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Isolation and Characterization of Dental Pulp Stem Cells from Primary and Permanent Teeth

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Abstract: Dental pulp stem cells (DPSCs) share similar gene expression profiles and differentiation capabilities to that of bone marrow derived stem cells (BMSCs). DPSCs are potentially superior to other types of adult stem cell, as teeth are easy to access and are extracted routinely throughout life. The permanent (4 premolars and 2 third molars) and deciduous (4 first molars) teeth were removed using local anesthesia. The dental pulps were exposed using a sterile hand-held low-speed disc and the teeth were split using a chisel along the groove. The pulps of the teeth were then extracted with endodontic files or pansies and digested in collagenase type I and dispase. The resultant cell suspensions from each tooth were then centrifuged, cultured and evaluated by flow cytometry. The DPSCs from both permanent and deciduous teeth proliferated rapidly whilst attaching to the floor of the flask with a spindle-shaped morphology. More than 80% of the cell population exhibited the expression of surface antigens known to be found on mesenchymal lineages such as CD105, CD90, CD166, and CD73, whilst only less than 4% expressed endothelial-hematopoietic epitopes including, CD11b, CD34, CD133, CD64, CD106, CD31 and CD45. DPSCs and BMSSCs, which can differentiate into multiple mesenchymal cell lineages, are putative candidate cells for tooth and bone-tissue engineering, respectively. Considering the results, dental pulp stem cells can be thought of as being part of the MSC population and may be considered suitable for use in regenerative medicine.
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Keywords: dental pulp stem cell; primary teeth; permanent teeth

1. Introduction

Mesenchymal stem cells (MSCs) are a promising tool for cell based treatment of a variety of tissue defects due to their capacity of extensive replication and potential for multilineage differentiation. These cells were first isolated and described from bone marrow as a colonogenic fibroblastic cell population capable of producing deposits of bone and cartilage-like tissues in culture (Friedenstein *et al*., 1966). Due to the disadvantages associated with marrow MSCs including the burden of marrow harvest, the limited number of MSCs in marrow tissue and the in vitro ageing problems of the cells, many attempts have been made in the search for other potential sources of MSCs (Liu *et al.,* 2009; Baxter *et al*., 2004). Dental pulp stem cells (DPSCs) share similar gene expression profiles and differentiation capabilities to that of bone marrow derived stem cells (BMSCs) (Gronthos *et al.,* 2000; Gronthos *et al*., 2002). DPSCs are potentially superior to other types of adult stem cell as teeth are easy to access and are extracted routinely throughout life (Huang *et al*., 2008).

DPSCs are a relatively convenient stem cell resource, resulting from pulp exposure and endodontic treatment. Not only can they be obtained from primary incisors and permanent third molar teeth, in accordance with existing literature, but can also are sourced from supernumerary teeth (Huang *et al.,* 2009) and crown fractured teeth without the need for extraction.

DPSCs are potentially more accessible and may be easier to obtain compared with adult bone marrow stem cells after pulp exposure and endodontic treatment. Many reports have recently discussed pulp tissue engineering with the stem cells of human dental pulp (Cordeiro *et al*., 2008; Wei *et al.,* 2007). Therefore, banked DPSCs may provide a good prospective in future regenerative dental and medical treatment. This may involve the controlled direct employment of the cells in situ and the possible seeding of stem cells at areas of injury for regeneration or use with appropriate scaffolds for tissue engineering solutions (Cordeiro *et al.,* 2008; Wei *et al*., 2007; Huang *et al*., 2006).

2. Material and Methods

All participants have given an informed written consent and the study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (TUMS) which was in compliance with Helsinki declaration. The permanent (4 premolars and 2 third molars) and deciduous (4 first molars) teeth were removed using local anesthesia. For the isolation of the DPSCs, teeth were rinsed for a minute in a washing solution containing Dulbecco's phosphate buffered saline (DPBS; Invitrogen, Carlsbad, CA, USA) and 3X antibiotic (X= 100 units⁄ml penicillin (Sigma), and 100 µg ⁄ ml streptomycin (Sigma)) and delivered to the laboratory in transfer solution containing DMEM and 1X antibiotic (pen str) and 1X amphotricin B (Gibco 250µg/ml). The surfaces of both teeth were first cleaned with DPBS and a 0.5–1.0 mm deep groove was cut around the teeth using a sterile hand-held low-speed disc. The dental pulps were exposed by splitting the teeth with a chisel along the groove. The pulps of the teeth were then extracted with endodontic files or pansies. The extracted pulp tissues were then divided to small pieces using surgical scissors and subsequently digested in collagenase type I (3 mg⁄ml, Invitrogen) and dispase (4 mg⁄ml, Invitrogen) for 40 min at 37_C. The resultant cell suspensions from each tooth were then centrifuged by (sigma 2-16PK) in 1200 speed for 5 mins. The single cell suspensions were then cultured in a DPSC medium containing D-modified Eagle's medium (GIBCO 1g/l glucose, pyruvate +), 10% fetal bovine Serum (Hyclone), 100 units⁄ml penicillin (Sigma), and 100 µg ⁄ ml streptomycin (Invitrogen). DPSCs were cultured at 37_C with 5% CO2. Fresh medium was replaced every 2 days and at 100% confluence for optimal cell harvesting. DPSCs of each tooth were divided into 6 fluorescence-activated cell sorting round-bottom tubes (Becton Dickinson Falcon, Sunnyvale, CA) at 2 _ 10^5cells/tube and stained with immunoglobulin G–fluorescein isothiocyanate –-conjugated or phycoerythrin-conjugated anti- CD105, CD90, CD166, CD73, CD11b, CD34, CD133, CD64, CD106, CD31 and CD45 (Beckman Coulter, Villepinte, France, 20 mL each). After a 20-minute incubation interval at ambient temperature in the dark, cells were washed twice with 2 mL of fluorescence-activated cell sorting wash solution of phosphate-buffered saline containing 0.1% phosphate buffered solution (PBS) FBS and 0.1% NaN3 and centrifuged for 5 minutes at 230g. Supernatant was removed, and cells were fixed with 1% formaldehyde (in phosphate-buffered saline). Respective immunoglobulin G isotype-matched controls (Beckman Coulter) were used as negative controls. All of the resultant data wasacquired using a Coulter Epics XL (Beckman Coulter) and analyzed using EXPO32 ADC software (Beckman Coulter) and WinMDI version 2.8 (Windows Multiple Document Interafce for Flow Cytometry; freeware developed by Joe Trotter, downloadable at methods.info/software/ flow/winmdi.htm

3. Results

The DPSCs from both permanent and deciduous teeth proliferated rapidly whilst attaching to the floor of the flask with a spindle-shaped morphology (fig.1). After the third passage, homogenous populations of DPSCs from the permanent and deciduous teeth were obtained and maintained in culture based on the cell morphology. More than 80% of the cell population exhibited the expression of surface antigens known to be found on mesenchymal lineages such as CD105, CD90, CD166, and CD73, whilst only less than 4% expressed endothelial-hematopoietic epitopes including, CD11b, CD34, CD133, CD64, CD106, CD31 and CD45 (fig.2). The cells exhibited a relatively high proliferation capacity with a population doubling time of approximately 16 hours.

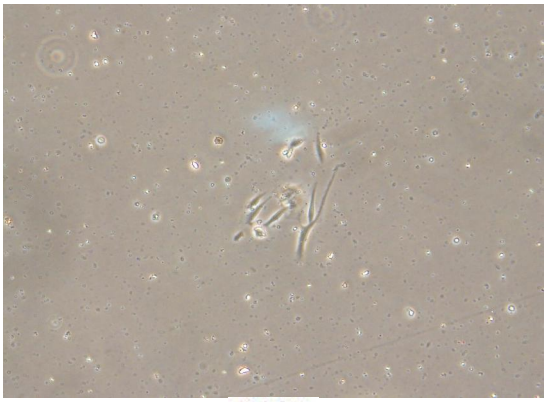

Fig1 A

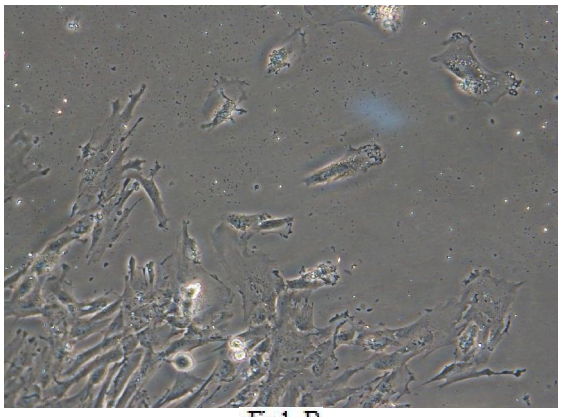

Fig1 B

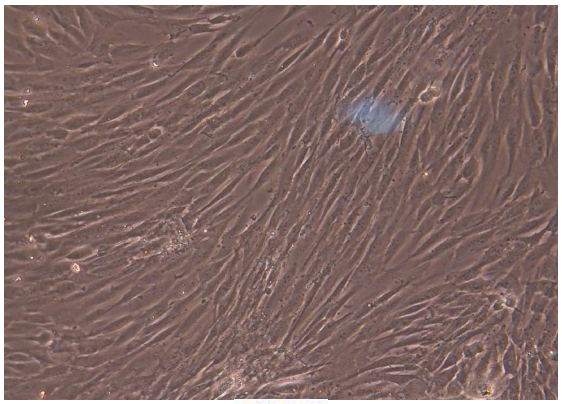

Fig1 C

Figure 1. Cultured human pulp cells (original magnification x10): (A) Cells on day 1; cells have attached to the culture dish. (B) Cells on day 5; showing confluence of cells (C) Cells on day 14.

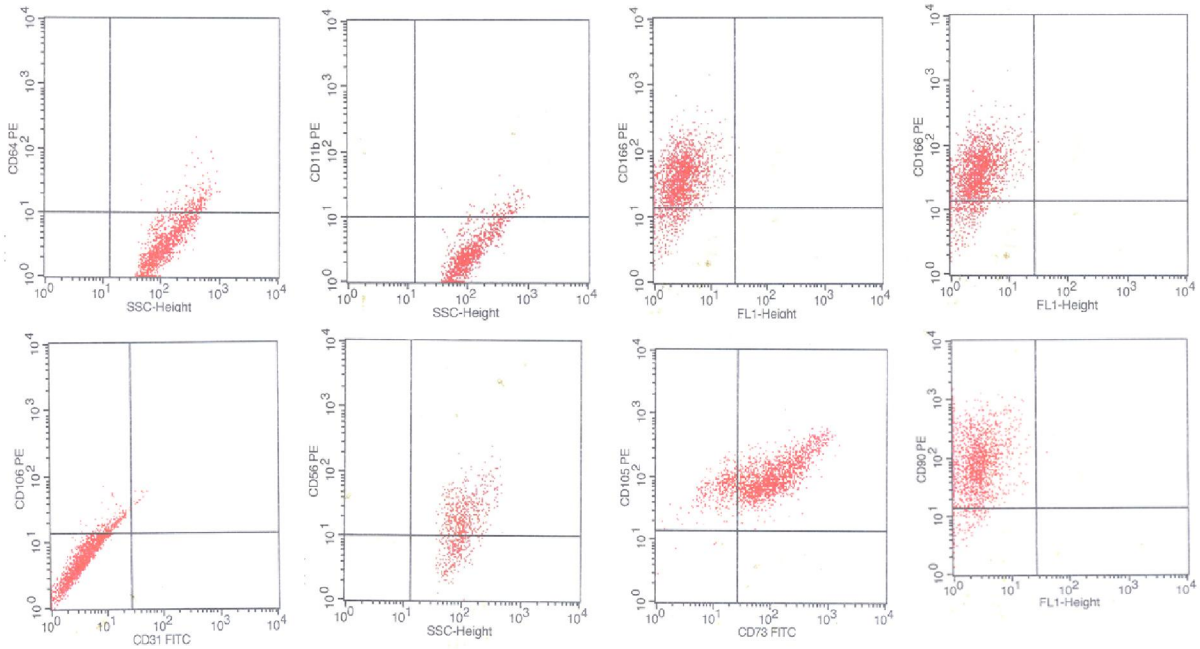

Figure 2. Flow cytometry of the mesenchymal-related antigens in dental pulp stem cells (DP-SCs).

4. Discussions

Adult stem cells, with the capacity of self-renewal and multi-lineage differentiation, play a crucial role in postnatal tissue development and provide an attractive progenitor cell source for tissue engineering and regenerative medicine (Mimeault *et al.,* 2006). Stem-cell-based tissue engineering has been performed in animal models for many types of tissue regeneration, such as articular cartilage, bone, tendon, muscle and adipose tissues. Studies, including direct cell-pellet implantation (Iohara *et al.*, 2004; Ohazama *et al.,* 2004) and tissue engineering, in combination with biocompatible scaffolds, have enabled us to contemplate new and promising strategies for hard tissue repair, particularly that of tooth reconstruction (Young *et al*., 2005; Rezwan *et al.*, 2006). DPSCs (dental pulp stem cells) and BMSSCs (bone marrow stromal stem cells), which can differentiate into multiple mesenchymal cell lineages, are putative candidate cells for tooth and bone-tissue engineering, respectively (Gronthos *et al*., 2000; Gronthos *et al.*, 2002; Gronthos *et al.*, 2006; Bianco *et al*., 2001; Batouli *et al*., 2003; Yeon *et al*., 2006). Several tissues including adipose, cord

blood, peripheral blood, amniotic fluid, cartilage and bone have been shown to contain MSCs (Nöth *et al.*, 2002; Eslaminejad *et al.,* 2007). Dental pulp is another exciting tissue type which may contain MSCs. This tissue type has been the subject of a number of investigations attempting to isolate and characterize stem cell populations. The evidence indicating the existence of a stem cell population in pulp tissue originates from the observations made of the repair process occurring in dentin, following carious lesions. The cells responsible for reparative dentin formation are thought to arise from the precursor cells, residing somewhere within the pulp tissue (Ruch 1998; Kitamura *et al.*, 1999). In this regard, a study by Tsukamoto and colleagues showed that fibroblastic cells from the pulp of deciduous and supernumery teeth were able to produce dentin-like nodules at culture (Tsukamoto *et al.,* 1992).

Similar results were reported by Couble et al (2000) in an explant culture of pulp tissue in a medium containing glycerol phosphate. Definitive evidence for the existence of stem cells in pulp tissue was provided by Gronthos et al (2000), who culture-expanded fibroblastic cell populations from pulp tissue and examined their differentiating potential both in vitro and in vivo. According to their reports, the cells tended to differentiate into bone and neural cell lineages in vitro and produced dentinproducing cells in vivo. Subsequent investigations also indicated the bone differentiating potentials of the dental pulp stem cells (Laino *et al*., 2006; d'Aquino *et al*., 2007).

Several studies evaluate the surface antigens known to be found on mesenchymal lineages such as CD29, CD90, CD105 (Huang et al. 2009), CD13, CD90, CD146, CD 166 (Karaöz *et al*., 2010), CD105, CD90, CD44, CD73 (Eslaminejad *et al.,* 2007) CD73, CD90, CD105 (Mashadi Abbas *et al*., 2011).

In DPSCs there is a low expression of CD14, CD34, CD45 (Huang *et al*., 2009), CD3, CD8, CD11b, CD14, CD15, CD19, CD33, CD34, CD45, CD117, HLA-DR (Karaöz *et al*., 2010), CD56, CD11b, CD31, CD34, CD33, CD45 (Eslaminejad *et al.,* 2007) and CD34, CD45 (Mashadi Abbas *et al*., 2011).

Several publications have stressed the importance of the expression of pluripotentiality associated markers. The transcription factors Nanog, Sox2, Oct3/4, SSEA4, SSEA-3, TRA-1-60 and TRA-1-81 are indispensable for the stem cells to divide indefinitely without affecting their differentiation potential, i.e. maintaining their self-renovation capacity (Kerkis *et al*., 2006).

To date, human dental mesenchymal stem cells have mostly been derived from primary and permanent third molar teeth. Dental pulp stem cells are part of the MSC population and may be considered suitable for use in regenerative medicine, owing to their relatively rapid rate of in vitro propagation (Baghban *et al*., 2010).

They may even be suitable for the regeneration of functional and living teeth, which potentiates possible future therapeutic strategies for the replacement of diseased or damaged teeth.

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