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Short Note

A New Device for Preparing Complementary Replicas¹

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Summary The construction and method of use of a new device for preparing complementary replicas are described. The device is of a simple construction and provides highly satisfactory and reproducible results.

Introduction

Since the introduction of the method by Steere (6) and Moor *et al* (4), freeze-fracturing has been extensively employed for examining the details of cell and membrane organizations. It is now generally believed that the process of freeze-fracturing reveals internal hydrophobic planes of the membrane (1, 2), and exposes two membrane fracture faces, PF and EF-faces according to the convention by Branton *et al* (3). The conventional freeze-fracture preparation allows visualization of only one half of the fracture faces, but examination of the complementary faces revealed by the complementary replica method (7) is necessary for a correct understanding of the freeze-fractured ultrastructures.

The present short technical note describes a new device for preparing complementary replicas which can be installed in a JEE-AFE freeze-etching apparatus which is currently used in our laboratory. The device described here is of a simple construction and provides highly satisfactory and reproducible results.

Method

Device Figure 1 shows a unit constructed for preparing the complementary replicas. Figure 1-a shows a block-holder for fixing a fracture-block to a specimen stage of the freeze-etching apparatus (JEE-AFE). Figure 1-b shows a fracture-block in closed state and Fig. 1-e shows the same block in open state. The upper and lower block are joined with a hinge. Holes for holding specimens are bored through the upper block to the lower block. There are three holes and three specimens can be fractured at the same time. Figure 1-c shows a pair of specimen holders consisting of two

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fine tubes with holes in semi-circular form. Figure 1-d shows the cap for securing the specimen holder to the fracture-block. Except for a part of the block-holder which is made of teflon (* in Fig. 1-a), all parts of the unit are made from brass.

Procedure [1] A pair of fine tubes of the specimen holders is joined as shown in Fig. 1-c, and the holes of the tubes are loaded with specimens. They are frozen by the appropriate cooling rate (generally very rapidly), depending upon the experimental purpose, and stored in a liquid nitrogen bath. [2] The fracture-block is cooled down to -196°C by immersing it in liquid nitrogen. The specimen holders are inserted into the holes of the closed block and covered with caps in liquid nitrogen. [3] The loaded block (b in Fig. 2) is immediately transferred onto the block-holder (h in Fig. 2) which was previously installed on the specimen stage (s in Fig. 2) of the freeze-etching apparatus and a copper-constantan thermocouple (t in Fig. 2) is fixed to the lower block for measuring the temperature of the block itself. [4] After instalment of the fracture-block to the freeze-etching apparatus, the chamber is evacuated to approximately 1×10^{-5} torr. During evacuation, the liquid nitrogen tank (l in Fig. 2) is cooled for preventing the contamination of the specimens. The temperature of the fracture-block is gradually raised at a rate of approximately $10^{\circ}\text{C}/\text{min}$. The size of both the upper and lower block is designed in such a way as to attain the same rewarming rate. [5] When the temperature of the fracture-block reached -100°C , the upper block is forced open by the knife-arm (a in Fig. 2) to an open state, and as a result the specimens are fractured. [6] The fracture surfaces are replicated by platinum-carbon immediately after fracturing or after etching for a few minutes. Then, the replicas are prepared followed by routine method (5). Since the form of replicas is semi-circular, it is quite easy to match the complementary portions.

Figures 3 and 4 show some results obtained using this device and the specimens show good complementation. It is expected that this device will be applicable for preparing the complementary replicas and will give a correct understanding of the freeze-fractured ultrastructures.

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Explanation of Figures

- Fig. 1.** A unit for preparing complementary replicas. a, a block-holder; b, a fracture-block in closed state; c, a pair of specimen holders; d, a cap; e, a fracture-block in open state. $\times 1$.
- Fig. 2.** A photograph of the fracture-block (b) and block-holder (h) installed in the freeze-etching apparatus. (t) thermocouple, (a) arm of knife, (l) liquid nitrogen tank, (s) specimen stage.
- Fig. 3.** Electron micrographs of two complementary replicas of a red blood cell, suspended in isotonic saline solution, frozen by direct immersion in liquid nitrogen, fractured at -100°C and etched to -94°C and immediately replicated. a, PF-face; b, EF-face. $\times 53,000$.
- Fig. 4.** Electron micrographs of two complementary replicas of a yeast cell, suspended in distilled water, frozen by direct immersion into Freon 22 kept at approximately -160°C , fractured at -100°C , and immediately replicated. $\times 32,000$.



