Fetal stem cells obtained from Amniotic Fluid and Wharton´s Jelly expanded using platelet lysate for tissue engineering applications

A. R. Costa-Pinto[1,2], I. Aleixo[1,2], A. M. Frias[1,2], S. Fernandes[3], L. Rocha[3], R. L. Reis[1,2], N. M. Neves[1,2].

[1] 3B's Research Group, University of Minho, Avepark, Zona Industrial da Gandra, S. Claudio do Barco, 4806-909 Guimarães, Portugal
[2] ICVS/ 3B´s Associate Laboratory, Braga/ Guimarães, Portugal
[3]Genetics Department, Faculty of Medicine, University of Porto, Portugal

Extra-embryonic tissues, such as amniotic fluid (AF) and Wharton´s Jelly (WJ) of umbilical cord, offer many advantages over both embryonic and adult stem cell sources. These tissues are routinely discarded at parturition and the extracorporeal nature of these cell sources facilitates isolation, as well as the comparatively large volume and ease of physical manipulation theoretically increases the number of stem cells that can be isolated.

Autologous approaches to use MSCs, namely from bone marrow, have difficulties regarding the limited availability of large amounts of cells from the patient. Fetal stem cells appear to have even more pronounced immunomodulatory properties than adult MSCs (1, 2). This allogeneic escape mechanism may be of therapeutic value, because transplantation of allogeneic human MSCs in stock would be readily available, as opposed to the culture of autologous cells for subsequent transplantation.

Cell expansion protocols are based on the use of media supplemented with fetal bovine serum (FBS) as a source of nutrientes and growth factors. The animal serum is not completely safe, once there is a possibility of contamination by animal viroses, prions or others contaminants and it is described that FBS used systematically in MSCs subcultivation induces more humoral immune response (3). Additionally anti-FBS antibodies could be detected in patients after receiving MSCs expanded in FBS (4). Platelet lysate (PL) has enormous possibilities in cell therapy, namely because of the high concentration of growth factors that promotes higher cell expansion, such as tissue regeneration (5). A recent study showed that proliferation of MSCs was much higher on PL gel compared to tissue culture plastic (6). The immunomodulatory properties of MSCs are maintained when expanded in culture medium supplemented with PL (7)

Based on these premises we isolated fetal stem cells from AF obtained from amniocentesis and WJ from umbilical cords. These cells were plated and expanded in

low density numbers in basal culture medium with FBS or either supplemented with PL. In each passage cells were counted for proliferation kinetics and prepared for flow cytometry analysis. Expanded populations were analysed both population size and complexity and for the MSCs well-known surface markers (CD34, CD45, CD73, CD44, CD106, CD105, CD29, CD90, CD31) and markers related with immune response (HLA-DR, 80, 83, 86) and embryonic markers SSEA-4 and TRA-1-60.

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