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Isolation of mesenchymal stem cells from the tooth pulp and cell characteristics

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Background: In recent years, a sufficient amount of data has been obtained on the possibility of isolating stem cells from the teeth pulp, both children and adults. The main population of cells that can be isolated from the tooth pulp is mesenchymal stem cells. As a possible source of MSC for tooth regeneration, several types of cells are considered today: tooth MSC, MSC from non-dental intraoral sources, primarily the mucous membrane of the cheek, gums, periosteum, and MSCs from other sources such as bone marrow, adipose tissue and umbilical cord.

Objective: To develop a protocol for MSC isolation from the pulp of third molars removed by orthodontic indications and characteristics of isolated MSC.

Material & Methods: Third molars without visible carious lesions and signs of inflammation (n=20) of patients of both sexes aged 16-35 years were removed by orthodontic indications. All patients signed informed consent form allowing the use of isolated MSC for research purposes. Cells were isolated enzymatically. The tooth was placed in 2 ml of a 0.5% collagenase II type for 1 hour at 37°C, followed by addition of 3 ml working medium (1:1 DMEM/F12) to stop the fermentation and subsequent centrifugation of cell suspension (5 min, 1000 rpm, room temperature). The supernatant was carefully selected. The cell pellet was re-suspended in 1 ml of the complete culture medium (1:1 DMEM/F12, 10% FBS, 100 μM L-ascorbic acid, 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin and 0.25 mg/ml amphotericin B). The resulting suspension of dental pulp cells was placed on 25 cm2 culture plates with the complete culture medium and subsequently incubated at 37°C and 5% CO2. The culture medium was replaced every 2-3 days until confluence reached 80%. Isolated passages were performed according to standard protocol (1:3 ratio, every 7-8 days). Further analysis included cells on 2-3 passages. Analysis of the cell population characteristics was conducted by the following MSC criteria: morphologic criteria, expression of typical cell surface markers, ability to differentiate into specific cell types. Evaluation of the cell shape was performed by light microscopy. Analysis of MSC markers expression (presence of CD73, CD90, CD105, absence of CD14, CD20, CD34, CD45) was performed by flow cytometry (Beckman Coulter, FC500 assay kit, MSC phenotyping kit: human #130-095-198, Miltenyi Biotec) according to the manufacturer's instructions. We analyzed the ability of cells to differentiate into osteo-and chondrogenic cell lines, and the ability to differentiate into specific tooth cells, odontoblasts. Osteo- and chondrogenic differentiation was carried out by the addition of standard differentiating media (StemPro, Chondrogenesis Differentiation Kit, # A1007101, Gibco StemPro, Osteogenesis Differentiation Kit, # A1007201, Gibco) for 30 days with the following histologic staining. Odontoblastic differentiation was assessed by expression of characteristic markers - dental sialofosfoproteina (DSPP) (primary antibody anti-DSPP, Abcam, ab216892; secondary antibody - goat anti-IgG, IgA, IgM rabbit FITC (f-GAR Iss), Imtek) with immunocytochemistry standard protocol.

Results: The MSC isolation protocol is prepared inclusive of modern data on the base of own experience for human tooth. All procedures for cells isolation (n=20) from the removed teeth were successful. Dynamic observation of cell cultures after 24h of culturing showed the formation of cell colonies and most of the cells acquired the typical fibroblast-like form of MSC already. After culture medium replacement, all cells obtained characteristic fibroblast-like shape. The level of CD90 and CD105 expression was 98.5±3.8% and 96.8±2.9%, respectively, and CD14, CD20, CD34, CD45 markers were absent. The success of osteo- and chondrogenic differentiation was confirmed by the histochemical staining. Analysis of odontoblastic differentiation showed that use of standard differentiating osteogenic medium can lead to specific tooth cell differentiation.

Conclusion: The pool of cells from third molars of adult patients isolated according to the developed technique is characterized by the basic features of mesenchymal stem cells isolated from other sources and described according to the passports of the cell lines - the ability to self-renew and multilinear differentiation, has an osteo-odontogenic and chondrogenic potential.

Biography

Svetlana Lyamina has completed her Cand. of Med. Sci. (equal to PhD) at the age of 25 years from Saratov State Medical University, Saratov, Russia, and Doct. of Med. Sci. at the age of 31 from Moscow State University of Medicine and Dentistry, performing postdoctoral studies from Moscow State University of Medicine and Dentistry. She is the professor of pathophysiology department of Moscow State University of Medicine and Dentistry. She has published more than 48 papers in reputed journals and she is the author of 2 invention patents

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