Low temperature preservation of mesenchymal stromal cells seeded in various scaffolds for tissue equivalent development

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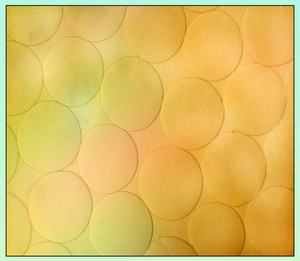
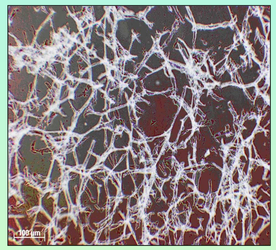
Introduction

Mesenchymal stromal cells (MSCs) can differentiate into various cell types, which make them attractive for regenerative medicine. Preservation of MSCs seeded and cultivated in scaffolds at cryogenic or hypothermic temperatures can serve as ready-to-use transplantation units for tissue repair.

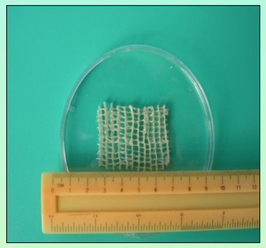
The aim: investigation of ability to proliferation and multilineage differentiation of MSCs within alginate microspheres (AMS) and porous scaffolds before and after different approaches of low temperature preservation.



AMS (Ø 0.5 mm)



AMC (Ø4×1 mm)



DCS (5×3 mm)

Scaffolds: alginate microspheres (AMS), alginate-gelatin macroporous cryogel (AGMC) sponges, plane demineralized chitinous skeletons (DCS) of marine sponge Ianthella basta

Methods

MSCs were obtained from adult human dermal tissue or bone marrow.

For encapsulation MSCs were resuspended in 1.2% solution of sodium alginate (Sigma) and sprayed in a solution containing 100 mM CaCl2.

AGMC were provided by Dr. Lozinsky (Institute of Elementoorganic compounds, RAN, Moscow).

AGMC and DCS of marine sponge Ianthella basta were seeded by MSCs by perfusion method.

MSCs in scaffolds cultivated in α-MEM, supplemented with 15% fetal bovine serum at 37°C and 5% CO_2.

For conventional cryopreservation scaffolds with cells were placed into 1.8 ml cryovials, equipped with medium contains 10 % dimethylsulfoxide (DMSO) in culture medium. After 5 min incubation at 4ºC, the vials were transferred into Mr. Frosty-Boxes, cooled with the rate of 1 ºC/min down to -80ºC and were transferred into liquid nitrogen.

For vitrification MSCs in AMS were placed into cryovial equipped with medium contained 10 % DMSO, 20% ethylene glycol, 20% 1,2-propandiol and 0,5 M sucrose in culture medium and transferred into liquid nitrogen.

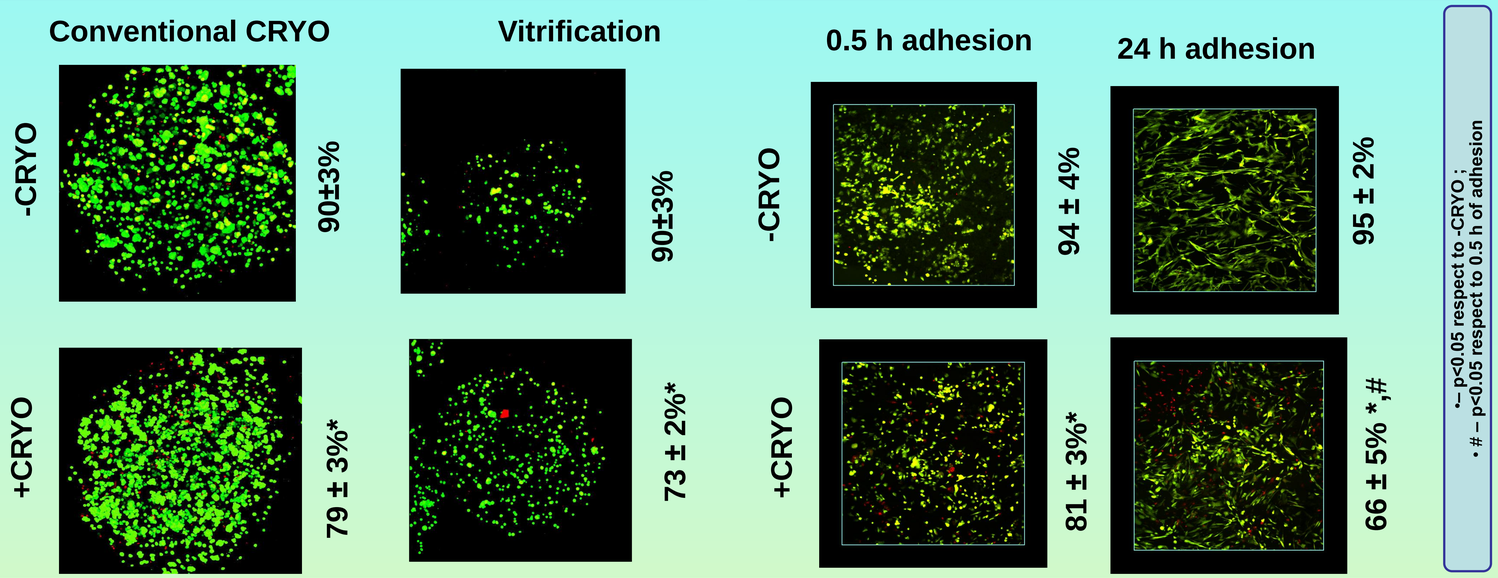
The samples were thawed in a 37ºC water bath.

Viability and metabolic activity of cells were determined by FDA/EB staining, MTT- and Alamar Blue (AB)-tests.

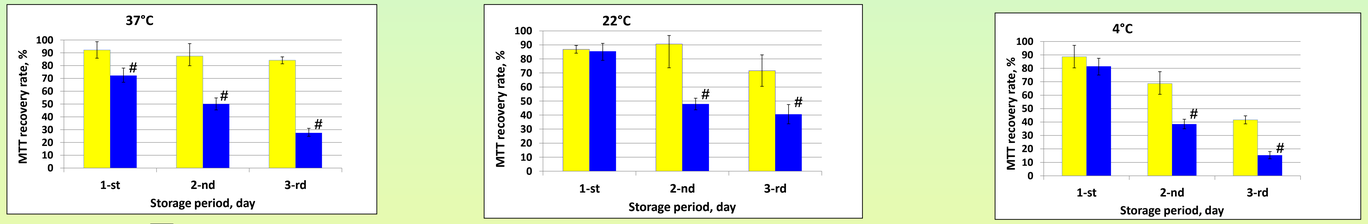
Results were expressed as mean ± SEM.

MSCs encapsulated in AMS can be preserved by Conventional cryopreservation and vitrification (Cryobiology 2013, 66 (3), 215-222).

MSCs spreading on macroporous scaffold surface are more sensitive to conventional cryopreservation (J. Mat. Sci 2014, 25 (3), 857-871).



Encapsulation in AMS delayed cell death during storage in ambient temperatures

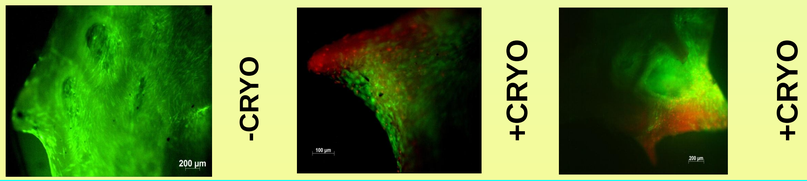


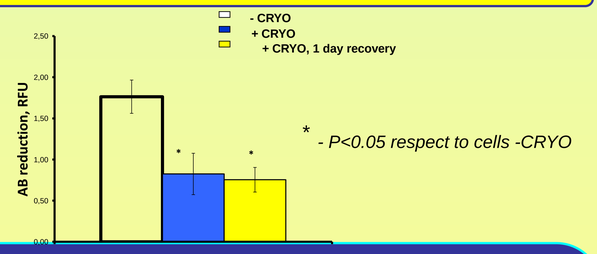
- MSCs in AMS

- MSCs in suspension

- *P<0.05 respect to cells in AMS*

Response of MSCs growing on DCS of marine sponge Ianthella basta on conventional cryopreservation





* - *P<0.05 respect to cells -CRYO*

Conclusions

✓ Response of MSCs seeded and cultivated into porous scaffolds to conventional cryopreservation protocol depended on cell adhesion and spreading as well as structure of scaffolds per se.

✓Between observed three types of scaffold the lowest cryoresistance had MSCs growing as sheets on chitinous marine demosponge Ianthella basta.

✓After cryopreservation in various scaffolds survived MSCs retained abilities to proliferation and differentiation into osteogenic and adipogenic lineages.

✓The data obtained indicate that cryo-banking of MSCs cultivated into tissue engineered scaffolds is feasible for the future regenerative medicine projects.