DMSO-FREE METHODS OF PRESERVING MESENCHYMAL STEM CELLS (MSCs) THAT RETAIN HIGH LEVELS OF POST THAW FUNCTION

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A novel, biologically-inspired strategy was developed to improve the preservation of mesenchymal stem cells (MSCs). MSCs are being investigated for the treatment of cardiovascular disorders, diabetes, connective tissue disorders, acute lung injury, amyotrophic lateral sclerosis, kidney diseases and more. To date, over 300 clinical trials involve the use of MSCs, with well over 2000 patients safely treated. Current methods of preserving MSCs are inadequate/ suboptimal. Concerns over poor post thaw function have become so pervasive that it is now common for MSCs to be cultured for 24-72 h prior to administration. These MSCs have a short shelf life (< 24 hours), require special FDA permission, and the process increases cost and reduces access.

The research described here utilizes an evolutionary algorithm to identify combinations of naturally occurring osmolytes that yield high cell recovery post thaw and optimize the composition of a DMSO-free, protein-free medium for cryopreservation of the cells. Additionally, we demonstrate that these novel solutions maintain MSC functionality when evaluated using surface markers, attachment, proliferation, actin alignment, RNA expression, and DNA hydroxymethlyation.

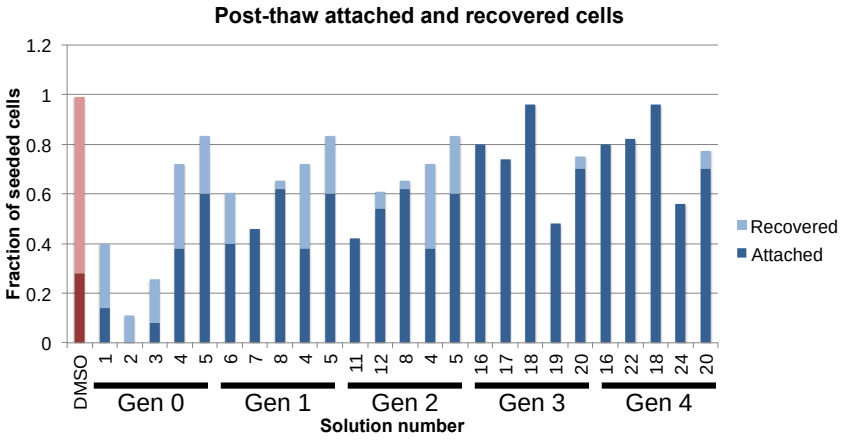

Figure 1 Algorithm optimization of experimental solutions for attachment. With increasing generation (advancing optimization), recovered cells and the fraction of those cells that attach increases.

MSCs were combined with algorithm dictated concentrations of different combinations of osmolytes. Samples were incubated at room temperature to allow for equilibration. Samples were frozen in a controlled rate freezer using a 3°C/min cooling protocol, and stored at -196°C. Samples were thawed in a water-bath at 37°C and immediately enumerated using AO/PI stain on a hemocytometer to calculate recovery. Conventional post thaw characterization was performed to quantify (1) attachment and proliferation; (2) alignment of the cytoskeleton; and (3) flow cytometry cell surface marker expression for normal MSC phenotype (CD73+, CD90+, CD105+, CD45-). Additional molecular analysis was performed to probe RNA expression levels for key cellular function (i.e. IDO) as well as epigenetic characterization of the cells to determine whether a normal methylation state of the cells has been maintained with the new DMSO-free solutions.

The post thaw function of cells improved as the algorithm optimized solution composition for a given combination of osmolytes (Figure 1) suggesting that there is a 'sweet spot' for the solution composition. Conventional post thaw characterization demonstrates that samples of MSCs frozen in DMSO-free formulations perform as well (proliferation, flow cytometry) or better (attachment, RNA, DNA) than DMSO frozen samples, and more closely mimic fresh cell behavior than MSCs cryopreserved in DMSO. We believe that this approach can transform preservation of cell therapies by eliminating adverse effects of DMSO on both cells and patients, streamlining process of cells by eliminating complicated introduction and removal steps.