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Three-dimensional culture system by magnetic levitation for the study of hematopoietic stem cell microenvironment

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Background: Hematopoietic stem cells (HSC) proliferation, differentiation, self-renewal and survival are regulated by specific characteristics of their microenvironment. Bone marrow (BM), HSC's niche, is composed by different cell populations that by their signaling and interactions regulate HSC. Attempts to recreate this complex microenvironment have been devised in various studies using diverse co-culture models with different types of cell populations.

Objective: The objective of this study was to propose a 3D magnetic levitation culture, free of any exogenous structures and substances, in which HSC are co-cultured with mesenchymal stem cells (MSC) and endothelial cells (EC), where a multicellular sphere is formed, providing an organoid model to be analyzed.

Methods: For the conformation of the multicellular spheres, MSC were isolated from human BM, HSC from human umbilical cord blood and EC line Lonza CC.2811 was used. System standardization was accomplished by size and shape spheres evaluation; percentage of cell aggregation; viability assay and HSC CFU-assay; followed by immunohistochemical staining and immunofluorescent analysis of histological sections.

Results: Nanoparticles density was stablished at 1µL/10.000 cells. The optimum ratio for the culture of the three cell populations was 1MSC: 2HSC: 2EC. Multicellular spheres were completely formed at day 10 on culture, accomplishing a sphericity >0.8 and an aggregation percentage of 70-90% on day 5. The HSC isolated form the multicellular spheres preserve their multipotent function.

Discussion: The multicellular spheres obtained with this methodology are very suitable in evaluating proliferation, differentiation, clonogenic potential and other aspects of HSC when in contact with other BM cell populations, thus allowing for further studies to evaluate how HSC and its niche respond to different treatments, drugs and stress caused by infections, myelosuppression, neoplasms and aging, among other factors, so that future therapies for hematological diseases can be developed.

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