Expansion of Hematopoietic Stem Cells from Cord Blood in Culture in vitro

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Important biotechnological challenge is prolonged expansion of hematopoietic stem cells (HSC), without losses in their functional activity and high levels of proliferation. Cord blood is recognized as promising source of HSCs. However, the numbers of cells obtained from cord blood are insufficient for transplantation in adults. Therefore, the aim of research project focused on ways to enrich the stem cell population for use as a potential transplant. Long-term culture in vitro (5 weeks) was performed on 25 samples of cord blood. 46 experiments included: 10 - with different compositions of hydrogels, 15 - with different growth factors, the rest - under different cultivation conditions. Methods included long-term culture of mononuclear cord blood in vitro (Dexter) with the addition of cytokine complexes and cultivation in a semi-liquid agar (Pike and Robinson). Polyarcylamide hydrogel substrates allowed optimization of culture conditions. Additional washing step with saline allowed removal of toxic substances from the gels. Toxicity of gels was determined by MTT assay and a DNA comet method was used to assess damage DNA damage. By comparing different conditions for the cultivation of hematopoietic progenitor cells, we conclude that cultivation on hydrogel substrates, washed in a saline solution for 24 hours, is suitable for HSC expansion. We showed that the addition of a combination of cytokines (IL-3, IL-6, GM-SCF) to the culture yields a fivefold increase in hematopoietic cells with a high degree of functional activity. Cultivation on hydrogel substrates in the presence of cytokines allowed long-term support for proliferative activity of hematopoietic cells.

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