Sample Preparation For Scanning Electron Microscopy Using Home-Made Freeze-Fracture Devices

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For observing intracellular structures by scanning electron microscopy, several methods including manual fracture of completely dried tissue blocks, teasing of tissues, and cutting with sharp razors have been used. However, those techniques have some limitations in the application. Recently, the authors made a freeze-fracture device for fracturing frozen tissues. The fractured tissues can easily be subject to chemical etching to disclose the cytoplasmic structures. In this experiment, as a first step, the investigators tested the effect of glutaraldehyde fixation in freeze-fracture-etching using rat liver tissue.

Liver tissue was obtained from a rat euthanized according to the animal experimental protocol. The tissue was cut into small peieces and pre-fixed with 2.5% glutaraldehyde (0.1M phosphate buffer, pH 7.4) for 0, 5, 10, and 15 minutes, followed by a postfixation with 1% buffered osmium tetroxide for 1 hour at room temperature. After cryprotection with DMSO (25%, 50%) for 30 minutes, the tissue was inserted into the hole of the home-made specimen holder and quick frozen in liquid Freon22[1] (Chlorodifluoromethane), and transferred into the liquid nitrogen. Fracture was made with a razor blade set up in a home-made freeze-fracture device (KMEM No.1-K2), and thawed. Chemical etching was done according to the Tanaka's method[2] with 0.1% osmium tetroxide for 72 hours at room temperature with a gentle shaking. Osmium was changed every 12 hours. The etched tissues were then conductively coated with 1% osmium tetroxide and 2% tannic acid, dehydrated, and dried by t-butyl alcohol freeze drying method (Hitachi, ES-2030). Dried sample was attached to the aluminium stub and coated with a osmium plasma coater (OPC 60N).

The fractured sites revealed some organelles and filaments, the structures of which were best preserved in the weakly prefixed samples. Prefixation more than 5 minutes hamper from observing the cytoplasmic details due to the insufficient etching.

Our preliminary results show that the freeze-fracture with a home-made device may be

useful if specimen preparation protocol is refined.

Reference

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