

Stem cells derived from bone marrow cultured on a multilayer film and subsequently analyzed by scanning electron microscopy (SEM)

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Abstract

Mesenchymal stem cells (MSC) have been widely studied due to their broad potential of multi-differentiation and self-renewal. It is known that the microenvironment is highly correlated to their biological behavior. Thus, several *in vitro* protocols aim to mimic the tissue microenvironment and complexity. Therefore, in this experiment we choose two biopolymers to create a support environment to the MSC. The cells used were mesenchymal cells obtained from bone marrow due to their rapid development *in vitro*, and their healing potential. The main objective of this protocol was to test a multilayer biofilm to mimic the tissue microenvironment for MSC, maintaining physiological pH and temperature. During the experiment the growth and behavior of these cells in this environment, as well as their ultrastructure were analyzed. As base for the culture we chose a paper sheet with a pore size of 0.40 microns, negatively charged on their surface, which was covered by a multilayer biofilm. The biopolymers of choice were chitosan (material monitoring in biotechnological studies, widely found in nature, biodegradable and biologically active at physiological pH, presenting also a bactericidal action) and hyaluronic acid (mimics the tissue environment, since it is widely found in human and animals tissues as well as being biodegradable). The preparation of the material was done through a layer-by-layer technique developed by Descher et al. in 1992. The cells were plated on this surface and cultured for five days with medium change every 48 hours. The material was fixed with 2,5% glutaraldehyde for 24 hours and processed through standard procedure for scanning electron microscopy. Five samples were analyzed and all presented continued growth, with the cell intertwining between the paper fibers, forming colonies in the material. A relevant fact noticed in this protocol was that the cells remained round, with no morphological changes to fibroblastoid conformation. The ultrastructural analyzes showed the presence of round cells adhered to the paper fibers. Those cells were morphologically different from red blood cells since their format was round and not flattened. It could be observed that the created microenvironment gave full condition for MSC to grow, multiply and adhere on the paper surface, maintaining the characteristic of colony forming. However, the cells remained a round shape with no fibroblastoid morphology as usually observed in



plastic. This particular morphology may be due to the pores of the material. Although the cultured cells did not resemble blood red cells, more tests need to be made to discard the presence of other hematopoietic cells.

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