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New Preparation Method using Ionic Liquid for Fast and Reliable SEM Observation of Biological Specimens

Mari Sakaue[1], Masamichi Shiono[1], Mami Konomi[1], Junichiro Tomizawa[2], Eiko Nakazawa[1], Koji Kawai3, Susumu Kuwabata[4]

[1.] Tokyo Solution Lab., Hitachi High-Technologies Corp., KANAGAWA SCIENCEPARK, R&D BUSINESSPARK BLDG C-1F, 3-2-1, Sakado, Takatsu, Kawasaki, Kanagawa 213-0012, Japan
[2.] Hitachi High-Technologies Corp., 882, ichige, hitachinaka, Ibaraki 312-8504, Japan
[3.] Miyoshi Oil & Fat CO., LTD. 4-66-1, Horikiri, Katushika, Tokyo 124-0006, Japan
[4.] Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871 Japan

For SEM observation, it is necessary for biological specimens to be treated with several types of preparation media to preserve their shape under vacuum. Ionic liquids are unique materials because of their natural incombustibility, non-volatility, and high ionic conductivity. Here, we used an ionic liquid to prepare samples for EM observation [1] [2]. The ionic liquid, IL1000 has been designed for use in EM sample preparation with a high level of safety and high solubility [3]. Figure 1(a) shows the SEM images of a *Helicobactor Bilis* sample prepared by conventional procedures. To preserve the flagella structure, the sample was immobilized on the cover slip coated with poly-L-lysine, and freeze-dried after fixation with 2 % glutaraldehyde (GA) in 0.1 M phosphate buffer and dehydrated with acetone in descending concentrations. The conventional sample preparation method takes approximately 8 hours. The surface of the cell body and some flagella are clearly observed (figure 1(a)). On the other hand, in the ionic liquid (IL) method, the sample fixed by the buffered 2 % GA is immersed in 10 % IL1000 solution for 15 minutes and dropped onto filter paper. The sample is then directly transferred into the SEM without further drying. The resulting SEM image of this sample clearly shows the helical shape of the bacteria and flagella (Figure 1(b)). Figure 2 shows the SEM images of three-dimensionally cultured hepatocytes on a nano-pillar prepared by different procedures. The spheroid is fixed with 2 % GA in 0.1 M phosphate buffer. The IL method sample was treated with 5% IL1000 solution for 15 minutes and the excessIL1000 liquid was then absorbed away. Figure 2(a) shows the fixed cell observed at low vacuum SEM. The cell structure is observed without charging. However, figure 2(b) shows that the cell is not fully attached to the nano-pillar due to slight tissue shrinkage (→). As shown in figure 2(c, d), the SEM image of the spheroid treated with IL1000 indicates that the connection between tissue and nano-pillar by the fine pseudo-pod (○) can be imaged under high vacuum conditions without any shrinking or charging.

Figure 3 shows the SEM images of mold growing on a rice cake. The square cut sample x5mm^2 , was immersed in the 10 % IL1000 solution for approximately 4 hours, and then directly transferred into the SEM. Fine threads protruding from the rice cake are clearly observed (fig.3a) and the higher magnification image shows the clear and smooth surface of the spores. These results indicate that the IL method for biological sample preparation greatly reduces preparation time, and is additionally better at preserving the sample's original shape in the SEM.

References

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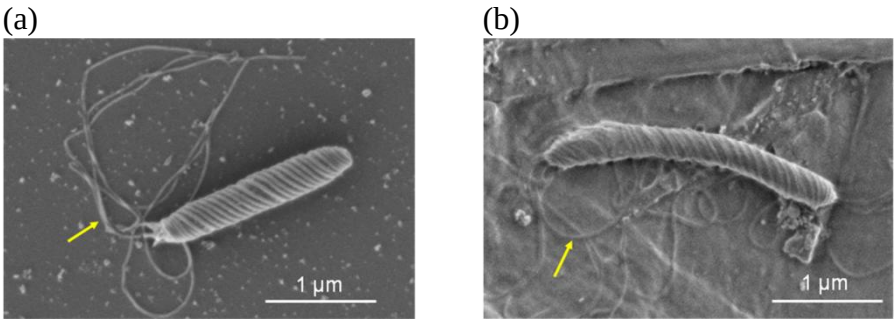

Figure 1. SEM images of *Helicobacter Bilis*
(a) Frozen dried sample fixed by GA. (b) Ionic liquid IL1000 treatment Instrument: SU6600, Acc. Volt. 1 kV, Magnification: x 30,000(a), x 25,000(b).

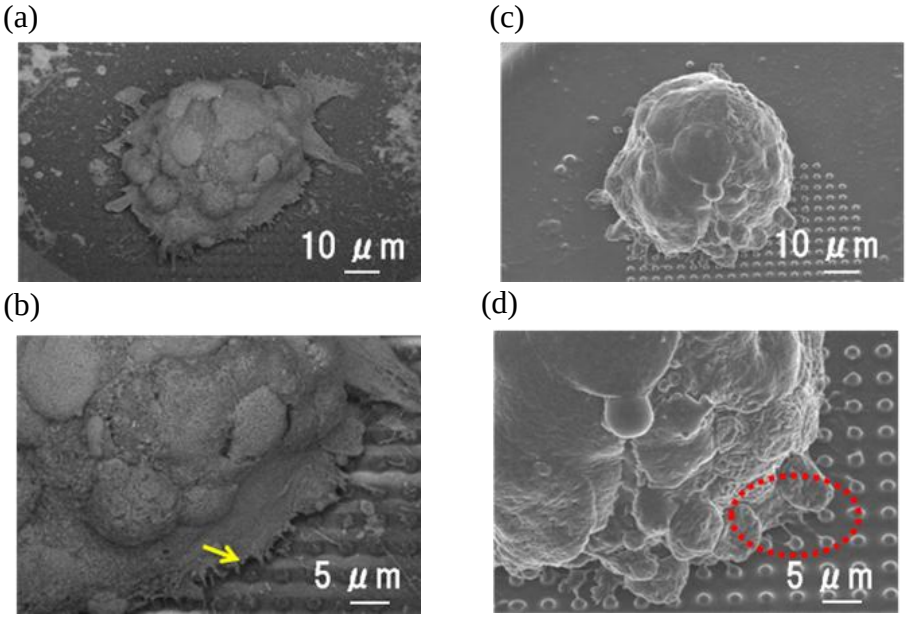

Figure 2. SEM images of rat hepatocyte spheroid cultured on nanopillars.
With no ionic liquid and under low vacuum observation (a,b), 5 % ionic liquid treatment (c,d). Instrument: SU6600, Acc. Volt. 5 kV, Magnification: x 1,000(a,c) x 2,500(b,d), Tilt 35°.

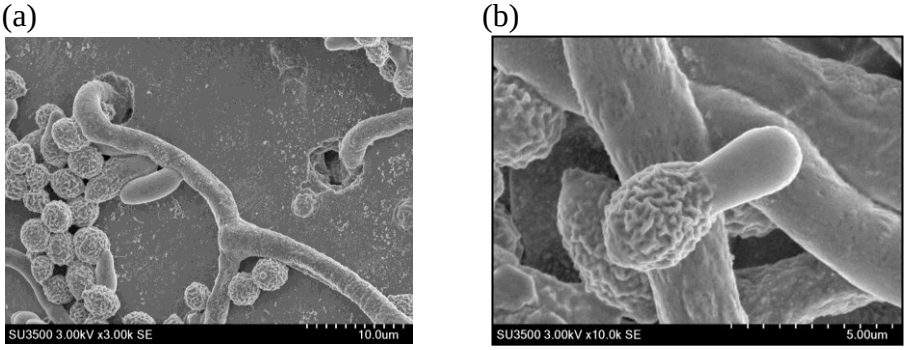

Figure 3. SEM images of mold growing in the rice cake. 10 % ionic liquid treatment
Instrument: SU3500, Acc. Volt. 3 kV, Magnification: x 3,000(a), x 10,000(b).

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