Supplement 1.

**IFN-γ Stimulation of Dental Follicle Mesenchymal Stem Cells Modulate Immune Response of CD4+ T Lymphocytes in Derp1+ Asthmatic Patients in vitro**

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**Material and Methods**

***Isolation of Dental Follicle Mesenchymal Stem Cells***

Isolation of mesenchymal stem cells was performed as previously described (21). Briefly, dental follicle tissues were transported in Dulbecco’s phosphate-buffered saline (DPBS) (Gibco, USA) containing 1% penicillin/streptomycin (Gibco, USA). Follicles were cut into 0.5 mm pieces and enzymatically digested with 3mg/mL collagenase type I (Gibco, USA) for 45 minutes at 37∘C. They were then inactivated with 3mL of Dulbecco’s modified Eagle’s medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin and centrifugated at 1200 rpm for five minutes. Cell pellets were transferred to T-25 flasks containing 5x103cells/cm2 and incubated at 5% CO2 atmosphere at 37∘C in culture medium composed of DMEM, 10% FBS, and 1% penicillin/streptomycin. The stem cells were allowed to reach 80-90% confluency by changing culture medium freshly prepared twice a week. The cells were detached with 0.25% trypsin-EDTA (Gibco, USA) and passaged until the third passage. DF-MSCs were analyzed for the positive and negative marker expression for characterization of MSCs using flow cytometry (FACS Calibur, BD)

The third passage cells were analyzed for surface antigen expressions. DF-MSCs were incubated with antibodies of positive markers for mesenchymal stem cells of human CD29 allophycocyanin (APC), CD73 phycoerythrin (PE), CD105 PE, CD146 fluorescein isothiocyanate (FITC), and negative markers CD14 PE, CD34 FITC, CD20 APC and HLA-DR PE (BD Biosciences, USA) for 15 minutes at room temperature in the dark. The flow cytometry results were analyzed using BD FACS Calibur.

To induce osteogenic, adipogenic and chondrogenic differentiation, human MSC stimulatory kits (StemPro) were used. The differentiation assay was performed in six-well plates (5 × 104 cell/well), and the differentiation media were prepared according to the manufacturer’s instructions and changed three times per week. After 14 days, the osteocytes, adipocytes and chondrocytes were stained with Alizarin Red, Oil Red O and Alcian blue, respectively, and after 21 days and cell types were evaluated under microscope.

**Results**

***Isolation, Characterization and Multipotency of DF-MSCs***

DF-MSCs attached and formed colonies with fibroblast-like morphology in the culture flasks during the early days of incubation (P0; Figure S1A). DF-MSCs reached 80% confluency in five to six days after being plated and exhibited fibroblast-like morphology in the later passages (P1, P2, and P3; Figure S1A). In the third passage immunophenotyping and differentiation potential of cells to osteogenic, chondrogenic and adipogenic lineages were examined.

DF-MSCs were analyzed by flow cytometry. These cells were evaluated with positive markers for CD29 (96,84±2,92), CD73 (94,29±1,87), CD105 (95,02±1,19), and CD146 (97,41±2,06) and were negative for CD14 (2,21±0,43), CD34 (2,57±0,86), CD20 (2,85±0,41), and HLA-DR (2,51± 1.38) (Figure S1B).

DF-MSCs were differentiated into osteocytes, adipocytes, and chondrocytes. The osteogenic differentiation capability was investigated in vitro during a 21-day culture period in osteogenic induction medium. DF-MSCs were stained with Alizarin red, and the osteocytes formed calcified bone nodule structures in the matrix. The adipogenic differentiation capability was evaluated by culturing the cells in adipogenic induction medium for fourteen days and Oil Red O and hematoxylin-eosin staining. Intracellular oil droplets were observed at the end of the culture period. The chondrogenic differentiation potential was evaluated by culturing with chondrogenic stimulation in vitro during a 14-day culture period and cell differentiation into chondrocytes and cartilage forming was confirmed with Alcian blue staining. Chondrocytes and intracellular proteoglycans were observed after culture period (Figure S1C).