Simple Cyropreservation Techniques to Preserve Cellular Ultrastructure of Freeze Fracture or Sectioning Prior to Image Analysis.

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Abstract

Computer-assisted computer imaging techniques are limited in their value by the extent to which the structure being analyzed deviates from its native state. For intracellular structures, traditional chemical fixations have been well established to leave substantial room for deviation from reality. For example, membrane-bound vesicles can be relocated or abnormally fused and molecules can be extracted. In addition, traditional techniques can substantially diminish antigenicity in immunocytochemical localization protocols. Although the alternative of freeze-substitution and ultra-low temperature embedding are established in the literature, many people have not switched to these techniques. For structures deep within a tissue (up to about 25 μm), these techniques will require an expensive high-pressure freezer (approximately $150,000.00). However, for structures on the surface of a tissue block, small organisms, or single cells in suspension, we will describe fairly simple apparatus and reliable techniques to achieve ultra-rapid freezing routinely (up to about 30 μm). Examples will be shown of the information revealed by application of freeze substitution protocols and of well-preserved structure and antigenicity in tissues that were only exposed to acetone as a fixative after ultra-rapid freezing and embedding at -35°C. In addition, we will describe preliminary efforts (in collaboration with Mr. Chuck Butterick, Director of the Electron Microscopy Center, TTU-Health Sciences Center) to use computer-assisted electron tomography to reconstruct three dimensional structures from freeze fracture and sectioned images. Lists of equipment and detailed protocols will be available upon request. This research was supported by NSF grants (RIR #9016892 and DCB #91-06704) to CHH and the Department of Biological Sciences, Texas Tech University.