

sucrose. Samples dried at 22 °C (65% RH, 2 h) in the presence of sucrose had a cooperative phase transition of 37 °C, while those dried without had a phase transition temperature of 46 °C. For the samples dried at 30 °C, the phase transition temperatures for the samples containing sucrose increased to 43 °C, while the media dried bacteria had a phase transition at 53 °C. At higher drying temperatures ($T > 30$ °C) no cooperative phase transition was observed for the media samples while those in sucrose demonstrated a weak cooperative transition around 40 °C. Initial results showed that the temperature-induced protein denaturation kinetics follow a significantly different time constant than the membrane phase transition, pointing to two distinct mechanisms of damage during desiccation. Viability studies confirmed the protective potential of sucrose during desiccation. (Conflict of interest: None declared. Source of funding: None declared).

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69. *Cryobiology of the fresh water rotifer Brachionus calyciflorus*. K.S. Lekha, N. Munuswamy, Department of Zoology, University of Madras, Chennai 600025, India

Cryobiological studies on the embryos (neonates) and adults of *Brachionus calyciflorus* were attempted. After screening a variety of cryoprotectants, a protocol for the cryopreservation has been achieved using cryoprotectants like Me₂SO and methanol. There is an associated depression in the freeze points of the rotifer after equilibration with these two cryoprotectants. Cryoprotection using 10% Me₂SO and 15% methanol was seen to promote various degrees of dehydration of the rotifer on external freezing compared to freezing in a freshwater medium. Low molecular weight cryoprotectants like methanol seem to permeate into both embryos and adults more efficiently. Cryo-microscopic observations revealed variations in the occurrence and location of internal freezing, depending on the cryoprotectants used. The pattern of ice formation was different; with Me₂SO ice formation was unidirectional and blade like, while with methanol the crystals were blunt, posing less threat of the ice crystals penetrating the animal. Using 10% Me₂SO as a cryoprotectant and a cooling rate of -1 °C/min, there was a recovery of 20% of viable amictic females when subjected to a temperature of -30 °C. Use of 15% methanol as a cryoprotectant under similar conditions gave 70% recovery of viable amictic females. The results suggest that a low molecular weight cryoprotectant like methanol affords better cryoprotection than the widely used Me₂SO for cryopreserving the rotifer *Brachionus calyciflorus*. (Conflict of interest: None declared. Source of funding: Department of Science and Technology, Government of India, SR/SO/AS-03/03.)

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70. *Electrofusion of Jurkat cells and giant unilamellar liposomes loaded with trehalose*. Ryo Shirakashi^a, Kazuhiro Yamauchi^a, Randolph Reuss^b, Ulrich Zimmermann^b, Vladimir L. Sukhorukov^b,^a Institute of Industrial Science, The University of Tokyo, 153-8505 Tokyo, Japan; ^b Lehrstuhl für Biotechnologie, Universität Würzburg, 97074 Würzburg, Germany

Native cells introduce large molecules by endocytosis or fusion of vesicles (liposomes). These phenomena are utilized in the field of drug delivery systems where artificial vesicles are fused with biological cells. There are some reports proving that liposomes containing cryo- or lyo-protective sugars have been successfully introduced into living cells, although the uptake or fusion rates were not sufficiently high. This study proposes an effective method for improving the uptake (fusion) rate of cryo-lyoprotective saccharides by cell-vesicle electrofusion. Electrofusion is a conventional technique to enhance the cell fusion rate by dielectric breakdown of the membrane in the contact zone between dielectrophoretically aligned cells or vesicles. In the present study, giant unilamellar vesicles (GUV) made of 1- α egg phosphatidylcholine (PC) and loaded with 300 mOsm trehalose solution were successfully fused with human leukemic cells (Jurkat line), by means of electrofusion. The fusion process was monitored by fluorescent video-microscopy. The GUVs were prepared by the electroformation method. To this end, egg-PC was dissolved in methanol/chloroform containing 0.1 mM octadecyl rhodamine B chloride, a hydrophobic fluorescent dye to stain the GUV membrane. The electroformation chamber consisted of two conductive, ITO coated glass plates. One of the ITO plates was covered with a thin PC layer produced by complete solvent evaporation using dry nitrogen gas. The chamber was filled with 300 mOsm trehalose solution containing 1 mM KCl. Subsequently, an electric field of 10 Hz frequency and 3.3 V/cm intensity was applied for 2 h to produce a dense suspension of GUVs containing 300 mOsm trehalose and 1 mM KCl. The GUVs were then mixed (1/3, v/v) with a suspension of Jurkat cells (10^5 cells/ml) in 300 mOsm trehalose medium (KCl free), in order to reduce the external conductivity below that of the intra-GUV solution. GUV-Jurkat cell suspension was pipetted between microstructured electrodes mounted on a glass slide. An AC electric field (5 MHz, 100 V/cm) was used to align cells and GUVs between the electrodes by positive dielectrophoresis, which also gave rise to tight GUV-cell contacts. Electrofusion was triggered by exposing the contacted cells and GUVs to a DC electric pulse of 2 kV/cm strength and 40 ms duration. Fusion resulted in a rapid cell enlargement and cell membrane staining with the fluorescent dye due to the incorporation of GUVs into cells. Under appropriate field conditions the whole fusion process required less than 1 min. No fusion was induced by field strengths less than 2 kV/cm, whereas higher field intensities (e.g. 4 kV/cm) induced cell lysis. The electrofusion method presented here allows the rapid delivery of large quantities of membrane-impermeable cryo-lyoprotective sugars into living cells. (Conflict of interest: None declared. Source of funding: JSPS.)

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71. *Adapted freezing and thawing procedures to improve the cryopreservation of cell seeded scaffolds*. Inga Bernemann, M. Kuberka, Birgit Glasmacher, Institute for Multiphase Processes and Biomedical Engineering, University of Hannover, 30167 Hannover, Germany

Functional tissue engineering implies the development of engineered cell constructs for reconstructive surgery. Cryopreservation offers the possibility of off-the-shelf-availability of those cell-seeded, 3D scaffolds for example to be independent of fixed surgery dates. To guarantee a high quality of cell seeded constructs after