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Characterization of human stem cells of the apical papilla

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Abstract

Introduction: Dental tissues represent an alternative and promising Mesenchymal stem cells (MSCs) source for tissue engineering. Recently, a new population of MSCs residing in the apical papilla of permanent immature teeth has been discovered and was termed SCAP.

Aim: This work is aimed to characterize human stem cells of the apical papilla to provide new insight on SCAP research for future biomedical applications.

Methods: SCAP cultures were established from impacted third molars of young healthy donors at the stage of root development. Cultures were analyzed for stem cell markers, including CD73, CD44 and CD45 using flow cytometry.

Results: The obtained results revealed that 43.9% and 75.9% of the CD44 and CD73 positive cells; respectively didn't express CD45.

Conclusion: the isolated stem cells are isolated from non-hematopoietic but mesenchymal source. These results suggest that SCAP represent a very promising adult mesenchymal stem cells source with enhanced properties that could be isolated and characterized by flow cytometric methods to be used for tissue engineering applications

Key Words: Regeneration, mesenchymal stem cells, immature teeth, flowcytometry, stem cells from the apical papilla.

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Introduction:

Endodontic regenerative procedure is such a biological procedure to replace diseased or damaged structures with healthy tissues to restore the normal physiologic functions of the pulp. A successful endodontic regenerative therapy is attained necessary by stimulation of dental stem cells (1).

During this procedure, the bleeding step delivers the stem cells of the mesenchyme into canal systems of the roots. These stem cells have anti-inflammatory ability which can survive in acidic inflammatory media and have an important role during the re-apexogenesis procedure of immature roots that affected by periapical diseases(2)(3).

Different stem cells' sources have been identified including stem cells from human exfoliating deciduous teeth (SHED), stem cells of the apical papilla (SCAP), and dental pulp stem cells (DSPSCs). These cells origin is the mesenchyme. In vitro, they undergo multilineage differentiation and have magnificent clonal expansion capacity(4)

Stem cells in the apical papilla of permanent immature teeth have been discovered in 2006 by Sonoyama et al, who were first isolated SCAP from the apical papilla of immature tooth.(5)

SCAP considered to be primary odontoblast's source that form root dentin, where the source of replacement odontoblasts is DPSCs. Hence, when treating immature teeth conservation of SCAP allow continuation of the root formation.(6)

SCAP is one of sources in regenerative therapy (stem cell-based therapy) as their ability for proliferation, self-renewal, low immunogenicity and differentiation (7). they have great osteogenic/odontogenic capacity and powerful dentinogenesis.(8)These cells can be obtained from the wisdom tooth which is frequently extracted for orthodontic reasons. However, the apical papilla is that loosely attached soft tissue at the root apexes of the immature permanent teeth. The difference between the apical papilla and the pulp is that contains less cellular and vascular components.(9)

Although both pulp and papilla being a continuity of one another, the apical papilla combats the pulp when it became necrotic , and can still vital because it has an access to the apical collateral circulation . (10)

SCAP are considered a type of mesenchymal stem cells, hence they sharing a lot of surface markers. In this study we will confirm that SCAP is one of dental derived stem cells which have similar characteristics and exclude hematopoietic stem cells.

Methodology:

Harvesting of stem cells and cell culture:(11)(7)(12)

This work was approved by the research ethics committees Faculty of Dentistry Ain Shams University with approval number: (FDASU-Rec IM112105). All experiments were performed according to the committee guidelines of the stem cells experiment. For this in vitro testing, SCAP was isolated and cultured in stem cell unit, Global lab, Cairo, Egypt.

Patient recruitment:

Three healthy patients aged between 17 and 18 years were scheduled for wisdom teeth extraction in the Oral and Maxillofacial Surgery Department of Ain Shams University. After gaining verbal and written informed consent from these patients, the extraction was done. Three fully impacted sound immature third molars (incomplete root) were used in the

study. The extracted teeth were immediately rinsed with sterile PBS (PH 7.4) and transferred in transfer solution (PBS + 10000 U penicillin/streptomycin + preservative media 1%DEMSO) stored cold in ice box until being transferred to the laboratory for isolation of stem cells within 30 min. (figure1)

Isolation of SCAP:

Apical papilla was detached with a pair of forceps. Then, SCAP were isolated from this tissue using enzymatic digestion method by the following protocol: 1) The dental tissue was minced into small pieces approximately, $2mm^3$ diameters in Petri dish containing PBS (pH 7.4) and antibiotics. 2) The tissue was digested by collagenase type I with continuous agitation (in water bath and shaker) for 1 hour at 37°C.After digestion, the cell pellets were harvested by centrifugation. 3) To achieved single cell suspension of SCAP, cells passed through a cell strainer. 4) Cells were seeded in T- flask 75 cmm^3, in complete culture media DMEM [Gibco, Thermosientific, Germany] containing [(PSA) 1% penicillin G sodium (10.000 UI), streptomycin (10 mg) and amphotericin B (25 µg) and 10% fetal bovine serum]. 5) At 37 °C in an atmosphere of 5% CO2 flasks were incubated. 6) Isolated stem cells were monitored for infection and growth every 24 (figure 1) hours using inverted phase contrast microscope.

Figure 1 photomicrograph showing: a) extracted teeth in a transfer solution. b) extracted teeth in a petri dish showing the apical papilla. c)the dental tissue minced into small pieces. d)the cell pellet after tissue digestion

characterization of SCAP:

Characterization was done using flow cytometric analysis. The immunoassaying stains [CD45-PC5 (Phycoerythrin Cynin), CD44-FITC (fluorescein Isothiocyanate) and CD73-PE (phycoerythrin)] are used to stain the SCAP cells. The cells are suspended in PBS and the count is adjusted to 10^6 /ml. After centrifugation at 800xg for 10 minutes, the supernatant was discarded and the cell pellet is washed two times with PBS. Five µL of each antibody was added directly onto the cell pellet. To avoid intense autofluorescence signals emerged from higher number of cells, only one antibody is used per tube. Cells were incubated at 4°C for 45 minutes, washed, and resuspended in binding buffer. Finally, cells were gated based on their monoclonal antibody staining, and the data was processed for flowcytometric analysis by Navios software (Beckman Coulter).

Culture of SCAP:

Human stem cells that were isolated from apical papilla Stem cells was cultured in flask 75 cmm^2, in complete culture media. Flask was incubated at 37 °C in 5% CO2. Every 24 hours, the media was changed. At 3rd passage (passage = 70%-80% confluency), cells were harvested. The harvested cells were cryopreserved in % DMSO and FBS and stored in -80°C for further analysis. Inverted phase contrast microscope with digital camera [LABOMED inverted microscope, LABOMED Vega digital camera, Labmed-USA] was used to monitor the growth and infection.\

The data was introduced to a PC using Statistical package for Social Science (SPSS 24 for windows; SPSS Inc, Chicago). All graphs were plotted by GraphPad Prism Software 8.4.2 (San Diego, US).

Results:

Characterized SCAP:

Morphological appearance:

At the apex of the extracted teeth, a smooth soft tissue was observed and can detached with a pair of tweezers. (Figure 2)

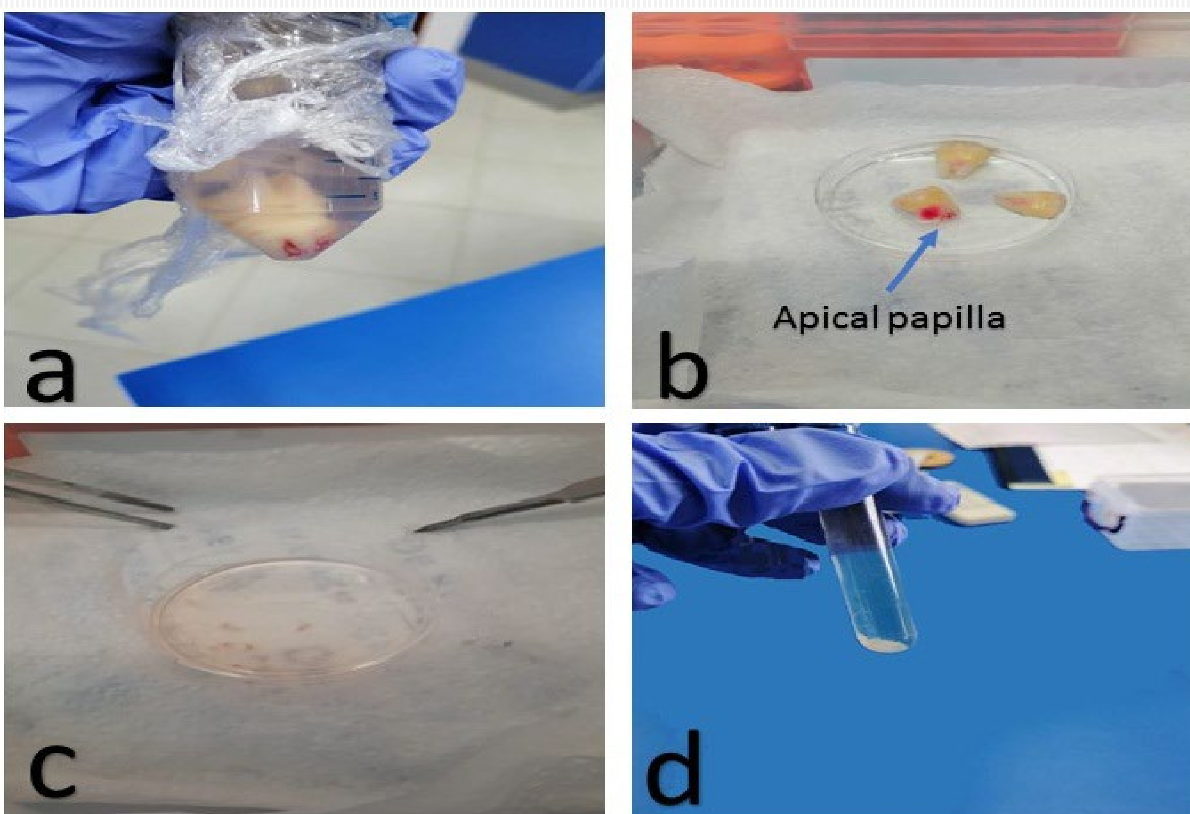

Figure 2 photomicrograph showing: a) extracted teeth in a transfer solution. b) extracted teeth in a petri dish showing the apical papilla. c)the dental tissue minced into small pieces. d)the cell pellet after tissue digestion.

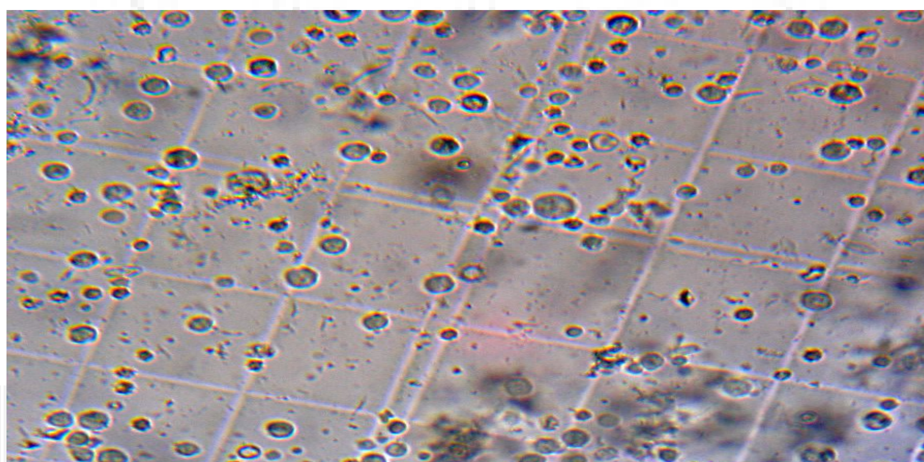

Figure 3: phase contrast photo of isolated stem cells after 24 hours culture.

Result of flowcytometry:

The observed results revealed that 67.7% of cells showed double bright surface expression of CD44/CD43 in contrast to only 5% of cells were double negative for both biomarkers. In addition, 26.7% 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 43.9% and 75.9% 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**. (Figure 3)**

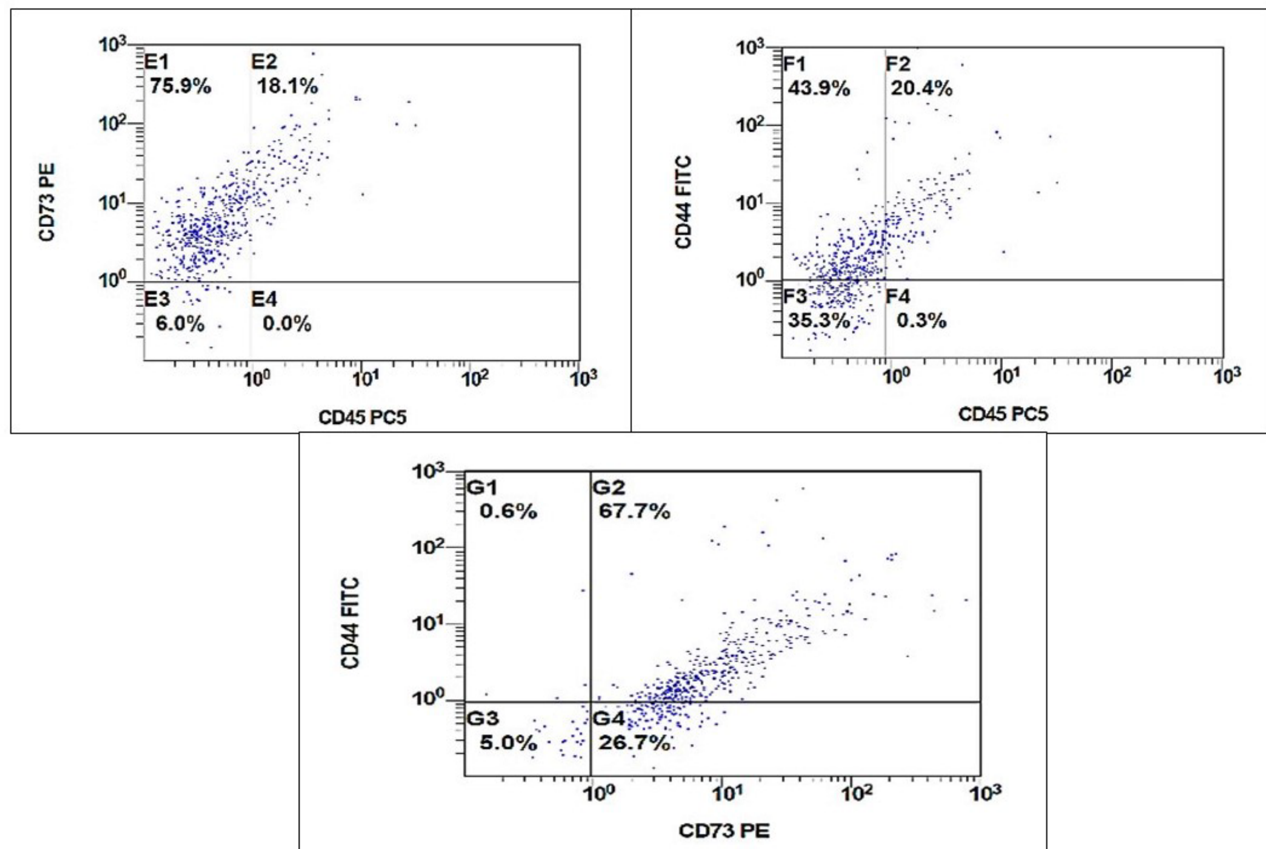

Figure 3 Characterization of SCAP cells using Multiparametric analysis: Representative FCM dot plots showing the gate protocol for SCAP cells. The SCAP stem cells were stained with stem cell markers (CD73, CD44 and CD45). The CD73 and CD44 positive cells were gated in corresponding to CD45.

Discussion:

SCAP, like other mesenchymal stem cells (MSC), express markers of the MSC and have a capability of proliferation and multilineage differentiation. SCAP express lots of markers such as CD24, CD44, CD49, CD51, CD56, CD61, CD73, CD90, CD105, CD106 and CD166. While, SCAP are found to be negative for the expression of CD34, CD45, and CD150, indicating that they are not of hematopoietic origin. CD24 is a specific marker for SCAP, which is undetectable in dental pulp stem cell. (7)(5)

SCAP can be isolated from permanent immature teeth's apical papilla and characterized by flowcytometry. These cells are considered as a source for endodontic regenerative treatments avoiding the ethical limitations and risks of other stem cell (13)

In the present study, the apical papilla tissue has been easily detached from the apex. Then isolation and culturing of SCAP from this tissue was done. For accurate measurement, labeled antibodies were used to stain antigens such as CD45, CD 73 and CD 44 by the flowcytometry.

Regarding cell transferring to the lab, the cells were preserved in DMEM containing DEMSO and stored cold for less than 30 min. as showed in studies which measured the cell viability after melting the ice, the cells decreased in viability to 80% after 2 hours. Which is agree with our finding.(14)

In our study, after SCAP characterization by Flowcytometry, the observed results revealed that 67.7% of cells showed double bright surface expression of CD44/CD73. 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 43.9% and 75.9% 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. This result come in agreement with Kang et al.(7)

On the other hand, there are studies used different surface markers for dental stem cells like Stro-1, CD146, and 3G5. They were positive to them. These markers confirmed that they are dental in origin. (15)(16)

Conclusion:

Stem cells of apical papilla are mesenchymal stem cells of dental source could be isolated from human immature teeth and characterized by flow cytometry for the expression of CD44, CD73 and negative for CD45.

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