cartilage regeneration, emerging as a new alternative stragegy to treat knee ostoearthitis. We have developed an allogeneic and biomimetic bioscaffold composed of Platelet Rich Plasma and synovial fluid that preserve and mimics the natural environment of MSCs isolated from knee. However, cryopreservation of knee-isolated MSCs embedded within the aforementioned biomimetic scaffold to create a reserve of young autologous embedded knee MSCs for future clinical applications remained unsolved. Thus, we tested several cryoprotectant solutions combining dimethyl sulfoxide (Me_2SO) and sucrose, quantifying MSCs viability and functionality after thawing. MSCs embedded in bioscaffolds cryopreserved with Me_2SO 10% or the combination of Me_2SO 10% and Sucrose 0,2 M displayed the best cell viabilities and functionality after thawing, allowing their future clinical use in patients with cartilage defects.
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TOWARDS LARGE-SCALE CRYOPRESERVATION: STERILE VITRIFICATION OF ADHERENT HUMAN INDUCED PLURIPOTENT STEM CELLS AND THEIR NEURAL DERIVATES

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Cryopreservation is still the only possibility to store viable cells for long periods. In general, conventional slow freezing methods are efficient enough when it comes to banking of single cells in suspension for subsequent expansion (e.g. human induced pluripotent stem cells, hiPSCs). However, considering adherent cells or multicellular systems that are increasingly relevant for biomedical research and application, slow freezing shows major limitations. (1) Usually the adherent cells have to be enzymatically or mechanically dissociated to single cells or small aggregates prior to freezing, (2) crystallization-induced damaging mechanisms additionally disrupt cadherin- and integrin-mediated cellular contacts, and especially for hiPSC, (3) the recovered viable cell numbers is dramatically reduced compared to the control. Vitrification provides the possibility to overcome these limitations, but requires skilled handling especially regarding sterile procedures, imply small sample sizes and therefore is considered as unsuitable for bulk storage. To launch vitrification for large cell numbers and thus enabling ready-to-use cryopreserved adherent cell systems, we introduce a sophisticated multi-usage cell culture disposable covering comprehensive cell-based workflows from cultivation/differentiation to sterile vitrification. We validated this disposable by a comparative multi-centre study, examining adherent vitrification in the disposable and of suspension-based conventional slow freezing of six hiPSC lines and the accordant hiPSC-derived neural progenitor cells (NPCs). Viability, cell number, immuno staining and FACS as well as gene expression and raster electron microscopic analysis were performed as post thaw quality controls after one day and four days, respectively. Our data shows superior performance of vitrification over slow freezing of both cell systems. Higher numbers of viable cells and metabolic activity could be detected, while the functionality maintained. Together with the option to parallelize the disposable in multi-well formats, vitrification is applicable for large-scale cryopreservation of adherent multi-cellular systems and enables ready-to-use formats for a variety of biomedical purposes.
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Conflict of Interest: None to declare

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ADVANCES IN CRYOPRESERVATION OF ALGINATE-ENCAPSULATED STEM CELLS AND ANALYSIS OF CRYOPRESERVATION OUTCOME

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Cryopreservation of clinically relevant cells and biologics encapsulated in alginate hydrogels is an efficient methodology to preserve their functionality for further application in regenerative medicine and transplantation. The main advantages of alginate encapsulation for cryopreservation include possibility for process scaling up, encapsulation of required cell numbers within viscous environment, which in turn serves as a reservoir for cryoprotective agents, protects cells from mechanical and osmotic stress as well as provides controlled membrane permeability. In this work, we report on our success in cryopreservation of multipotent stromal cells within different alginate hydrogel formulations (solid and coaxial beads of different sizes), analysis of cell viability using the designed μVision software allowing for spatial reconstruction of cryomicroscopic images as well as ice recrystallization using μ Crystal software. The developed approach for cell encapsulation in coaxial alginate beads with varied membrane thickness provides effective formation of tissue-like 3D structures within the core. This approach could serve as a model for cryopreservation of self-assembled structures with cell-cell contacts. For solid alginate beads, optimization of cryopreservation parameters yielded intact alginate hydrogels after thawing according to cryomicroscopic data. High cell viability and recultivation efficiency 24h after thawing (viability 83% by Calcein-AM/EthD-1 staining and 70% by recultivation efficiency) have been achieved. In addition, optimal cryopreservation protocol has been successfully validated for freezing of cell structures within coaxial alginate beads, whereas μCrystal software proved to be efficient for analysis of cryomicroscopic images of ice recrystallization. Taken together, our work provides a comprehensive overview of the main results achieved by the group on cryopreservation of stem cells within alginate solid (microbeads) and coaxial hydrogels.
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ME2SO- AND SERUM-FREE CRYOPRESERVATION OF MESENCHYMAL STROMAL CELLS USING ELECTROPORATION OF SUGARS

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Cryopreservation is the universal technology enabling continuous availability of cell aliquots to meet regenerative medicine demands. However, safety concerns over Me2SO-induced side effects and immunogenicity of animal serum (main components of standard freezing media), support their replacement with non-toxic substances. Due to multiple cryoprotective properties, selected disaccharides, such as sucrose and trehalose, are widely used as additives to various freezing solutions. Conceptually, combined introduction of sugars into cryopreservation media and their pre-freeze loading into cells serves as a novel alternative to conventional cryopreservation workflow. Among diverse techniques for sugar loading (e.g. fluid-phase endocytosis, genetically engineered proteins or nanoparticle-mediated delivery) electroporation is a preferred method in cryopreservation owing to its high-performance speed, safety and accuracy. In this study, we investigated the effect of electroporation-assisted