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Preparation of mesenchymal stem cells for transplantation: cultivation and quality control

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Abstract

Mesenchymal stem cells (MSCs) are the precursors of most human tissue. They can be efficiently applied for treatment and prevention of such serious diseases as myocardial infarction, diabetes, liver failure, cardiomyopathy, and several autoimmune diseases. However a sufficient amount of MCSs are necessary for successful clinical use, namely 1.5–2.0x10^6 cells/kg of patient weight. This amount is not possible to obtain without cultivation.

We selected optimal conditions for human MSC cultivation that provide their maximal viability and proliferative activity. MSCs were cultured on AdvanceStem Media (Hyclone, New Zealand) for 25–40 days for no longer than 4 passages. The first passage was on day 10–12 after explantation. Then the cell culture was seeded every 5–9 days with an initial density of 1.3x10^3 cells/sm^2.

In order to control the quality of cells, cytogenetic analysis and flow cytometry analysis with specific antibodies to cell surface markers were applied at every passage on one section of the MSC cell culture. After each passage the conditional media was tested for sterility.

Unambiguous MSC populations were obtained; their phenotype doesn't change during cultivation and is described as CD34-, CD45-, CD44+, CD90+, and CD105+. In karyotyping at least 15 metaphase plates per each sample were analyzed. No alteration in chromosome structure or amount was detected in the samples. No bacterial neither fungal contamination was detected in the samples.

Keywords: mesenchymal stem cells, cultivation, quality control, immunophenotyping, karyotyping

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