P-555: Cryopreservation of Cell Sheets of Adipose Stem Cells: Limitations and Successes

F. P. Prata[1], M. T. Cerqueira[2], J. Moreira-Silva[2], R. P. Pirraco[2], R. L. Reis[2], A. P. Marques[2];

[1]3B's Research Group - Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine; ICVS/3B's - PT Government Associate Laboratory; Department of Biomedical Sciences and Medicine, University of Algarve, Guimarães-Braga, Portugal, [2]3B's Research Group - Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine; ICVS/3B's - PT Government Associate Laboratory, University of Minho, Guimarães-Braga, Portugal.

Cell Sheets of hASCs (hASCs-CS) have been previously proposed for wound healing applications (1, 2) and despite the concern for production time reduction, the possibility of having these hASCs-CS off-the-shelf is appealing. The goal of this work was to define a cryopreservation methodology allowing to preserve cells viability and the properties CS matrix.

hASCs-CS obtained from three different donors were created in UP-cell thermoresponsive dishes(Nunc, Germany) as previously reported(1,2). Different cryopreservation conditions were considered: i)FBS plus DMSO(5% and10%); ii)0.4 M of Trehalose plus DMSO (5% and 10%); iii)cryosolution PLL (Akron Biotech, USA); and iv)vitrification. The cryopreservation effect was first assessed for cellular viability by flow cytometry using 7-AAD, and after dissociating the hASCs-CS with collagenase and trypsin-EDTA 0.25%. The expression (RT-PCR) and deposition (western blot and immunocytochemistry) of collagen type I, laminin and fibronectin, and the organization (TEM) of the extracellular matrix was further assessed before and after hASCs-CS cryopreservation to determine a potential effect of the method over matrix composition and integrity. The obtained results confirmed that cell viability is affected by the cryopreservation methodology, as shown before for different CS (3). Interestingly, the matrix properties were not significantly altered and the typical cell sheet's easiness of manipulation for transplantation was not lost. Acknowledgments: RL3 - TECT - NORTE-01-0124-FEDER-000020 co-financed by ON.2 - O Novo Norte, under the NSRF, through the European Regional Development Fund (ERDF); SFRH/BPD/70230/2010.

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