GLASS VISUALIZATION IN CELLS AND TISSUES BY LOW-TEMPERATURE SCANNING ELECTRON MICROSCOPY (CRYO-SEM)

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Many cryopreservation protocols rely on vitrification, after water being removed by evaporation, cryoprotectant addition or external ice formation followed by quench-cooling. Vitrification guarantees long-term survival because ice nucleii formation is impaired in these extremely viscous solutions. Specimen vitrification monitoring allows optimizing cryopreservation protocols for different species and tissues. However, physical changes associated to glass transition, T_g , are small and difficult to detect. Differential scanning calorimetry (DSC) is frequently used: T_g appears as a small jump in the thermogram base line. DSC yields global information for the whole pan content, and the absence of ice in the particular cells required for survival cannot be acknowledged. When observing biological samples by low-temperature scanning electron microscopy (cryo-SEM), the "etching" protocol increases contrast: after initial sample cooling at liquid nitrogen conditions, temperature is raised under high-vacuum to induce ice sublimation. After metallization, image appears as a distribution of black "indented" regions (ice crystals), separated by clearer areas, not sublimating. An application of etching-cryo-SEM can detect vitrified areas within a specimen. If solutions in its tissues are viscous enough to become vitrified upon introduction in the microscope cryo-chamber, etching is not effective, as vitrified water sublimation rate is very low. As illustrated with both models (vitrification solutions at different dilutions) and real samples (mint axes at different stages of cryopreservation protocols), the "border" between ice forming and vitrifying conditions shows extremely clear. Increasingly dehydrated and viscous tissues produce increasingly clear and detailed images (due to the smaller size of their ice crystals). When dehydration is enough to allow vitrification upon cooling, this detailed image is briskly lost. There is a perfect agreement between the dehydration conditions producing ice thermal events or T_g base line-displacement by DSC with those yielding detailed images or not-etched ones. For real mint samples, there is also agreement for specimen survival.

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