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An efficient procedure of isolation, cultivation and identification of bone marrow mesenchymal stem cells

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Abstract

Background: Bone marrow mesenchymal stem cells (MSC) have a wide application in domain of Regenerative Medicine. Of a great importance is utilization of a suitable bone marrow extraction technique that can provide a sufficient number of MSC to perform laboratory tests without seriously affecting the health of the laboratory animal. At the same time, before using in researches and clinical application, the MSC needs to be identified.
Material and methods: The study was conducted in rabbits (n = 9), in which, from one iliac bone, by aspiration were taken 3.39 ± 1.27 ml of bone marrow. The nucleated bone marrow cells were separated through centrifugation using concentration gradient. The specific for stem cells culture medium was used, and MSC were multiplied during 2 passages. From the obtained MSC, $1x10^6$ cells were subject to differentiation by chondrocytes lineage for other 20 days. The obtained chondrocytes aggregates were morphologically examined by Hematoxylin–Eosin staining and specific cartilage staining with Safranin O and Toluidine blue/fast green.
Results: There was a strong correlation between the volume of collected bone marrow and the time required to achieve a 70-80% of MSC confluence (p=0.01). Also, the MSC isolated from bone marrow extracted from rabbit iliac bone were differentiated successful on chondrocyte line in all cases, confirmed through the specific cartilage staining with Safranin O and Toluidine blue/fast green (p<0,001).
Conclusions: The volume of 3.39 ± 1.27 ml of bone marrow, harvested from rabbit iliac bone is sufficient to obtain a large number of MSC for the laboratory tests *in vitro* and *in vivo.* As a standard method for MSC identification could be used just the capability of the cells to differentiate in the specialized cell, including chondrocytes.
Key words: mesenchymal stem cells, bone marrow, rabbits, cellular identification, iliac bone, autocells.