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IT-4-P-5966 Effect of hydroquinone treatment on OTO en bloc stained biological specimens.

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INTRODUCTION

In recent three dimensional (3D) ultrastructural reconstruction techniques such as serial block face scanning electron microscopy (SBFSEM), TEM-like ultrastructural images of biological specimens are directly obtained from block surface of resin embedded specimens. Since this TEM-like block face images (BFI) is usually obtained using backscattered electrons (BSE) as a material contrast image, specimens are stained strongly by heavy metals prior to embedding into resin. In order to enhance the membrane contrast for BFI, we usually stain specimens by the method of Deerinck (2010). As a recent large volume reconstruction requires very long time to obtain image sets, we need a new staining method which provides much higher contrast that enable to acquire images in a shorter time. Takahashi et al. (1986) have reported that hydroquinone (HQ) treatment during the traditional electro-conductive staining increases specimen conductivity and drastically reduces charge problem for SEM observation. They concluded that HQ treatment might increase the efficient secondary electron (SE) generation. As BFI could be obtained not only by BSE but SE, we examined whether HQ treatment in en bloc staining protocol increased the contrast for BFI using SE in this study.

MATERIALS & METHODS

C57BL/6 mouse liver was used. The animals were deeply anesthetized with diethyl ether and sodium pentobarbital, and fixative of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) was transcardially perfused through the left ventricle with heparin containing saline. After perfusion, liver tissues were removed and cut into small cubes about 1 mm^3 in the fixation, and were further fixed in the same fixative for 2h at 4℃. After that, en bloc staining was performed as follows: The specimens were treated with reduced-OTO staining method (1.5% potassium ferrocyanide and 2% OsO_4, 1% TCH, 2% OsO_4). Subsequently specimens were treated with 1% HQ solution. Some specimens were skipped this step as a control. Then, they were further stained by 4% uranyl acetate and Walton’s lead aspartate solution. After staining, specimens were dehydrated in an ethanol series and were embedded in epoxy resin (EPON812, TAAB). The surface of resin block with specimens was observed by SEM (Quanta 3D FEG, FEI).

RESULTS AND DISCUSSION

The contrast of SE images was drastically increased by HQ treatment, although there is no effect for BSE images. This result suggests that HQ treatment effectively enhances SE generation from specimens. This enhancement may accelerate data acquisition speed for SBFSEM 3D reconstruction.