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An effective Model for the Expansion of human Mesenchymal Stem Cells

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Bone-marrow derived human mesenchymal stem cells (hMSCs) will probably be a valuable tool for regenerative medicine in the future. In-vitro cultured hMSCs show restricted proliferation and differentiation capability. In this study we demonstrate, that age of donors is a main limiting factor for in-vitro expansion and differentiation of hMSCs, but extracellular matrix (ECM) proteins (ECM-gel, Laminin-1, -5, -S, collagen IV, fibronectin), hypoxic conditions (1% and 5% O₂), the use of human serum instead of fetal calf serum (FCS), and CD 271+ selection have positive effects on growth rates and differentiation capacity.

Cells were analyzed via histochemical and immunohistochemical staining procedures, telomerase activity detection, different flow cytometry protocols (incl. STRO-1, Oct-3/4 and Alkaline Phosphatase expression), quantitative photometrical and fluorometrical assays, and RT-PCR.

hMSCs, selected from young donors (age 21-38), had significant higher proliferation and differentiation potential, compared to a group of elder (44-89) donors. Basement-

membrane ECM proteins lead to a 103 fold higher in-vitro expansion and each protein had defined ascending effects on the process of differentiation; these effects were inhibited by antibodies against receptors of the particular protein. Hypoxic conditions, human serum, and CD 271 positive selection had also direct, significant effects on proliferation and the maintenance of differentiation capacity, and the combination of mentioned factors yielded again in a significant higher cumulation for the contemplated attributes. Cultivated samples were investigated for proliferation behaviour. The subunit of so-called RS-cells (flow-cytometry: light forward and side scatter population; small cells: 7-13 µm diameter) was exclusively responsible for growth of hMSCs cultures and showed better differentiation features. Each detected subpopulation originated from RS cell subunit. At least this in-vitro model was used for the expansion of peripheral blood extracted circulating cells after G-CSF stimulation, and CD 133 positive selection and/or Interleukin-6 (IL-6) treatment. Selected cells showed flow cytometric characteristics of hMSCs and could successfully cultivated, and differentiated into osteoblasts, chondrocytes and adipocytes.

Without positive selection or IL-6 usage no cells with hMSCs properties were detected.

ECM-components, hypoxia, human serum, and magnetic bead associated cell selection are valuable constituents of an model for effective cultivation of mesenchymal stem cells extracted from different sources.

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