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Characterization of Human Dental Pulp Stem Cells

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Abstract

Aim of the study was to isolate and characterize human dental pulp stem cells.

Materials and methods: Human dental pulp stem cells (DPSCs) were isolated from three sound fully impacted third molars. DPSCs were isolated from dental pulp tissue using enzymatic digestion method. The immunophenotype of isolated cells was analyzed by flow cytometry using anti-human antibodies directed to mesenchymal cell markers CD44 and CD73 and hematopoietic cell marker CD45.

Results: The obtained results revealed that 51.5% and 76.1% of the CD44 and CD73 positive cells; respectively didn't express hematopoietic cell marker CD45, which confirm that the isolated stem cells are isolated from non-hematopoietic source.

Conclusion: Dental pulp stem cells (DPSCs) are part of the mesenchymal stem cells (MSC) population and may be considered suitable for use in vital pulp therapy, regenerative medicine and tissue engineering.

Keywords: Characterization, Dental pulp cells, Regeneration.

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Introduction

The idea of stem cells was introduced at the beginning of 20th century. The researches have shaped the perception of these cells as main contributors to organogenesis and regeneration process. This resulted in introducing tissue engineering technology around the end of twentieth century. It had been noticed that the tooth has the ability to form reparative dentin following injuries; that's why, it was suggested that the pulp tissue contains resident stem cells(1).

One of the goals of endodontic treatment is keeping the dentition in a physiologically functional state for the maintenance of oral and systemic health. Teeth with immature apices present challenges in cleaning and shaping, obturation of canals, and potential root fractures caused by weakened root dentinal walls(2). Hence, preservation of pulp vitality is important in order to maintain its function as a biosensor that provides nutrition to dentin, moreover dentin formation especially for immature permanent teeth helping in complete root formation and dentinal wall development(3). Vital pulp therapy and the regenerative endodontics help for preserving and regenerating the vitality of the dental pulp and restoring the physiologically functional dentition(1,4).

Human dental pulp stem cells (DPSCs) can differentiate into various types of cells, such as odontoblasts, adipocytes, chondrocytes, and osteoblasts(5). Following stimulation or injury, stem cells in the pulp tissue may be stimulated to proliferate and differentiate into odontoblasts by morphogens released from the surrounding dentin matrix. Odontoblasts secrete the extracellular dentin matrix which act as a barrier for dental pulp protection from external stimuli(6). In addition, hDPSCs interact with various biomaterials and are effective in mineralized tissue formation(7).

Dental pulp stem cells (DPSCs) have same characteristics of mesenchymal stem cells (MSCs) including plastic adherence, clonogenicity and multilineage differentiation in vitro(8,9). DPSCs have the ability to proliferate and differentiate into odontoblast like cells(10). Dental pulp cells are excessively used in tissue regeneration especially dental pulp and long bone(1). Also, they are considered the main candidate cells in vital pulp therapy(3).

The aim of the study was to isolate and characterize human dental pulp stem cells.

Materials and Methods

1- Harvesting of Dental Pulp Stem Cells

This study was approved by Research Ethical Committee (REC), Faculty of Dentistry, Ain Shams University with approval number (FDASU-Rec IM112106). The experiments were performed according to the committee guidelines of the stem cells experiments.

A. Patients Recruitment

Three healthy adult patients aged from 18 to 20 years, each with sound fully impacted wisdom tooth indicated for extraction, were selected from oral and maxillofacial surgery department, Faculty of Dentistry, Ain Shams University. Patients had standard surgical procedures done after informed consent. Molars were directly immersed in phosphate buffered saline (PBS) (pH:7.4), antibiotics including Penicillin, Streptomycin and antimycotic, in addition to 1% dimethyl sulfoxide (DMSO) as preservative media (Figure 1). Then, the molars were immediately transferred to the stem cell unit within 30 to 40 minutes.

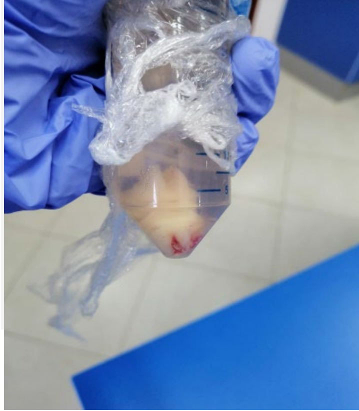

Figure 1 photograph showing extracted molar immersed in PBS, antibiotics and preservative medium

B. Isolation and culture of dental pulp stem cells:(5,11)

All isolation and experimental procedures were done at stem cell unit, Global Research Laboratory.

The pulp tissue was isolated by sectioning each tooth into two halves at cementoenamel junction (CEJ) using chisel and mallet and the pulp tissue was gently extirpated by barbed broach.

DPSCs were isolated from dental pulp tissue using enzyme digestion method by the following protocol:

The dental pulp tissue was minced into small pieces approximately, 2mm3 diameter in Petri dish containing PBS (pH 7.4) and antibiotics. (Figure 2). The pulp tissue was digested into collagenase type I and dispase solution with continuous agitation for 5 minutes at 37°C. (Figure 3). Tissue clumps are passed through a cell strainer to obtain single cell suspension(12). Cells were cultured in a flask 75 cmm2, in DMEM (Gibco Dulbecco's Modified Eagle Medium) containing ten percent fetal bovine serum (FBS) and one percent of penicillin G sodium (10.000 UI), streptomycin (ten mg) and amphotericin B (twenty-five μicrogram) (PSA). Flask was incubated at thirty-seven °C in an atmosphere of five percent CO2 for 48 hrs before cell characterization. The culture media was changed every 24 hours.

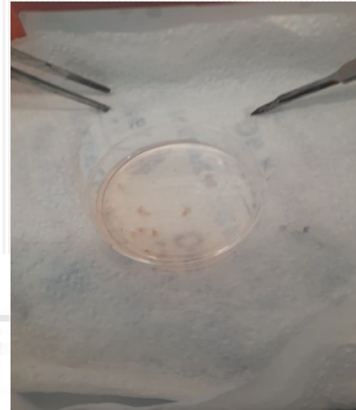

Figure 2 photograph showing pulp tissue fragments in petri dish containing PBS and antibiotics

Figure 3 photograph showing cell pellet after pulp tissue digestion

C. Flow cytometric characterization of isolated cells:

The immunophenotype of isolated hDPSCs was analyzed by flow cytometry(10) using anti-human antibodies directed to mesenchymal cell markers CD44 and CD73 and hematopoietic marker CD45(13,14). The CD45-PC5, CD44-FITC and CD73PE were used to stain the hDPSCs cells. The cells were suspended in PBS and the count was adjusted to 106 /ml. After centrifugation at 800xg for 10 minutes, the cell aspirate was discarded and the cell pellet was washed two times with PBS. Five μL of each antibody was added directly onto the cell pellet. To avoid intense autofluoresence signals emerged from higher number of cells, only one antibody is used per tube. Finally, the data was processed for flowcytometric

analysis, and cells were gated based on their monoclonal antibody staining.

Results

Human dental pulp stem cells characterized by flowcytometry:

The observed results revealed that 76.1% (G2 area) of cells showed double bright surface expression of CD44/CD73 in contrast to only 3.9% of cells were double negative for both biomarkers. In addition, 19.4% (G4 area) were positive for CD73 and 0.6% express CD44. In order to confirm the non-hemopoietic source of stem cells, the CD73 and CD44 cells were gated with CD45' hemopoietic stem cell marker. The obtained results revealed that 51.5% (F1) and 76.1% (E1) of the CD44 and CD73 positive cells; respectively didn't express CD45, which confirm that the isolated stem cells are isolated from non-hematopoietic source (Fig.4).

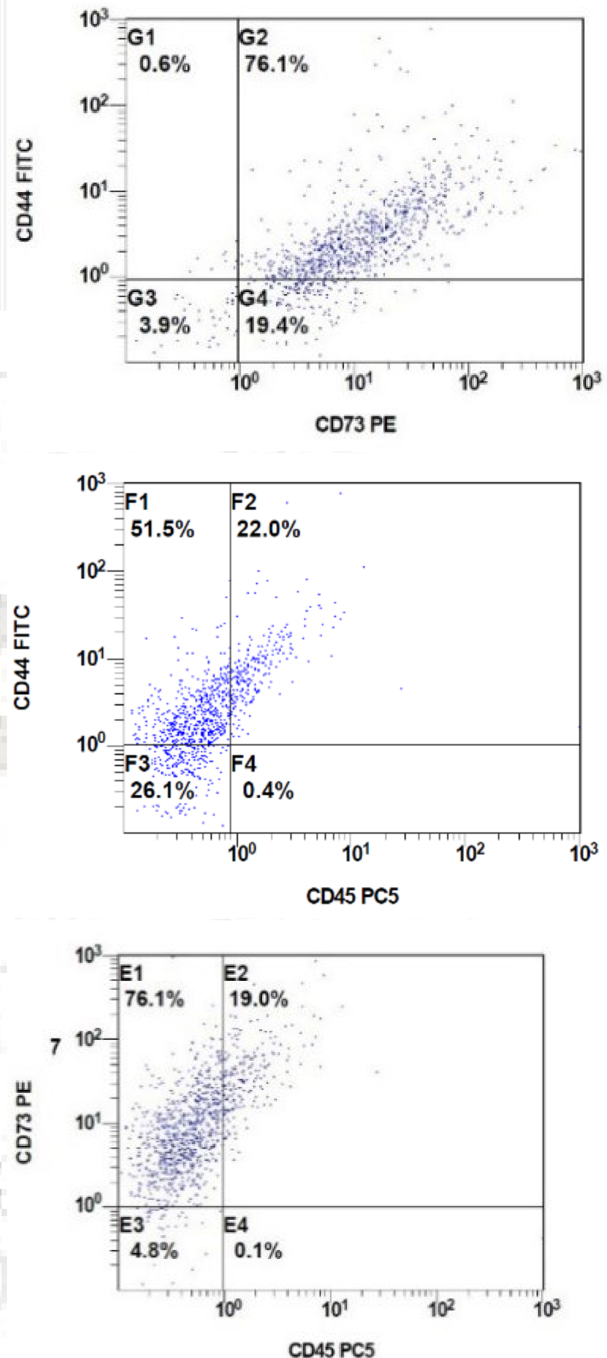

Figure (4): Characterization of hDPSCs cells using Multiparametric analysis: a Representative FCM dot-plots showing the gate protocol for hDPSCs cells. The hDPSCs stem cells were stained with stem cell markers (CD73, CD44 and CD45). The CD73 and CD44 positive cells were gated in crossponding to CD45.

Discussion

Regenerative endodontics is a biological treatment method aims to replace damaged dentin, root structures, and pulp–dentin complex cells (15,16). The most promising cell sources for tissue engineering are stem cells. A stem cell is an undifferentiated cell, which has the potential to proliferate and generate progenitor cells that can differentiate into specialized cells throughout postnatal life. Although there are unsolved questions and usage limitations regarding stem cells, stem cell research remains one of the most active academic fields. Based on their origin, there are two main types of stem cells-embryonic stem cells (ES cells) and postnatal or adult stem cells (AS cells)(3).

Adult stem cells (AS cells) provide a good cell source for tissue regeneration and have an important role in tissue engineering (8). AS cells are found in almost all kinds of tissues, many tissues as bone, adipose, peripheral blood and cartilage proved to contain mesenchymal stem cells (17,18). Also, AS cells have been isolated from different dental tissues, including dental pulp (DPSCs), periapical follicle (SCAP), and periodontal ligament (PDLSCs)(3). Many investigations had been done aiming to isolate and characterize stem cells from dental pulp which was suggested to contain mesenchymal stem cells. This suggestion originates from the observed reparative dentin formation following caries. The cells forming reparative dentin are suggested to arise from precursor cells present in the dental pulp(19). Definitive evidence for stem cells' existence in dental pulp was introduced in 2000 by Gronthos et al(5). They concluded that the cells in vitro had the ability to differentiate into bone and neural cell lineages while in vivo differentiated into dentin producing cells. The differentiation potential into bone cell lineage of the dental pulp cells was also suggested by later investigations(20,21).

DPSC surface markers are similar to the mesenchymal stem cell surface markers and include CD twenty-nine, CD forty-four, CD seventy-three, CD ninety, CD105 and CD146. It is necessary to know that dental pulp stem cells do not express all of these surface markers, with each cell expressing some of these markers at different ratios(9).

Conclusion

Isolation of DPSCs can be done from extracted fully impacted third molars. Dental pulp stem cells (DPSCs) are part of the mesenchymal stem cells (MSCs) population and considered suitable for use in regenerative medicine. The gathering of basic information and clinical data regarding dental pulp stem cells will hopefully result in the application of these cells in specific tissues regeneration.

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