The widespread use of mesenchymal stromal cells (MSCs) in tissue engineering requires the development of the cryopreservation methods for MSC-based tissue equivalents, which would provide a high cell viability. We have previously shown the positive effect of pretreatment with sucrose on the post-thaw survival of MSCs in the suspension without dimethyl sulfoxide (DMSO) and fetal calf serum.

The aim of this study was to examine the effect of sucrose pretreatment on the efficiency of MSCs cryopreservation in two- and three-dimensional carriers in presence of DMSO. Two-dimensional culture of MSCs was a cell monolayer on coverslips, three-dimensional one was represented by the cells in the chitin carriers. The samples were cultured in 0.1–0.2 M sucrose and frozen with 1 deg/min rate in a medium with 0.1–0.5 M sucrose and/or 10% DMSO. The survival of the cells was assessed by trypan blue staining (monolayer or suspension), the metabolic activity was estimated by Alamar Blue test (carriers).

It has been found that cryopreservation using 10% DMSO enabled to preserve (92.0 ± 2.0)% of cells in suspension. Cryopreservation of the MSCs adhered and spread on the coverslips allowed to preserve (15.8 ± 5.8)% cells, the metabolic activity of cells within chitin matrices made (40.1 ± 8.9)%.

In order to elucidate the mechanisms of pretreatment effect we have studied the penetration of [14C]-sucrose into MSCs, the behavior of cells in culture and the expression of mitogen- and stress-activated p38 kinase. It has been shown that sucrose penetrates into cells, where it obviously renders a cryoprotective effect. The presence of sucrose in the culture medium does not affect the MSC morphology and their movement on the tissue culture plastic surface. Moreover, the pretreatment results in a time-dependent change in p38 activation, indicating a possible involvement of stress proteins into sucrose cryoprotective effect.

Thus, the pretreatment with sucrose is a promising approach which could be used for further development of the methods to cryopreserve the MSCs on two- and three-dimensional carriers.