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Differentiation of adult marrow stem cells of the rat in coculture with adult cardiomyocytes

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Marrow stem cells (MSC) are pluripotent progenitors of a variety of cells. Recently, the possibility of implantation of MSC in the infarcted myocardium has been extensively studied with promising results in the regeneration of the necrotic area and in the improvement of the cardiac function. After implantation in the myocardium, MSC undergo a milieu-dependent differentiation to express phenotypes similar to the cells in the immediate microenvironment. Among the points not yet elucidated, we wanted to investigate whether or not the differentiation can be initiated *in vitro* by coculture of adult MSC with adult cardiomyocytes.

The aim of this study was to characterize the morphological and biochemical changes occurring in adult MSC cocultivated with adult cardiomyocytes. Stem cells were obtained from the bone marrow of the femurs of humanely killed 6 to 12 months old green fluorescent protein (GFP) transgenic rats by medium flushing into the shaft of the bone, plated at 2000 cells/cm², grown to 90 % confluency and passed at least three times before the experimental use. GFP rats were used to better recognize MSC in coculture. In the pre-coculture stage, confocal microscopy revealed 100 % GFP-positive MSC.

Cardiomyocytes were obtained by enzymatic dissociation of ventricles from humanely killed young adult rats. About 150.000 suspended cardiomyocytes were plated on a glass-bottom dish with 90 % confluence (about 300.000 cells/dish) GFP-positive MSC and kept in 1 % serum M1018 medium plus Insulin-Transferrin-Selenium (ITS) solution (1:1000) and 2, 3-butanedione monoxime (10 mM).

Preliminary experiments revealed in confocal microscopy a good condition of both MSC and cardiomyocytes for at least 48 h after the beginning of the coculture. Moreover immunohistochemistry revealed positive staining against sarcomeric myosin and sarcomeric actin. In cocultures we also observed positivity for connexin 43 both between MSCs and cardiocytes and between MSCs. Intracellular calcium measurements in confocal microscopy suggested also a functional role for these junctions. Further investigation will be planned in cocultivated MSC to identify the expression of specific early-stage cardiac differentiation marker genes and to perform careful morphological characterization of MSC and cardiomyocytes interactions with confocal-deconvolution microscopy. Coculture-committed MSC cells will be also used for biochemical and electrophysiological studies.

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All procedures accord with current national and local guidelines