Archives of Oral Biology 68 (2016) 131–141

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Attachment and growth of dental pulp stem cells on dentin in presence of extra calcium

F. Özdal-Kurt[a,*], B.H. Şen[b], I. Tuğlu[c], S. Vatansever[c,e], B.T. Türk[b], I. Deliloğlu-Gürhan[d]

[a] Celal Bayar University, Faculty of Life Science, Department of Biology, 45140, Manisa, Turkey
[b] Ege University, School of Dentistry, Endodontology, Izmir, 35100, Turkey
[c] Celal Bayar University, Faculty of Medicine, Department of Histology and Embryology, 45020, Manisa, Turkey
[d] Ege University, Faculty of Engineering, Department of Bioengineering, 35100, Izmir, Turkey
[e] Near East University, Experimental Health Science Research Center, Lefkosa, North Cyprus

ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form 29 November 2015
Accepted 26 April 2016

Keywords:
Stem cell
Dentin
Surface
Odontoblast-like cells
Scanning electron microscopy

ABSTRACT

Objective: We aimed to differentiate dental pulp stem cells (DPSC) to odontoblast-like cells (ODPSC) and to investigate their attachment and growth on dentin in the presence of extra calcium by colorimetric assay and scanning electron microscopy (SEM).
Methods: After isolation of DPSC, they were differentiated to ODPSC. Standard dentin discs from human molar teeth were prepared. While the dentin discs in Group 1 did not receive any extra treatment, the discs in Group 2 were treated with acidic calcium phosphate precipitation (CPP) solution. In Group 3, the discs were suspended in phosphate buffered saline containing calcium. DPSC or ODPSC (3×10^4 cells/mL) were seeded on all discs and incubated for 7, 14 or 21 days. Attachment and growth of 7-day cell cultures on extra dentin samples were examined by SEM. MTT assay showed that number of cells on dentin surfaces was increased by time periods regardless of type of treatment and cells ($p < 0.05$).
Results: While DPSC and ODPSC showed similar proliferation rates at 7 and 14 days ($p > 0.05$), the number of ODPSC was higher than DPSC in 21-day samples ($p = 0.039$). MTT assay showed that number of cells on dentin surfaces was increased by time periods regardless of type of treatment and cells ($p < 0.05$). Calcium-treated dentin surfaces always had lower number of cells; being significant for only CPP-treated surfaces ($p < 0.01$). Both types of cells demonstrated good attachment and proliferation on dentin surfaces regardless of type of dentin treatment.
Conclusions: Because the nature of dentin surface itself showed good adhesive characteristics with ODPSC and DPSC, additional calcium treatment of dentin surfaces may not be necessary.

1. Introduction

Pulpal disease is one of the most prevalent illnesses that millions of people are suffering from all around the world (Baelum, van Palenstein Helderman, Hugoson, Yee, & Fejerskov, 2007; Wang et al., 2010a). The current therapy is to remove the pulpal tissue and replace with synthetic materials such as resin and gutta-percha. However, these materials are not capable of replacing biological functions of lost tissue, leading to the reduced mechanical properties and reduced vitality of the teeth. It has been shown that cells from injured pulp cells had lower proliferative capacity, but more capacity of calcium deposition. These injured cells expressed stronger osteogenic markers like bone morphogenic protein (BMP-7) and less odontogenic proteins Dentin sialoprotein (DSP). In addition, they used NF-kB pathway where the cells demonstrated significantly more odontogenic differentiation. Moreover, they upraised DSSP expression. It was also shown that EphB-EphrinB interactions controlled odontogenic differentiation through calcium hydroxide. These receptors were localized at the areas where the DSP was stained (Nakashima, Iohara, & Sugiyama, 2009; Lovelace, Henry, Hargreaves, & Diogenes, 2011; Wang et al., 2010a, 2013).

As the dental pulp has a critical role in the homeostasis of teeth and essential for longevity and quality of teeth life, stem cell-based therapies would be an improving approach to repair or replace the damaged or lost tissues of teeth in order to recover the morphological and biological functions. There is many source to obtain stem cells from dental tissues such as DPSCs: dental pulp stem cells; SHED: stem cells from human exfoliated deciduous teeth; PDLSCs: periodontal ligament stem cells; DFSCs: dental

* Corresponding author at: Assoc. Prof. Dr. Feyzan Özdal-Kurt, Celal Bayar University, Faculty of Life Science, Department of Biology, 45140, Manisa, Turkey.
E-mail address: feyzanozdalkurt@yahoo.com (F. Özdal-Kurt).

http://dx.doi.org/10.1016/j.archoralbio.2016.04.008
0003-9969/

follicle stem cells; TGPCs: tooth germ progenitor cells; SCAP: stem cells from the apical papilla, OESCs: oral epithelial progenitor/stem cells; GMSCs: gingiva-derived MSCs, PSCs: periosteum-derived stem cells, SGSCs: salivary gland-derived stem cells (Egusa, Sonoyama, Nishimura, Atsuta, & Akiyama, 2012). Following the discovery of adult stem cells in teeth, it has been generally accepted that DPSC are more ethically agreeable cells and they are easily acquired in comparison to the other mesenchymal stem cells derived from bone marrow, adipose tissue, peripheral blood and umbilical cord blood (Friedlander, Cullinan, & Love, 2009; Jiang, Peng, Li, Yang, & Zhu, 2012; Pierdomenico et al., 2005).

DPSC can differentiate into various cell types like odontoblasts, osteoblasts, adipocytes, muscle cells, endothelial cells, neural cells (Huang, Snyder, Cheng, & Chan, 2008; Yang, Zhang, Pang, & Fan, 2012; Zhang, Walboomers, Shi, Fan, & Jansen, 2006) by signalling with different mediums that contains different cocktails (Kadar et al., 2009; Karaöz et al., 2010). In addition to morphogens including growth and differentiation factors, specific scaffolds are needed for dental pulp tissue engineering (Nakashima, 2005; Sloan & Smith, 2007). Scaffold plays a critical role in tooth tissue engineering. From an endodontic point of view, it should be possibly similar to natural dentin for pulp derivated stem cells (Ma, 2008; Lovelace et al., 2011). In order to achieve regenerative endodontic therapy, it is necessary to stimulate growth of DPSC and their attachment to root canal dentin. It has been already established that natural components of dentin may be inductive for stem cells (Casagrande et al., 2010; Demarco et al., 2010; Liu et al., 2004). The factors in the environment and different treatment modalities of root canal dentin may amplify or decrease the attachment of DPSC. For example, it has been recently reported that presence of smear layer on dentin surface and types of irrigants affect the attachment of DPSC to the root canal dentin (Ahangari et al., 2012; Ring, Murray, Namerow, Kuttler, & Garcia-Godoy, 2008).

Calcium plays an important role in hard tissue metabolism (Ma et al., 2005; Kim, Camata, & Lee, 2006; Zayzafoon, 2006) and it is one of the important elements of dentin. It is known to influence cell adhesion (Hirano, Nose, Hatta, Kawakami, & Takeichi, 1987) as well as various other aspects of cell physiology (Huang & Miller, 2007; Hidalgo & Donoso, 2008). Effects of calcium have been investigated on osteoblast cells and reported that signalling by calcium enhances the osteoblast differentiation and proliferation (Jung, Park, & Han, 2010; Ma et al., 2005). Related with mesenchymal stem cells, calcium-coated surfaces improve human mesenchymal stem cell attachment (Kim et al., 2006). However, to our knowledge, no information is available regarding the effects of extra calcium treatment of dentin on growth and attachment of DPSC and odontoblast-like stem cells (ODPSC).

Therefore, the first aim of this study was to differentiate DPSC to ODPSC and to examine this differentiation by histochemical staining and immunohistochemistry. Then, the second aim was to investigate the attachment and growth of both cell types on dentin in presence of extra calcium by colorimetric assay and scanning electron microscopy (SEM).

2. Materials and methods

All procedures were approved by the Ethics Committee on Human Research, Celal Bayar University, Manisa, Turkey (Approval #028).

2.1. Stem cell isolation

DPSC were isolated from healthy adult human teeth. After extraction, teeth surfaces were cleaned and the crown was separated from the cervical region with sterilized diamond burs under water cooling under sterile conditions. The pulp tissue was gently separated by forceps and digested in a solution of 3 mg/ml collagenase type I (Sigma, St. Louis, MO) for 60 min at 37 °C. Cell suspensions were obtained by passing the digested tissues through a 70-μm cell strainer (Becton/Dickinson, Franklin Lakes, NJ). Single cell suspensions were seeded in 60- or 100-mm culture dishes with control medium (CM) containing;α-Minimum Essential Medium (α-MEM; Life Technologies/GIBCO BRL, Gaithersburg, MD) supplemented with 20% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml penicillin-G, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone (Gemini Bio-Products, Inc., Woodland, CA) and maintained in 5% CO2 at 37 °C. Colonies were allowed to form and cells were passed (1:3 ratio) at 80% confluence. Then, cells were continuously passed at 1:3 ratio when confluent. These pulp cells were designated as DPSC. The above cells were continuously passed or harvested and kept frozen in liquid nitrogen until experimentation.

2.2. Cell viability and proliferation tests

MTT assay (reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a purple formazan product) was used to estimate cell viability and proliferation. Cells were incubated with 0.5 mg/ml of MTT in the last 4 h of the culture period; the medium was then decanted. Formazan salts were dissolved with dimethylsulphoxide (DMSO) and the absorbance was determined at 570 nm in an UV–vis spectrophotometer multiplate reader (VersaMax, Molecular Device, USA). Cell numbers were measured by MTT for three times every other day during culture life.

2.3. Cell cultivation and differentiation

Cells were cultivated in twenty four-well plates (Greiner Bio-one, The Netherlands) and incubated in a humidified atmosphere of 95% air, 5% CO_2 at 37 °C (Hera Cell, Kendro Laboratory, Germany). After 3 days, the medium containing floating cells that composed of mainly blood cells and fat cells was replaced with CM for DPSC or fresh odonto-inductive medium (OM) for odontogenic differentiation. OM included CM plus ascorbic acid. The medium was changed every other day. Cells were microscopically observed under inverted microscope (IX71, Olympus, Tokyo, Japan) with a phase-contrast attachment and photomicrographs were obtained. The characterization of DPSC and ODPSC were done with immunohistochemically for DSP. For SEM examination and MTT assay, the cells were collected from the confluent cultures in which differentiation was completed.

2.4. Alkaline phosphatase (ALP) and Von Kossa (VK) histochemical staining

For ALP/VK histochemistry, the medium of confluent culture was removed and the DPSC or ODPSC layers were rinsed with PBS 3 times and fixed in cold 10% neutral buffer formalin (NFB) for 1 h at 4 °C. Then, the fixed cells were washed with deionized water and incubated with buffer containing 0.1 mg/ml naphthol AS-MX phosphate disodium salt and 0.6 mg/ml Fast Red TR salt. After 1 h at 37 °C, the cell layers were again washed with deionized water and observed with both naked eye and the inverted phase-contrast microscope. Calcium phosphate deposit visualization of the extracellular matrix (ECM) was detected by the VK method in which calcium phosphate deposits were stained brown to black.

2.5. Immunohistochemistry

DPSC and ODPSC of confluent cell culture were also assessed immunohistochemically using antibodies against osteocalcin (OC,