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The isolation of fibroblasts by volumetric regulation cycles

M. Jian, V. Cobzac and V. Nacu

Laboratory of Tissue Engineering and Cells Culture, State University of Medicine and Pharmacy "Nicolae Testemitanu", Chisinau, Republic of Moldova

The fibroblasts are the most used cells for *in vitro* testing of various substances. The explant modality of fibroblast isolation is a widely used method. In order to fix the explant to the cell culture surface are used various substances or are performed various manipulations, such as applying the mechanical force or explant sticking to cell culture surface after a short period of drying. This paper presents another way of fibroblast isolation using the explant modality, which consists in manipulation with the volume of cell culture media in a well of a 12-well plate during several cycles of cell isolation. From 3 domestic rabbits, under general anesthesia, pieces of dermis were harvested and cutted to 32±8 mm^3 (n=7), and placed by one per well, in a 12-well plate. For 3 days the explants were incubated in 3 ml of cell culture media to ensure the cellular multiplication on the explants. After removing the cell culture media, a small volume of medium was added to maintain the explants moist but fixed to the cell culture surface. When the explants self-attached to the cell culture surface, in the wells were poured 2 ml of cell culture media. After cellular colonies formation, the explants were transferred to another well in which the previous procedure was repeated, starting with the addition of a small volume of medium. The isolated cells from the wells, were cultured to a 80-90% confluence and subcultured in a 75cm^2 flask. As a result, at 16±2 days, from the attached explants (n=6), after isolated cells subculture to a confluency of 70-80%, were obtained $2.9x10^6 \pm 1.6x10^5$ cells per flask, which were identified as fibroblasts by Hematoxylin-Eosin and Masson Trichrome stainings.