CELL CRYOPRESERVATION ON NANOSTRUCTURED SURFACES: BLOCK-FACE SCANNING ELECTRON MICROSCOPIC METHOD FOR STYDYING OF THE CELL-SUBSTRATE INTERFACE

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Cell cryopreservation on nanostructured surfaces, as proposed by IBMT [1], may be beneficial for cell survival. This type of preservation modifies the cell-substrate interaction before and after freezing. Cell-substrate distances are sub-micron and must be studied by a high resolution method as TEM. However, cutting cross-sections of cells grown on nanostructures of silicon or metal is not always possible due to a hardness of these materials. Here, we present a block-face method for SEM investigation of cells grown on nanostructured substrates and the first results with nanostructured gold, carbon-nanotube-substrates and black silicon. The proposed method may be widely used for the study of cell- substrate–interaction in new substrates.

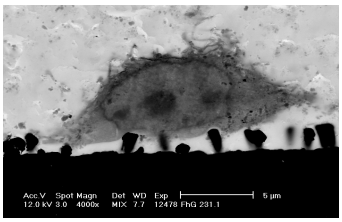

Fig1. Control

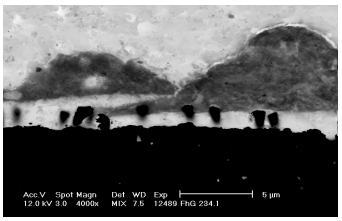

Fig.2. After freezing

L929 mouse fibroblasts were grown on nanostructured substrates mounted in micro-cryo-substrates [see 1]. After vitality tests, the substrates were washed; fixed for SEM; treated with tannic acid and osmium tetroxide and uranyl acetate as previously described [2]; critical point dried and coated with gold or platinum. The same procedure was used for cells after automized cryopreservation [1]. Samples were investigated by SEM in secondary electron mode and then embedded with epoxy resin. Preparations were cross-sectioned with a diamond saw and polished with a diamond spray to create a smooth surface. The embedded cells were then examined by SEM in BSE mode and by X-ray microanalysis.

The inverted BSE of block-face preparations permits study of the cell–substrate interface while simultaneously giving ultrastructural information comparable with TEM imaging (Fig.1: block- face imaging of control preparation, Fig.2: block-face imaging after freezing and thawing). Because of the heavy metal treatment, cell structure is well preserved and high contrast. First measurements of the distance between the cells and substrate ranged up to about 40 nm (Fig.1) in the controls, increasing in some cellular regions after cryotreatment to as much as 500nm (Fig.2). This may be caused of ice crystallization and corresponds to the thickness of one ice crystal layer. Other reasons are discussed. [BMBF grant no 03N8707].

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