CELLULAR MICROARRAY DESIGN FOR THE CULTURE AND DIFFERENTIATION STUDY OF MESENCHYMAL STEM CELLS

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Lately there has been an increasing interest in the development of tools that enable the high-throughput analysis of combinations of signalling factors and its effect on stem cell biology and differentiation. Taking advantage of the DNA microarray technology, combinations of proteins can be printed on substrates in a microarray format. These combinations of factors function as artificial microenvironments when cells are cultured on them. This technique is usually referred to as cellular microarrays [1, 2].

The aim of this work is to provide an insight into the key steps involved in the cellular microarray fabrication using mesenchymal stem cells (MSCs). The application of this technique for the study of MSCs differentiation appears as an interesting issue due to the promising, and to date mostly empirical, applications these adult stem cells are finding in therapeutics [3-5].

Here, the use of a microarray plotter machine to fabricate protein microarrays for the culture of MSCs is reported (Figure 1). The strategy involved the immobilisation of fibronectin within the spots, as an extracellular matrix protein which allow initial cell attachment (Figure 2.A & B). The whole process for microarray fabrication is reviewed and suitable alternatives for the key parameters are assayed.

Results show that parameters such as the protein density printed on the substrate, the size of the printed spot, the initial cell density per spot and the culture medium, all play an important role in the cellular microarray formation and long term culture of MSCs. Moreover, these parameters can be tailored by a collection of secondary parameters directly associated with the fabrication protocol such as the protein concentration in the solution printed, the inclusion of glycerol in the solution to increase protein stability, the number of solution drops dispensed on the substrate, the cell seeding time and the cell seeding density (Figure 3). In this work, the whole panorama is analysed to find optimised parameters to keep MSCs in culture for periods of time long enough to evaluate spontaneous cell differentiation to osteoblasts (Figure 4). Furthermore, cellular microarrays were successfully implemented using both chemically activated glass and poly(methylmethacrylate) (PMMA) substrates (Figure 1). The last option opens up a wide range of possible applications which combine both surface topography modification and biochemical surface signalling, besides the implementation into micro fabricated devices. The results described are intended to serve as a base for future development of this technique as a tool to study MSCs differentiation.

References:

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Figures:

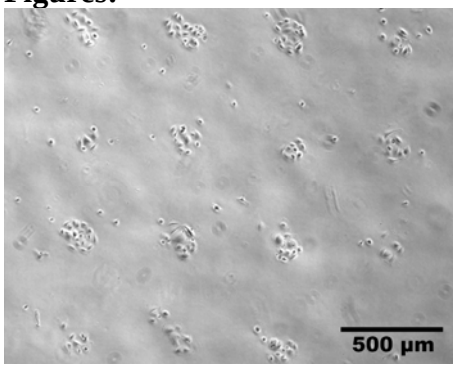

Figure 1: Cellular microarray formed with MSCs. Phase contrast microscopy image of 16 cell populations attached on the printed Fn spots. Microarray formed using PMMA substrates, Fn spots with a concentration of 40 µg/mL in PBS 2% Glycerol, 2% BSA passivation and seeding time of 10 minutes .

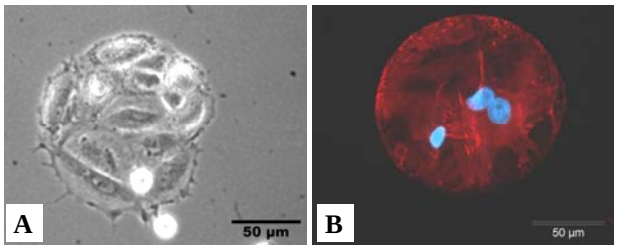

Figure 2. Detail of cells attached on single spots. A. Phase contrast image. B. Immuno fluorescence image of Fn (red) and cell nuclei (blue).

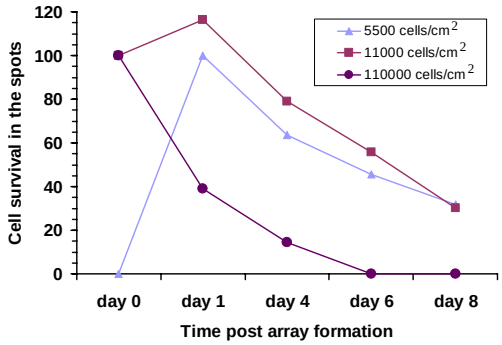

Figure 3. *Effect of cell seeding densities. Temporal plot of the number of cells per spot for increasing cell seeding densities, 8 days follow up. Results are for a spot size of 5 drops of Fn 200 µg/mL in PBS 2% glycerol. Cell survival temporal plot expressed as a percentage of the initial number of cells per spot at day 0 (for 11,000 and 110,000 cells/cm^2) or at day 1 (for 5,500 cells/cm^2).*

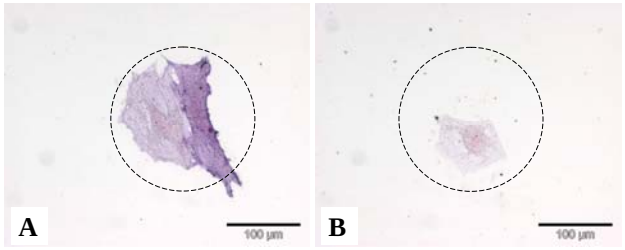

Figure 4. Differentiation. Immunohistochemical staining for ALP expression of MSCs cultured on a microarray for 15 days. Spontaneous differentiation to osteoblasts was shown by positive ALP staining (purple colour) in some of the microarrayed cells (A), while other cells remained unstained (B). Cellular microarray formed using PMMA substrates, cell seeding time of 15 minutes and Fn 40 µg/mL in PBS 2% Glycerol (printed at 1 drop per spot).