Expansion of Cord Blood Hematopoietic Progenitor Cells in Long-Term Culture on a Hydrogel Substrate in vitro

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Cord blood (CB) hematopoietic stem cells (HSC) are the most promising source for therapy purposes. Such cells are highly proliferative, have low immune reactivity and a lower risk of graft-versus-host reaction, when compared to adult bone marrow HSC. Major drawback of such cells is their low quantity that is insufficient to restore functional hematopoiesis in adults. Therefore, the aim of our work was to develop a method for the expansion of cord blood HSC ex vivo and to assess the functional activity and suitability as potential transplant material. To achieve this goal we used long-term in vitro HSC culture (7 weeks) on hydrogel substrates following with assessment of colony forming unit (CFU) activity in semi-liquid agar as well as immunophenotyping, cytology, light and inverted microscopy and statistical analysis methods. Long-term cultivation of CB-HSC on a hydrogel substrate in the presence of cytokines (SCF, IL3) led to active proliferation of cord blood cells in cultures with expansion from 1x10^5 to over 4x10^6 cells, while on a solid substrate with same cytokine mix expansion was significantly less at 3.0x10^6. CFU assay from expanded suspension cultures resulted at 521.5±27.5 CFU-GEMM and CFU-G colonies, while is solid substrate expanded HSC, CFU-M and CFU-GM prevailed. Our results indicate that potency of HSC depends not only on the complex of growth factors in the nutrient medium, but also on the degree of substrate rigidity and its surface characteristics. Conditions of cultivation on a gel substrate of stiffness 0.6 to 0.8 kPa in the presence of a cytokine complex are most attractive for supporting hematopoiesis expansion in long-term in vitro cultures. This approach can be further developed for expansion of CB-HSC suitable for transplantation