Preparation of Biological Samples for SEM Observations using Ionic Liquid

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Wet biological samples generally require pre-treatment before SEM observations. It consists of laborious procedures and takes from several hours to a day or more. Recently, the use of Ionic Liquids (ILs), which are unique materials because of non-volatility and high electrical conductivity, was proposed for simple and rapid preparation of wet biological samples [1]. The shapes of small insects and bacteria were observed by SEM using the IL-treatment. In this work, the possibility of ILs for SEM observations of cells has been investigated.

As for the test samples, plants such as carrot roots and eggplants were used. Thin sections were hand-cut using a razor blade, and then fixed with 3% glutaraldehyde solution for 2 h. After washing with phosphate-buffered saline (PBS), the samples were stained with Platinum Blue. Then the samples were suspended in an IL solution. The IL used in this study was 1-ethyl-3-methylimidazolium tetrafluoroborate (EMI-BF4) diluted with distilled water. After the IL-treatment, the samples were placed on a silicon substrate with a conductive carbon tape in between. The edges of the samples were also covered with the conductive tape. After this, the IL solution remained on the sample surface was absorbed by patting with a filter paper. Then the whole samples were put in a vacuum chamber to see if deterioration occurs on the samples under a vacuum environment. It was found that the covering is effective to obtain samples with little shrinkage under a vacuum condition. After these pre-treatment, the samples were transferred to the SEM chamber (Hitachi S-4300), and observed with accelerating voltages of 3 to 5 kV.

First, the processing time and the concentration of the IL were clarified. SEM observations of the samples treated with dilute solutions (< 5% in volume) showed a sign of charging and dehydration. The samples treated with denser solutions (> 50 %) tended to be deteriorated. The treatments with 10-20% IL solutions in distilled water gave good results. The processing time was less sensitive to the result. Fig.1(a) shows the low magnification SEM image showing the cells of carrot root. With increasing the magnification larger than 5k, the presence of the IL was seen in places. These IL remains on the sample as a small droplet or a thin layer as shown in Fig.1(b). Therefore, care may be needed for a higher magnification work. But, the IL technique is easy and quick, may be useful for rapid SEM examinations of biological samples.

[1] S. Abe, et al, Nano Biomedicine 6(1), p.41-46, 2014.

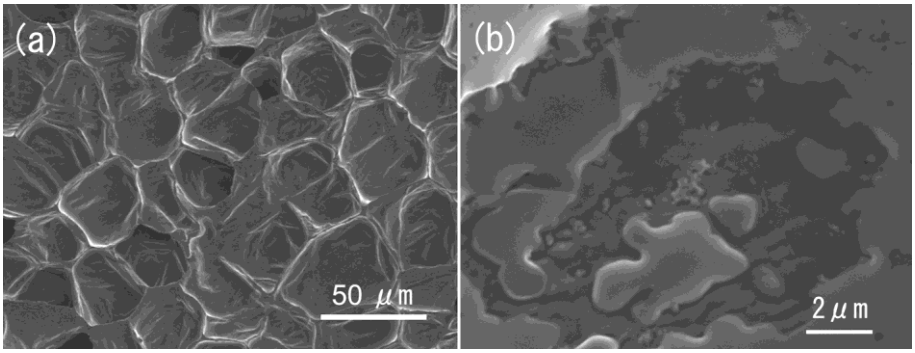

Fig.1 Low (a) and high (b) magnification SEM images of carrot root.

IL treatment condition: 20%, 40 min.