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ISOLATION, CHARACTERIZATION AND DIFFERENTIATION POTENTIAL OF ORAL MUCOSAL STEM CELLS

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Summary

Human oral mucosal stem cells (hOMSC) originate from the neural crest and possess multipotency, especially differentiation potential towards neuroectodermal lineage. They are easy to collect, as sampling does not result in irreversible destruction of oral tissues. Potential clinical use of hOMSC is also in the other medical fields; hOMSCs have been successfully transplanted to the cornea in patients with limbal defect, and similar cells can be differentiated into cardiomyocytes after myocardial infarction. hOMSC research in animal models of neurological diseases and injuries has shown their potential role in diseases which modern medicine has failed to treat. In this regard, we started the research of hOMSC and the possibility of their application in the model of ischemic brain stroke. After successfully isolating hOMSC and following their differentiation in the neuroectodermal and mesodermal directions, it was proved that it is possible to produce co-cultures of hOMSC mouse neural stem cells, which we see as an indicator that those cells will be able to coexist and communicate in the mouse model.

Keywords: human oral mucosal stem cells; mouse neural stem cells; co-cultures; neural differentiation

INTRODUCTION

The sources of dental- and oral tissue-derived stem cells (SC) may include pulp from deciduous teeth (Stem cells from Human Exfoliated Deciduous teeth (SHED)), permanent teeth indicated for extraction, e.g., third molars and supernumerary teeth (Dental Pulp Stem Cells (DPSC)), periodontal ligament cells, dental apical papilla and dental follicular cells (1). The most prominent differentiation potential of dental tissue derived stem cells is in their ability to form

dentin and periodontal tissues. DPSC, SHED and immature Dental Pulp Stem Cells (IDPC) are of ecto-mesenchymal origin. They can be easily and efficiently isolated and show high proliferation rate (2). They are multipotent and similar to mesenchymal bone marrow stem cells (3). It has been shown that DPSC may be differentiated in odontoblasts, chondrocytes, osteoblasts, adipocytes, neurons, smooth and skeletal muscle cells, endothelial cells and melanocytes. In vivo conditions, after implantation, they exhibit different potential for dentin formation, but also for bone, fat and nervous tissue (4). Not so well known oral source of SC is oral mucosa.

There are many important indications for possible application of stem cells in dentistry, such as bone defect regeneration, tooth regeneration with caries remineralization, tooth replantation and transplantation, pulp and dentin regeneration and formation, periodontal reconstruction, continuation of root formation, and finally, development of completely new tooth (5).

However, beside their dental applications, or their regional use in transplantation in maxillofacial surgery, oral stem cells can be useful for development or regeneration of other tissues and systems. They might also be used for the treatment of various systemic diseases, such as diabetes, muscular dystrophy, neurodegenerative diseases, myocardial infarction, arthritis, etc (6).

HUMAN ORAL MUCOSAL STEM CELLS

It is generally considered that dental SC are easily obtained, and thus dental tissues would provide convenient source for SC. However, harvesting SC from those sources represent irreparable and irreversible damage to those teeth. Using them as a source for SC are only appropriate if those teeth are scheduled for extraction for other reasons. Thus, in search for the oral SC source, we should be looking for tissue that harbors SC and that will not be irreparably damaged by process of obtaining SC. As mentioned, very important SC oral source is oral mucosa. Very little is known about human oral mucosal stem cells (hOMSC). These mesenchymal stem cells originate from the neural crest and therefore possess exceptional multipotency. hOMSC have shown that they can differentiate towards ectodermal, mesodermal and endodermal cell lineages (7). Their advantage is in that they are easy to collect, as sampling does not result in irreversible destruction of oral tissues. This is not the case with pulp or apical papilla stem cells, as tissue harvesting procedure inevitably necessitates irreparable damage.

USE OF ORAL STEM CELLS IN EXTRAORAL APPLICATIONS

Potential clinical use of hOMSC can be foreseen in disciplines beyond regenerative dentistry. Potential applications of oral stem cells are switching this field from regenerative dentistry to regenerative medicine (8). For example, in the field of ophthalmology, hOMSC have been successfully transplanted to the cornea in patients with limbal defect (9). Similar cells have shown that they can be differentiated into cardiomyocytes and repopulate the heart of mammals after myocardial infarction (10). Due to their neural crest origin, and their particular differentiation potential towards neuroectodermal lineage, perhaps the most important role for hOMSC would be in neural tissue regeneration. hOMSC research in animal models of neurological diseases and injuries has shown their significant potential role in diseases which modern medicine has failed to treat. There is published research on the regenerative ability of the same or similar tissues in different models of nervous system disorders. In the most recent research on parkinsonian models, SC originating from the neural crest have proven as interesting medium for regenerative neuroscience.

One group from Germany has demonstrated the effective use of nasal mucosa SC, which, like the oral cavity mucosa, also originates from the neural crest. The oral cavity as a source of SC is much more convenient and less painful to take biopsy than the nasal mucosa (11). The group from Israel has shown that hOMSC can differentiate in vitro into dopamine-producing cells and possess neuronal-dopaminergic phenotype. In the hemi-parkinsonian rat model, those cells have improved their performance deficit after transplantation in striatum (12). The same group showed that hOMSC could be differentiated into astrocytes-like cells, which have a neuroprotective effect in the experimental rat model of peripheral nerve (n. sciaticus) injury, thus improving the motor function after transplantation (13). The Korean group investigated the effect of hOMSC in spinal cord injury rat model. Spinal cord injury have induced tissue apoptosis, while hOMSC suppressed this injury-induced apoptosis. The discontinued spinal cord got improved after hOMSC transplantation and new neural tissue was formed around damaged area (14).

NEURAL DAMAGE MODEL FOR HOMSC RESEARCH

Mouse neural stem cells (mNSC) are multipotent cell population. They can be differentiated in astrocytes and neurons. In the rat model of amyotrophic lateral sclerosis, intravenously administered NSC have successfully migrated and differentiated (15). Experiments in a murine ischemic stroke model showed that mNSC, when transplanted in the brain, successfully differentiated into mature neurons (16,17). Thus, used model of murine ischemic stroke is possibly a good platform for investigating the neural regenerative potential of hOMSC.

In the process of planning the experiment, we wished to see what would be like to have hOMSC and mNSC cultivated together, in order to initially check if those cells can co-exist. The fact that they originate from different species would not necessarily be an obstacle to their co-existence, as SC exhibit immunomodulatory effect.

EXPERIMENTAL

Our aim was to see if hOMSC and mNSC can be co-cultured, so that we can anticipate that our planned in vivo model would be feasible. Having followed all ethical requirements, we have collected buccal mucosa biopsies from 6 healthy volunteers, mostly investigators themselves (18). After mincing the specimen and after enzymatic treatment and centrifuging, hOMSC were cultivated in low glucose DMEM medium with 10% fetal calf serum (FCS). Buccal biopsy procedure using 4 mm punch and tissue handling are shown in *figure 1*. Following 2-3 proliferation passages, hOMSC were plated on coverslips coated with fibronectin. They subsequently differentiated towards mesodermal and ectodermal cell populations. For the differentiation towards mesodermal lineage, we cultured cells in αMEM medium, supplemented with 12% FCS, 50 μg/ml vitamin C, 10^{-7} M dexamethasone and 10mM glycerol-2-phosphate. For ectodermal differentiation, we used DMEM medium supplemented with 10 ng/ml β-NGF, 50 ng/ml BDNF, N2-Supplement and antibiotics/antimicotics. Cultured cells were fixed on days 1, 3, 5, 7 and 14 of differentiation and immunocytochemistry was performed.

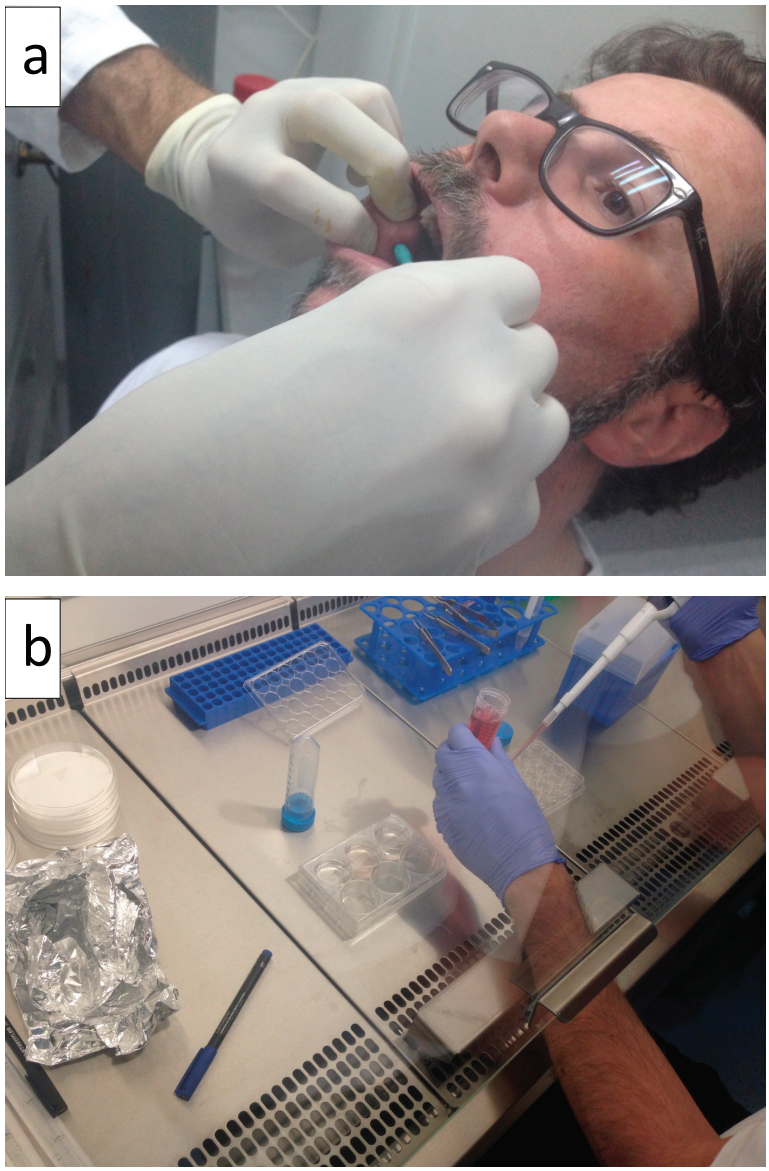

Figure 1. Taking a "punch" biopsy for isolation of hOMSC (a), and processing of tissue samples (b).

For obtaining mNSC we have used a GFP mouse strain, kept on an albino C57Bl6 background. In order to assess the best possible conditions for hOMSC and mNSC co-cultures, we have formed 4 groups of in vitro experiments: a)

hOMSC in human DMEM medium with factors for ectodermal differentiation, b) hOMSC and mNSC in the same human medium, c) hOMSC and mNSC in mouse DMEM / F12 medium with factors for ectodermal differentiation and d) mNSC in the same mouse medium (*Figure 2*). Whist experiments b) and c) were of our primary interest, we also performed a) and d) experiments to assess the proliferation and differentiation patterns of respective monocultures, which served as controls.

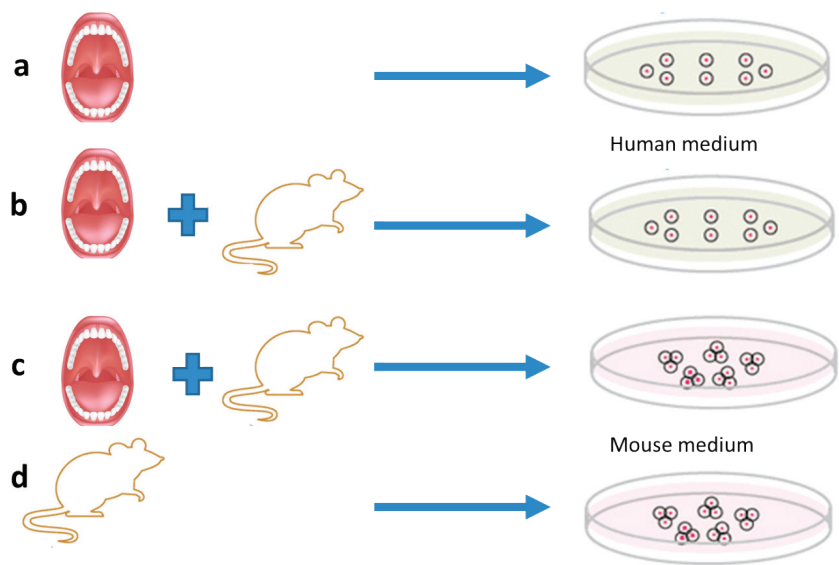

Figure 2. Schematic diagram explaining experimental groups of variously cultured cells :a) hOMSC in human medium; b) hOMSC and mNSC co-cultured in human medium; c) hOMSC and mNSC co-cultured in mouse medium and d) mNSC in mouse medium.

After isolation, we used cell markers in different time points (multipotency – Sox2, Mash1, Oct4, Nestin, ectodermal fate – Map2, β3-tubulin, NeuN and mesodermal – Osterix, Collagen 1 and CD 44). *Figure 3a* shows Oct 4 (green, fully pluripotent primordial cells) and Mash1 (red, expressed in proliferating neural precursors). *Figure 3b* shows Oct4 (green) + Sox2 (staining perinuclear spaces red and reveals early neural differentiation). *Figure 3c* shows Sox 2 (staining nuclei red) and Nestin (staining perinuclear spaces (granulated) green, which marks fully undifferentiated cells) expression in hOMSC. *Figure 3d* shows positive staining for MAP2 (staining cytoplasmic microtubules red, indicating formation of

differentiated neuron), and has lost positivity for Nestin (green), which signifies that differentiation towards neuron has taken place over time. A Chronology of expression was (in following order): Oct4, Mash1, Nestin, Sox 2 and MAP2. Some of the cells were still pluripotent (Oct4), Many retained features of SC (Nestin), we observed early neuronal differentiation (Sox2), and some have obtained features of mature neurons (MAP2), (ref ODI).

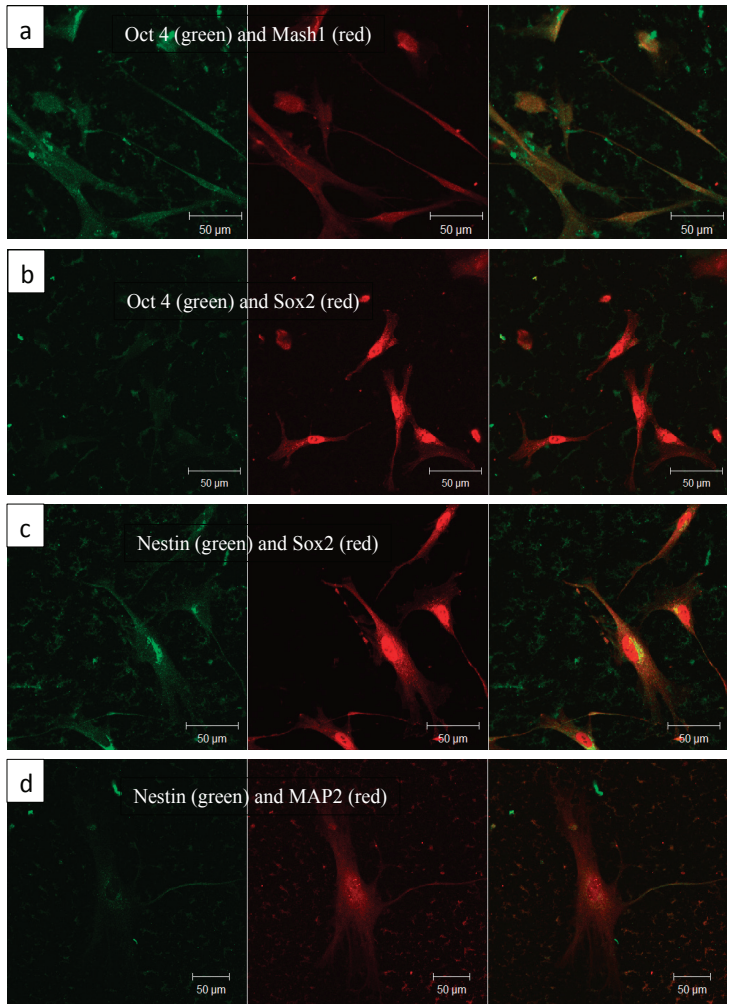

Figure 3. Immunocytochemistry staining of hOMSC characterizing different stages of differentiation. a) shows Oct 4 (green) and Mash1 (red); b) shows very weak of Oct4 signal (green) and Sox2 (red); c) shows Sox 2 (red) and Nestin (green); d) shows MAP2 (red) and virtual absence of Nestin (green).

hOMSC cultivated in medium supporting mesodermal differentiation were

expressing Osterix in 90%, which suggest that they can be differentiated into osteoblasts.. Co-cultures of hOMSC and mNSC, although able to grow in co-cultures, showed to behave differently depending upon the medium used. In co-cultures with human medium, hOMSC differentiated in the same manner as mentioned before. in those co-cultures over 90% of mNSC expressed GFAP, which labels astrocytes, thus showing that human medium isn't applicable for co-cultures as it will not allow differentiation of mNSC into neurons. When mouse medium was used, mNSC differentaiated well, and hOMSC were unaffected, but proliferated at much slower rate than mNSC. Thus hOMSC were overgrown by proliferating mNSC differentiating into neurons (18).

CONCLUSION

Apart from possibilities of their application for reconstruction of defects in oral and maxillofacial region, hOMSC can be used beyond that area, namely for neurological conditions. We have found robust results proving that transplantation of hOMSC into mouse brain stroke model can be performed successfully. This brings a new scientific direction for our research.

References

[1] Kang HK, Roh S, Lee G, Hong SD, Kang H, Min BM. Osteogenic potential of embryonic stem cells in tooth sockets. Int J Mol Med. 2008;21:539-44.

[2] d'Aquino R, Papaccio G, Laino G, Graziano A. Dental pulp stem cells: a promising tool for bone regeneration. Stem Cell Rev. 2008;4:21-6.

[3] Maria OM, Khosravi R, Mezey E, Tran SD. Cells from bone marrow that evolve into oral tissues and their clinical applications. Oral Dis. 2007;13:11-16.

[4] Perry BC, Zhou D, Wu X, et al. Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. Tissue Eng Part C Methods. 2008;14:149-56.

[5] Morsczeck C, Schmalz G, Reichert TE, Volner F, Galler K, Driemel O. Somatic stem cells for regenerative dentisty. Clin Oral Invest. 2008;12:113-8.

[6] Ishkitiev N, Calenic B, Aoyama I, Ii H, Yaegaki K, Imai T. Hydrogen sulfide increases hepatic differentiation in tooth-pulp stem cells. J Breath Res. 2012;6:e017103.

[7] Marynka-Kalmani K, Treves S, Yafee M et al. The lamina propria of adult human oral mucosa harbors a novel stem cell population. Stem Cells. 2010;28:984-95.

[8] Xiao L, Nasu M. From regenerative dentistry to regenerative medicine: progress, challenges, and potential applications of oral stem cells. Stem Cells Cloning. 2014 Dec 4;7:89-99.

[9] Chen HC, Chen HL, Lai JY, Chen CC, Tsai YJ, Kuo MT, et al. Persistence of transplanted oral mucosal epithelial cells in human cornea. Invest Ophthalmol Vis Sci. 2009 Oct;50(10):4660-8.

[10] Gafni Y, Rachima H, Marynka-Kalmani K, Blatt A, Vered Z, Pitaru S. A new in vivo/in vitro model for assessing the capacity of human derived oral mucosa stem cells to colonize the infarcted myocardium. Stem Cell Studies. 2011; 1:e6:42-7.

[11] Müller J, Ossig C, Greiner JF, Hauser S, Fauser M, Widera D, et al. Intrastriatal transplantation of adult human neural crest-derived stem cells improves functional outcome in parkinsonian rats. Stem Cells Transl Med. 2015;4(1):31-43.

[12] Ganz J, Arie I, Buch S, Zur TB, Barhum Y, Pour S, et al. Dopaminergic-like neurons derived from oral mucosa stem cells by developmental cues improve symptoms in the hemi-parkinsonian rat model. PLoS One. 2014 Jun 19;9(6):e100445.

[13] Ganz J, Arie I, Ben-Zur T, Dadon-Nachum M, Pour S, Araidy S, et al. Astrocyte-like cells derived from human oral mucosa stem cells provide neuroprotection in vitro and in vivo. Stem Cells Transl Med. 2014 Mar;3(3):375-86.

[14] Cho YS, Ko IG, Kim SE, Lee SM, Shin MS, Kim CJ, et al. Oral mucosa stem cells alleviates spinal cord injury-induced neurogenic bladder symptoms in rats. J Biomed Sci. 2014 May 13;21:43. doi: 10.1186/1423-0127-21-43.

[15] Mitrecić D, Nicaise C, Gajović S, Pochet R. Distribution, differentiation, and survival of intravenously administered neural stem cells in a rat model of amyotrophic lateral sclerosis. *Cell Transplant. 2010;*19: 537-48.

[16] Mitrečić D, Alić I, Gorup D. Stem cells and stroke-how glowing neurons illuminate new paths. *Neurogenesis.* 2017;4:e1304847. doi:10.1080/23262133.2017.1304847

[17] Alić I, Kosi N, Kapuralin K, et al. Neural stem cells from mouse strain Thy1 YFP-16 are a valuable tool to monitor and evaluate neuronal differentiation and morphology. *Neurosci Lett.* 2016; 634: 32-41.

[18] Alajbeg I, Alić I, Andabak-Rogulj A, Brailo V, Mitrečić D. Human- and mouse-derived neurons can be simultaneously obtained by co-cultures of human oral mucosal stem cells and mouse neural stem cells. Oral Dis. 2018;24(1-2):5-10.

Saţetak

Izolacija, karakterizacija i diferencijacijski potencijal matičnih stanica oralne sluznice

Ljudske matične stanice iz oralne sluznice (hOMSC) potječu od neuralnog grebena, posjeduju multipotentnost, naročito potencijal diferencijacije prema neuroektodermalnoj liniji. Jednostavno ih je prikupiti, jer uzorkovanje ne rezultira nepovratnim uništenjem oralnih tkiva. Potencijalna klinička upotreba hOMSC također se može naći u drugim medicinskim područjima; hOMSC se uspješno transplantira u rožnicu pacijenata s limbalnim defektom, a slične stanice mogu se diferencirati u kardiomiocite nakon infarkta miokarda. Istraživanje hOMSC-a na životinjskim modelima neuroloških bolesti i ozljeda pokazalo je njihovu moguću ulogu u bolestima koje suvremena medicina ne može adekvatno liječiti. U tom smislu, započeli smo istraživanje hOMSC-a i mogućnosti njihove primjene u modelu ishemijskog moždanog udara. Nakon uspješnog izoliranja hOMSC-a i praćenja njihove diferencijacije u neuroektodermalnim i mezodermalnim pravcima, dokazano je da je moguće proizvesti ko-kulture hOMSC mišjih neuralnih matičnih stanica, što vidimo kao pokazatelj da će te stanice moći koegzistirati i komunicirati u mišjem modelu.

Ključne riječi: ljudske matične stanice oralne sluznice; mišje neuralne matične stanice; ko-kulture; neuralna diferencijacija.

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