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Multicellular sphere model for hematopoietic stem cells microenvironment study

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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 of 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. Nevertheless, it has never been reported a co-culture system that uses simultaneously three BM cell populations in vitro that allows the study of the regulation of HSC in a closer way to it is *in vivo* microenvironment. The objective of this study is to propose a 3D magnetic levitation culture, free of exogenous structures and substances, in which HSC are co-cultured with Mesenchymal Stem Cells (MSC) and Endothelial Cells (EC), where a Multicellular Spheroid (MS) is formed, providing an organoid model to be analyzed.

Methods: For the confirmation of the MS, 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, the percentage of cell aggregation, viability assay, and HSC CFU-assay, followed by immunohistochemical and immunofluorescent analysis of histological sections.

Results: Nanoparticles density was established at 1uL /10.000 cells. The optimum ratio for the culture of the three cell populations was 1 MSC: 2 HSC: 2 EC. MS was 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 from the MS preserve their multipotent function.

Discussion: The MS 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.

Biography

Emilia Barreto-Duran is a last year M.Sc. student, with passion and interest for regenerative medicine research. She is actually working with stem cells, with the special interest in mesenchymal and hematopoietic stem cells. She has focused her research on the effect of the microenvironment (bone marrow cell populations and oxygenation) on the proliferation, self-renewal, and differentiation of hematopoietic stem cells.

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