figure 6. Bar chart for MTT assay for ASCs and OMSCs on day 14 and day 21.

Assessment of Stemness after Differentiation by Flow Cytometry

CD90 was negatively expressed in the cells (ASCs: 4±1% and OMSCs: 2±1%)(Figures 8) as well as CD105 was also negatively expressed in the cells (ASCs:0.04% and OMSCs: 3.14%) (Figures 7,8)

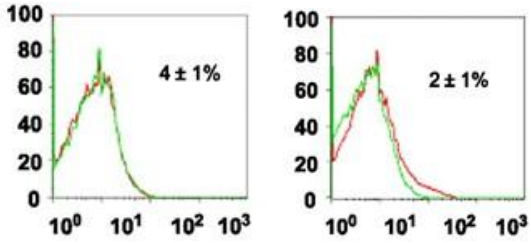


Figure 7. Flow cytometric analysis of ASCs (A) and OMSCs (B) for CD90 after differentiation showing negative expression.

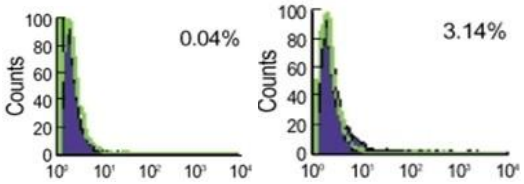


Figure 8. Flow cytometric analysis of ASCs (A) and OMSCs (B) for CD105 after differentiation showing negative expression.

Assessment of Differentiation

Alizarin Red Staining

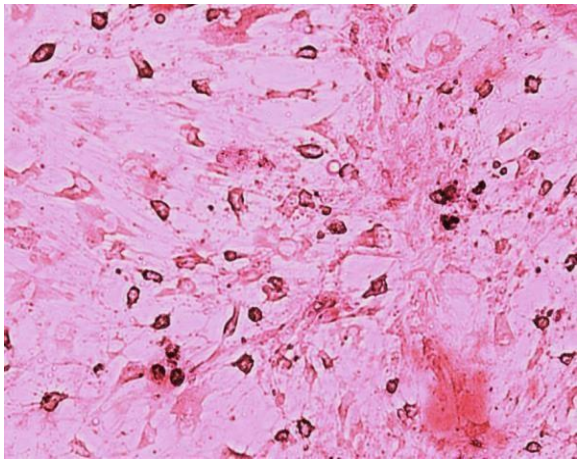
After applying the odontogenic induction medium, the cells were observed regularly for morphological changes. Within the first week, the cells started to change their spindle-shape into round shaped cells and migrate mainly from the periphery towards the center of the dish, giving rise to rounded aggregates.

By the 14th day, staining with calcium specific Alizarin Red stain was done and the aggregated cells were the first to be stained indicating beginning of mineralization in these cells. By the 21st day, the staining became more intense and multiple isolated mineralized extracellular nodules/sites appeared (Figure 9,10).

PCR

The odontogenic differentiation potential was assessed by the expression of DSPP using Real time PCR. A highly significant increase in the expression of DSPP from day 14 to day 21 was detected in ASCs and OMSCs.

It was recorded that the amount of DSPP expressed was significantly higher in OMSCs cultures than in ASCs and BMSCs at the period of 14 days as well as 21 days after odontogenic differentiation.



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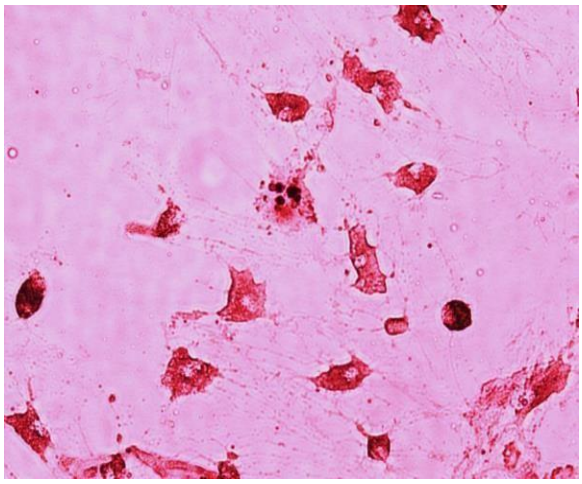
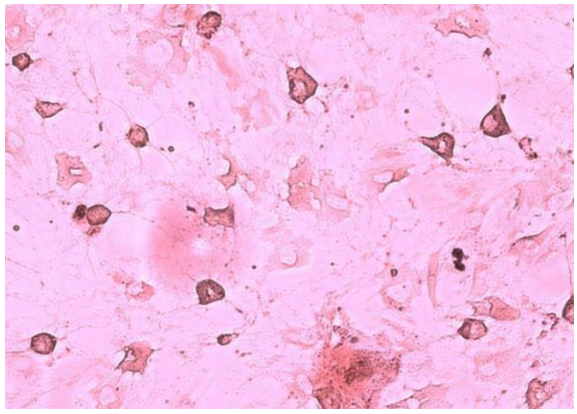


B

Figure 9. (day 14) showing ASCs (A) with intracellular staining(blue arrows) and beginning of extracellular mineralization (green arrows) and OMSCs (B) with dense intracellular staining and extracellular calcified nodules (arrows) (Alizarin Red; x200).



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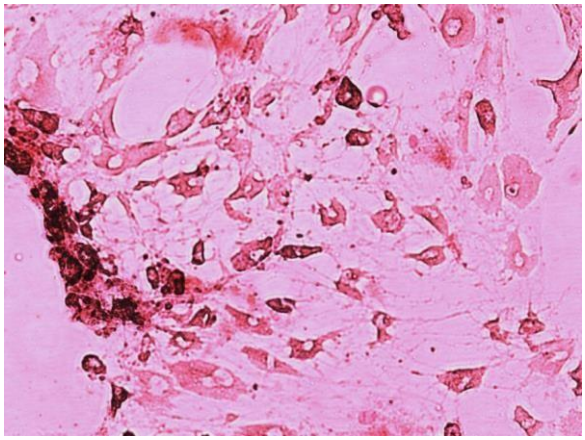

B

Figure 10. (day 21) showing ASCs (A) with extracellular mineralization(green arrow) and oval odontoblast- like cells with odontoblastic processes(blue arrows) and OMSCs (B) with dense extracellular Alizarin Red staining. Cells attain odontoblast- like appearances (arrows)(Alizarin Red; x400).

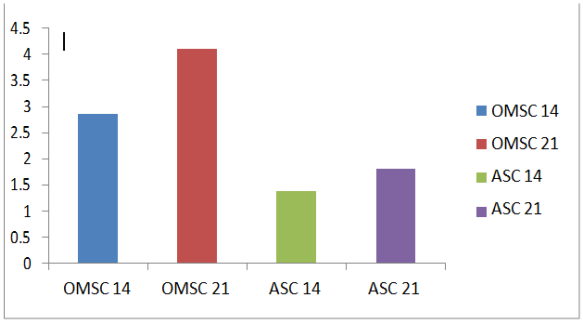

Figure 11. Bar chart showing expression of DSPP on day 14 and day 21.

Discussion

In recent years, there has been a remarkable interest in stem cells within the dental and medical community mainly because of their capability of self-renewal and multiple lineage differentiation [30]. Most recent studies of tissues regeneration aimed to use MSCs taken from sites that are even more accessible and rich in stem cells [20, 29]. Adipose tissue has been proven to be an alternative source of MSCs as it is characterized by stable proliferation doubling kinetics in vitro, good accessibility and tissue abundance [6].

The oral cavity is the richest stem cell source in the human body [31]. These oral stem cell populations have common cell properties including the capacity for self-renewal and multi-lineage differentiation potential as it is associated with a specialized environment that provides key signals to guide stem cell function. Fawzy K et al., [10] and Zhang Q. et al., [44] first characterized human gingiva-derived MSCs (GMSCs), which exhibited unique immunomodulatory functions, clonogenicity, self-renewal and multi-potent differentiation capacities similar to that of BMMSCs. GMSCs proliferate faster than BMMSCs, display a stable morphology and do not lose their MSC characteristics [11]. The present study focused on the ASCs and OMSCs regarding their characterization, proliferation and capability to differentiate into odontogenic lineages.

After the isolation procedure, different morphologies such as spindle and stellate-shaped cells were demonstrated in the cell culture. The cells were able to survive after several passages due to the use of mesenchymal cell promoting culture media as reported by Jin SH et al. [17].

In our research, different methods have been introduced to identify MSCs criteria in ASCs and OMSCs which detected that the cell were able to attach to the plastic flask floor. The cells also positively expressed the stem cell markers as CD90 and CD105 and negatively expressed hematopoietic cell marker as CD45. These results were in accordance with Angelova-Volponi A et al. [2] and Karim M et al. [20].

In the present study, during cell morphology analysis, all cell cultures showed diversity in morphology ranging from spindle to stellate appearance that was consistent throughout several passages. Our results were generally in agreement with Ge S., et al. [12] and Geetanjali B. et al. [13].

To assess the proliferation capability of the ASCs and OMSCs, MTT assay were chosen. Our results demonstrated that ASCs and OMSCs showed a significant increase in cell viability from day 14 to day 21, representing high cell proliferation rate(80-90%), where ASCs proliferated faster than OMSCs. Both tissue cultured cells proliferated faster in the first two weeks than they did in the third week. Our results were generally in agreement with Davies O et al. [6] and Mohamadreza Bet al. [29].

Flow cytometry was used to assess the loss of stemness of the cultured stem cells after induction of odontogenic differentiation [1]. The cultured cells were examined for expression of stem cell markers CD90 and CD105. Flow cytometric analysis revealed very much reduced levels of CD90 and CD105 and this suggested that the differentiated cells contain negligible proportion of stem cells indicating loss of stemness. Our results were in accordance with Seo M et al. [34] who found that stemness of dental pulp stem cells was almost lost by measuring the expression of stem cell markers (Stro-1 and CD146) which were expressed in a very much reduced levels indicating that cultured dental pulp cells had been already differentiated.

In the present study, Alizarin Red stain was used to assess the odontogenic differentiation ASCs and OMSCs as it is considered an early stage marker of matrix mineralization. These results were in accordance with Torkzaban P et al. [40] who induced odontogenic differentiation in DPSCs and SCAPs and observed that the cells migrate mainly from the periphery towards the center of the well, giving rise to rounded aggregates. These aggregates were the first to be mineralized, as shown by the calcium-specific Alizarin Red staining, but there were also multiple single mineralized nodules.

In the present study, to confirm odontogenesis, the odontoblast phenotype was also examined by RT-PCR for the expression of dentin sialophosphprotein (DSPP), since its expression marks the middle stage of odontoblastic differentiation. In our study, the results of RT-PCR further confirmed the findings established by Alizarin Red staining where DSPP gene was detected in ASCs and OMSCs on day 14 and day 21. These results were in accordance with Min et al. [27].

These results indicate that ASCs and OMSCs can efficiently differentiate into dentin forming cells expressing odontoblastic markers (DSPP), where OMSCs significantly expressed (DSPP) higher than ASCs. The results of the present study is in accordance with Martin et al. [26] and Shigeki et al. [35] who observed that the Alizarin Red staining showed a strong biomineralization in cell cultures after 28 days of differentiation. From the present study, it can be concluded that: ASCs possess a higher proliferative capacity than OMSCs as evidenced by MTT results, while OMSCs demonstrated a higher capability of differentiation as evidenced by DSPP expression.

References

1. Alfonso B. and Al-Rubeai M.: Flow cytometry. Compreh. Biotech. 2011; 1: 559-578.
2. Angelova-Volponi A, Kawasaki M, Sharpe PT.: Adult human gingival epithelial cells as a source for whole-tooth bioengineering. J Dent Res 2013; 92:329-334.
3. Bong A., Eun J., June H., Myeong G., Hyun J., Young S., Tae H., Mohana K., Sun A. and Gyu J.: In vitro and in vivo osteogenesis of human mesenchymal stem cells derived from skin, bone marrow and dental follicle tissues. Differentiation 2012; 83: 249-259.
4. Bright R, Hynes K, Gronthos S, Bartold PM: Periodontal ligament-derived cells for periodontal regeneration in animal models: a systematic review. J Periodontal Res, 2014.
5. Chen F., Sun H., Lu H. and Yu Q.: Stem cell-delivery therapeutics for periodontal tissue regeneration. Biomaterials 2012; 33: 6320-6344.
6. Davies O., Cooper P., Shelton R., Smith A. and Scheven A.: Comparison of the in vitro mineralization and dentinogenic potential of mesenchymal stem cells derived from adipose tissue, bone marrow and dental pulp. J. Bone Miner. Metab. 2014; 14: online springer.
7. Deepika N, Devadiga S, r Moidin T, Mittal S, Koshal N: Stem cells: A boon in dentistry, ournal of Dental Research and Review 2015; 2, 47-51.
8. Ekaterina I. and Vladimir P.: Photoacoustic flow cytometry. Methods 2012; 57: 280-296.
9. Ensanya A., Wojciech C., Vehid M., Hae W. and Jonathan C.: Tissue engineering in dentistry. J. Dent. 2014; 15: 915-928.
10. Fawzy K., Dorfer C., Fandrich F., Gieseler F., Moustafa M. and Ungefroren H.: Adult mesenchymal stem cells explored in the dental field. Adv. Biochem. Eng. Biotechnol. 2012; 10: 151- 166.
11. Fournier BP, Larjava H, Häkkinen L: Gingiva as a source of stem cells with therapeutic potential. Stem Cells Dev 2013; 22: 3157-3177.
12. Ge S., Mrozik K., Menicanin D., Gronthos S. and Bartold P.: Isolation and characterization of mesenchymal stem cell-like cells from healthy and inflamed gingival tissue: potential use for clinical therapy. Regen. Med. 2012; 7: 819-832.
13. Geetanjali B., Rupesh K., Navita G., Amruta P., Satish T., Hiral M., Gyan C. and Mohan R.: Human gingiva-derived mesenchymal stem cells are superior to bone marrow- derived mesenchymal stem cells for cell therapy in regenerative medicine. Biochem. Biophys. Res. Comm. 2010; 393: 377-383.
14. Gronthos S., Batouli, S. Miura, M. and Brahim, J.: Comparaison between stem cell based osteogenesis and dentinogenesis. J. Dent. Res. 2003; 82: 976-981.
15. Hakan O., Morikuni T. and Hiroshi M.: Mesenchymal stem cells isolated from adipose and other tissues: basic biological

properties and clinical applications. Stem Cells Int. 2012; 10: 1011- 1020.
16. Indumathi S., Rashmi M., Harikrishnan R., Rajkumar S., Venkata N. and Dhanasekaran M.: Human adult stem cells maintain a constant phenotype profile irrespective of their origin, basal media and long term cultures. Stem Cells Inter. 2015; 4: 1-29.
17. Jin SH, Lee JE, Yun JH, Kim I, Ko Y, et al.: Isolation and characterization of human mesenchymal stem cells from gingival connective tissue. J Periodontal Res, 2014; 17.
18. Jones KB, Klein OD: Oral epithelial stem cells in tissue maintenance and disease: the first steps in a long journey. Int J Oral Sci. 2013; 5: 121-129.
19. Kabir, R., Gupta, M., Aggarwal, A., Sharma, D., Sarin, A., and Kola, M. Z.: Imperative role of dental pulp stem cells in regenerative therapies: a systematic review. *Niger. J. Surg*. 2014; 20.
20. Karim M., Sebastian P., Stephan B., Neemat K., Hendrick U., Fred F., Jorg W. and Christ of D.: Isolation and characterization of multipotent postnatal stem/progenitor cells from human alveolar bone proper. J. Cran. Max. Fac. Surg. 2012; 40: 735-742.
21. Keishi Otsu K, Sakano M K, Fujiwara N, Kikuchi K, Keller L, Lesot H, and Harada H: Stem cell sources for tooth regeneration: current status and future prospects. Front Physiol. 2014; 5: 36.
22. Kerkis I. and Caplan A.: Stem cells in dental pulp of deciduous teeth. Tissue Eng. 2012; 18: 129-138.
23. Lee A., Jienny L., Chan L., Keum S., So H., Na Y., Jeong M., Byeong C., Ok J., Jae Y. and Sang H.: Comparative studies on proliferation, molecular markers and differentiation potential of mesenchymal stem cells from various tissues (adipose, bone marrow, ear skin, abdominal skin and lung) and maintenance of multipotency during serial passages in miniature pig. Res. Vet. Sci. 2015; 1: 1-10.
24. Marcella L., Francesca P., Anna S., Pasqualina N., Roberta M., Vincenzo D., Alfredo D., Gianpaolo P., Virginia T. and Luigi L.: Dental pulp stem cells: state of the art and suggestions for a true translation of research into therapy. J. Dent. 2014; 2: 761-768.
25. Marco T., Massimo M. and Francesco P.: The regenerative medicine in oral and maxillofacial surgery: the most important innovations in the clinical application of mesenchymal stem cells. Int. J. Med. Sci. 2015; 12: 72-77.
26. Martin G., Werner G., Oliver F., Tobias E., Andreas J. and Christian M.: Comparison of the differentiation potential of neural crest derived progenitor cells from apical papilla (dNC-PCs) and stem cells from exfoliated deciduous teeth (SHED) into mineralizing cells. Arch. Oral Biol. 2013; 58: 699-706.
27. Min S., Kyung G., Jaeseon L., Hyongbum K. and Seung H.: The effect of mineral trioxide aggregate on odontogenic differentiation in dental pulp stem cells. Basic Res. Biol. 2013; 39: 27-32.
28. Mizuno H., Tobita M. and Uysal C.: Concise review: adipose-derived stem cells as a novel tool for future regenerative medicine. Stem Cells 2012; 30: 804-810.
29. Mohamadreza B., Sima B. and Hamid N.: Odontogenic differentiation of dental pulp derived stem cells on tricalcium phosphate scaffolds. J. Dent. Sci. 2013; 8: 306-313.
30. Nguyen T., Mui B., Mehrabzadeh M., Chea Y., Chaudhry Z. and Chaudhry K.: Regeneration of tissues of the oral complex: current clinical trends and research advances. J. Canadian Dent. Assoc. 2013; 79.
31. Potdar, P. D., and Jethmalani, Y. D.: Human dental pulp stem cells: applications in future regenerative medicine. *World J. Stem Cells* 2015; 7:839.
32. Park Y J., Cha S., and Park Y S.: Regenerative Applications Using Tooth Derived Stem Cells in Other Than Tooth Regeneration: A Literature Review. Stem Cells International 2016; 2016.
33. Rouabhia M: Advantages and Limitations of Oral Stem Cell use for Oral Tissue Replacement. J Oral Bio. 2015; 2 (1): 9.
34. Seo M., Kyung-Gyun H., Hyongbum K. and Seung-Ho B.: Analysis of gene expression during odontogenic differentiation of

cultured human dental pulp cells. Restor. Dent. Endod. 2012; 37: 142-148.
35. Shigeki S., Naoto H., Fusanori N. and Ashok B.: Dentin sialophosphoprotein and dentin matrix protein-1: two highly phosphorylated proteins in mineralized tissues. Arch. Oral Biol. 2012; 57: 1165-1175.
36. Teti G, Salvatore V, Focaroli S, Durante S, Mazzotti A, Dicarlo M, Belmonte M and Orsini G: *In vitro* osteogenic and odontogenic differentiation of human dental pulp stem cells seeded on carboxymethyl cellulose-hydroxyapatite hybrid hydrogel. Front. Physiol. 2015; 27.
37. Stephens P. and Davies L.: Oral mucosal progenitor cells stem cell biology and tissue engineering in dental sciences. PART | IV Oral and Craniofacial Stem Cells for Tissue Engineering. 2015 Elsevier http://dx.doi.org/10.1016/B978-0-12-397157-9.00025-4.
38. Verma, K., Bains, R., Bains, V. K., Rawtiya, M., Loomba, K., and Srivastava, S. C.: Therapeutic potential of dental pulp stem cells in regenerative medicine: an overview. *Dent. Res. J.* 2014; 11:302–308,.
39. Tatullo M, Marrelli M, Shakesheff KM, White LJ: Dental pulp stem cells: function, isolation and applications in regenerative medicine. J Tissue Eng Regen Med 2014, [Epub ahead of print].
40. Torkzaban P., Saffarpour A., Bidgoli M. and Sohilifar S.: In vitro evaluation of isolation possibility of stem cells from intra oral soft tissue and comparison of them with bone marrow stem cells. J. Dent. 2012; 9: 143-156.
41. Vinicius R.: What and where are the stem cells for dentistry. Singapore Den. J. 2013; 34: 13-18.
42. Zuk P., Zhu M. and Mizuno H.: Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001; 7: 211-217.
43. Yu X, Ge S, Chen S, Xu Q, Zhang J, et al.: Human gingiva-derived mesenchymal stromal cells contribute to periodontal regeneration in beagle dogs. Cells Tissues Organs, 2013; 198: 428-437.
44. Zhang Q., Nguyen A., Yu W. and Le A.: Human oral mucosa and gingiva: a unique reservoir for mesenchymal stem cells. J. Dent. Res. 2012; 1: 91- 101.
45. Zhang Q., Shi S., Liu Y., Uyanne J., Shi Y. and Le A.: Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J. Immunol. 2009; 183: 7787-7798.