Fresh and Frozen micro-fractured human adipose tissue generates mesenchymal stem cells with higher differentiation potential and in vivo repair efficacy

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The differentiation potential and the ease of their isolation have made the multipotent mesenchymal stem cells very important for the development of a vast range of clinical applications in regenerative medicine. Adipose tissue is derived from the embryonic mesoderm, and the human adipose tissue derived stem cells (hADSCS) are multipotent stem cells able to differentiate into different cell lineages such as bone, muscle and neural cells. Here we report our findings on human adipose tissue-derived stem cells (hADSCs) obtained through the Lipogems® device from micro-fractured lipoaspirate. This is a system the allows the fragmentation of adipose tissue into small clusters. The reduced particle size of adipose tissue enriches the content in available stem cells that are more able to creep out of the tissue and within 7 days in culture are ready for the first passage. The use of such a device allows the successful establishment of hADSCs colonies even without enzymatic treatment with Liberase H1(30 minutes at 37°C). Lipogems®-hADSCs cells grown in mesenchymal classic medium (αMEM) were flat, large with few short processes, while those grown in neural Stem Cell Medium (SCM) were slim and elongated with one or few prolonged processes. The growth rate was comparable in both media. These cells can also be obtained from lipogems preparation after preservation at 4°C for up to 72 hours and, even, cryopreservation at -80°C for over 1 month. Differently it is almost impossible to obtain hADSCs from cryopreserved lipoaspirate. The cell cycle analysis showed that 75% of cells are in G0/G1phase and 21% in S+G2/M, and only a marginal 0.2% apoptosis. No Chromosomal abnormalities were observed during maintenance in culture . By means of flow cytometric analyses we determined that these hADSCs are 100% positive to surface markers typical of mesenchymal stem cells such as CD44, CD73, CD90, CD105, CD146 and CD166 and negative for CD34, CD45. These hADSCs from either fresh or frozen lipogems preparations are bearer of typical mesenchymal markers at values above 90%, and express embryonic markers such as SOX2, NANOG and OCT4 and neural markers such as NESTIN, NEUROD1, PAX 6 and MUSASHI. The superficial epitopes are maintained even when hADSCs were grown in culture after storage at -80°C. Their driven osteogenic and adipogenic differentiation in vitro yields hADSCs with finer intracellular micro-organelles and fat deposits are more numerous and smaller in size. We shall also present how these lipogems-derived hADSCs can transdifferentiate in vivo in specific lineages determined by the site of transplantation and by the condition.