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Cryoresistance and potential vitrification in lichen thalli

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It is well known that lichens from polar regions can survive conditions of low temperatures and desiccation. However, interspecific differences in their cryoresistance do exist. Lichens in extreme environments must cope with repeated freeze-thaw cycles and very often with added desiccation stress. Lichens are capable of surviving these long periods of low temperatures and desiccation stress in anhydrobiosis, which is a metabolic state induced by desiccation. In this state of near total zero water content lichens are capable to retain some basic metabolic activity, thus surviving these extreme conditions. Yet, underlying mechanisms of lichen cryoresistance are not fully understood. Critical freezing point (CFP) of the lichen thallus, in which all water content of lichen freezes, is a key parameter for understanding cryoresistance of lichens. In addition to the classical cold tolerance mechanisms, there is the possibility of vitrification, which is glass-like non-crystalline solidification. This may represent a parallel mechanism that allows lichens to survive sub-zero temperatures.

To evaluate CFP and vitrification in Antarctic lichens, we chose a method of Differential Scanning Calorimetry (DSC). CFP and potential vitrification event were evaluated on the DSC curves in response to different of thallus hydration. Three lichen species were chosen for this experiment: *Umbilicaria umbilicarioides, U. antarctica,* and *Xanthoria elegans*, these species were selected for their diverse ecological origins and expected tolerance to low temperatures. DSCs were measured in three hydration states: fully hydrated, partially hydrated and fully desiccated to assess the effect of their hydration state on cryoresistance. All three lichen species exhibited considerably high tolerance to freezing temperatures with the critical freezing point ranging from –6.29 to –30.75 °C with relatively low share of crystalized ice. Partially hydrated lichens exhibited higher cryoresistance than lichens that were fully hydrated. Lichens that were fully desiccated showed that they were capable of vitrification, and glass transition was discovered in all three species. In *U. umbilicarioides* and *U. antarctica*, however, the vitrification was more apparent than in *X. elegans*. Overall, the hydration state of the thallus had a significant impact on freezing behavior and also some species-specific differences were observed suggesting differences in the efficiency or stability of these protective mechanisms.

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Optimization of the conditions for the formation and cryopreservation of compatible spheroids of rat mesenchymal stem cells and neural cells

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Under natural conditions, neural cells (NCs) function within a microenvironment formed by glial cells, vascular endothelium, and mesenchymal stromal cells (MSCs), particularly pericytes. *In vitro* modeling of such an environment by generating spheroids that combine NCs and MSCs enables the study of cellular behavior, differentiation, and neuronal survival. Co-culturing NCs and MSCs in spheroids enhances their regenerative potential, offering promising prospects for designing bioconstructs applicable in central nervous system (CNS) therapy, biotechnology, and neuroengineering.

MSCs were isolated from the liver of rat embryos on embryonic day 16 (E16), and NCs were obtained from the brains of newborn rats. Both MSCs and NCs were cultured in DMEM/F12 supplemented with 10% fetal bovine serum (FBS). MSCs of passages 3—4 and NCs of passage 1 were used for spheroid formation. Spheroids were generated using the hanging drop method (20 µl drop, 3000 cells per drop) for 72 hours. To promote self-organization into 3D structures, methylcellulose (MC) was added to the culture medium. Cryopreservation was performed in DMEM/F12 with 10% FBS and 10% DMSO, using a cooling rate of 1 °C/min to –80 °C, followed by immersion in liquid nitrogen.

To optimize spheroid formation, the media containing various concentrations of FBS (2.5, 5, 10%) and MC (0.125, 0.25, 0.5, 0.75%) were tested. Experimental results showed that the optimal combination for spheroid formation of both MSCs and NCs — individually and together in a 1 : 1 ratio — was 0.5% MC and 5% FBS. Other combinations resulted in instability of spheroid formation, loose cell packing, low mechanical resistance, and heterogeneity in size and shape. Under optimal conditions, compact spheroids formed within the first day of culture, exhibiting a regular spherical shape and uniform size (100 ± 20 µm). Viability of such spheroids was 80 ± 10% according to FDA/PI staining.

During further culture on adhesive surfaces, all spheroids attached, and their cells migrated and spread, forming a monolayer. Morphologically, the spreading cells of compatible spheroids were predominantly fibroblast-like, with a small proportion of neuron- and neuroblast-like cells.

Cryopreservation of both mono- and co-cultured spheroids resulted in a significant reduction in cell viability, indicating the necessity for further optimization of freezing and recovery protocols for spheroid cultures. The findings are promising for advancing research in 3D cell modeling and cryopreservation of three-dimensional cell structures.