

DERIVATION OF ENDOTHELIAL CELLS AND FORMATION OF MICROVASCULATURE FROM MOUSE EMBRYONIC STEM CELLS

Alan Jesús Gómez-Calderon, Regenerative Medicine & Tissue Engineering Laboratory, Centro Médico Nacional "20 de Noviembre"- ISSSTE ; Facultad de Medicina- UNAM.Posgrado en Ciencias Biológicas

ajgc.022@gmail.com

Juan Antonio Suárez-Cuenca, Regenerative Medicine & Tissue Engineering Laboratory, Centro Médico Nacional "20 de Noviembre"- ISSSTE

Paul Mondragón-Teran, Regenerative Medicine & Tissue Engineering Laboratory, Centro Médico Nacional "20 de Noviembre"- ISSSTE

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Mouse Embryonic Stem Cells (mESC) are pluripotent cells derived from inner cells mass of blastocyst. mESC cells have the ability of in vitro self-renew, preserving the potential to generate the three embryonic layers, mainly due to the expression of important regulators of pluripotency such as Oct 3/4 and Nanog. mESC cells have been widely used in different experimental models of regenerative medicine and tissue engineering. The objective of this study was to derive a monolayer protocol to induce the differentiation of mESC into vascular precursor cells (VPC) and endothelial specialized cells (ESC). The mESC were maintained according to the protocol reported by Mondragón et al., (2011) and the differentiation method was modified of the protocols reported by McCloskey et al., (2006) and Hatano et al., (2013). We compared the differentiation and expansion of the VPC derived from mESC in Collagen Type I and type IV (used as extracellular matrix), as well as two VEGF concentrations (10 ng·ml⁻¹ and 30 ng·ml⁻¹). The collagen Type IV probes the optimal matrix, moreover no differences were found in the use of both VEGF concentrations. We assessed the expression of different proteins as markers for: pluripotent cells (Oct 3/4+), vascular precursor cells (Flk-1+ and PECAM+) and endothelial specialized cells (Ve-Cadherin+) by immunocytochemistry at different time points (120h, 360h, 600h and 840h) of differentiation. Population dynamics during differentiation kinetics, showed that there is an initial expansion of pluripotent cells (Oct4+) from 0 to 120 h; meanwhile the production and expansion of vascular precursor cells (Flk-1+ and PECAM+) was observed from 120 h to 360 h (Ve-Cadherin+); finally the "maturation" process and expansion of specialized endothelial cells was observed from 360 h to 840 h. At the end of the differentiation process (840 h), we detected pluripotent cells (Oct 3/4+: 3.1% ± 1.1%), vascular progenitor cells (Flk-1+: 20.2% ± 6.1%; & PECAM+: 16.4% ± 4.0%) and endothelial specialized cells (VE- Cadherin+: 23.85% ± 4.5%). The overall behavior of population dynamics throughout the differentiation process showed three stages: The expansion of pluripotent cells, the generation and expansion of endothelial progenitors and the maturation of endothelial cells. Specific growth rate (SGR) was calculated for each of the stages of the differentiation; importantly the specific growth rate of pluripotent cells (SGR= 0.0226 h⁻¹) was 1 log higher than the values of □ for the expansion of both endothelial progenitors (SGR= 0.0094 h⁻¹) and endothelial cells (SGR= 0.0011 h⁻¹). Microvasculature formation was observed at 840 h of differentiation process; transmission electron microscopy analyses allowed us to identify a large number of desmosome connections (adherent junctions) between endothelial cells, and the formation of communicative cell junctions, as well as the formation of small and wider intercellular channels at intercellular junction.

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