A Study on Biological Sample Preparation for High Resolution Imaging of Scanning Electron Microscope

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Abstract. Green chemistry has drawn attention for synthesizing nanoparticles as it utilizes an eco-friendly nontoxic chemical in the synthesis protocol. In addition, researchers start looking into the potential of organism such as bacteria, fungi and plants for synthesizing nanoparticles. Synthesis of nanoparticles requires details on structural analysis before/after synthesis in order to evaluate and control the quality of nanoparticles. Scanning electron microscope (SEM) has been widely used as an imaging instrument to structurally characterize nanoparticles before/after synthesis process. Maintaining the vacuum level in SEM system and controlling contamination originated from the sample are crucially important to enable high resolution imaging. In addition, sample surface need to be conductive to prevent image distortion due to the electron charging effect. Hence, raise the importance to study the method in preparing biological sample for SEM imaging. This work focuses on the three approaches of drying process for plant, bacteria and algae samples. Proper selection of preparation method on each samples involving air-drying using HMDS, CPD and FD methods have been done in order to prevent surface tension effect during drying process, which will result in the modification of the original structure. Comparison was made to get insight on the suitable sample preparation method for the specific biological sample. It is found that air-drying or CPD methods are suitable for the liquid-rich biological sample, while FD method can be a preference for biological sample that contains less liquid.

1. Introduction

Research in the synthesis of nanoparticles has experienced tremendous progress due to its application in various domain of science and technology. Composition, morphology and dispersity are some of an important factor for synthesizing nanoparticles in order to establish the functional properties of a specific material/application. Recently, green chemistry has drawn attention for synthesizing nanoparticles as it utilizes an eco-friendly nontoxic chemical in the synthesis protocol. In addition, researchers start looking into the potential of organism such as bacteria, fungi and plants for synthesizing nanoparticles [1–3].

In any researches on functional materials and devices, it is essentially important to analyze micro/nano-structures and to control them by nanostructural characterization [4–6]. Specifically, for the synthesis of nanoparticles, it requires details on structural analysis before/after synthesis in order to evaluate and control the quality of nanoparticles. For that purpose, scanning electron microscope (SEM) has been widely used as an imaging instrument to structurally characterize nanoparticles

before/after synthesis process. SEM imaging is performed in a high-vacuum chamber where a high-energy of focused electron beam scans across sample surface to generate a variety of signals from the interaction of electron-sample and reveal information of sample such as morphology and composition. During observation, it is very important to maintain the vacuum level of the chamber and prevent contamination originated from the sample. This becomes critical when it comes to the biological sample due to its nature of liquid-rich. If this liquid was not properly removed, it will evaporate in the vacuum chamber once the electron beam is focused onto the sample. As a consequence, it will create contamination and affect the quality of electron beam and chamber vacuum level. In addition, biological samples need to be conductive to prevent "charging effect" which is due to the accumulation of electron on the non-conductive sample surface. All of the aforementioned factors will consequently drop the quality of imaging in SEM.

Therefore, to enable a high quality of SEM imaging on biological sample, sample need to be dried with a proper selection of methods. However, the crucial problem during drying process is to prevent surface tension effect that will affect the integrity and original structure of the sample. And it should be noted that the protocol of sample preparation is different between each biological sample due to its original structure and the volume of fluid consists in the cell itself. Therefore, the aim of this work is to study on the sample preparation of a specific biological sample group. Three different type of methods involving air-drying method, critical-point drying method and freeze drying method were used in the study. The comparison was made to get an insight on the suitable sample preparation method for the high resolution SEM imaging in the specific biological samples.

2. Experimental Details

The biological samples that were involved in this study were divided into three main groups which is plant-based (fresh and none-fresh/dried) sample, algae and bacteria. Meanwhile, methods for the drying process were air-drying using hexamethyldisilazane (HMDS) solution, critical-point drying (CPD) using CO_2 at critical point of 1072 psi / 31°C, and freeze drying using liquid nitrogen. As for the air-drying and CPD methods, prior to the drying, sample was undergone a fixation using McDowell Trumps fixative, follows by dehydration using ethanol at different percentage. After the drying was completed, samples were then coated with platinum using auto-fine coater for 30 seconds to produce a conductive surface. SEM imaging was performed in MJIIT Microscopy Laboratory using field-emission scanning electron microscope (JSM-7800F) and low-vacuum scanning electron microscope (JSM-IT300).

3. Results and Discussion

Figure 1 shows the image of fresh crepe jasmine and its LVSEM images. In all images, the stoma of the leaf can be clearly seen, with opening size of stoma ranging from 1.5 – 4.2 μm. Minimal existence of mucus can be observed in the samples prepared by air-drying and CPD methods which is considered due to the fixation and dehydration solution. Meanwhile, for the non-fresh/dried rice hulls samples, slight different in sample integrity can be observed, as shown in FESEM images of Figure 2. The original round structure on the non-fresh/dried surface of rice hulls was slightly flattened when the sample was prepared using air-drying method. This might be considered due to the absorption of solution by the dried sample during its contact with fixation and dehydration solution. The rigidity of the round shape on the surface is maintained when the sample was prepared using FD method as the sample only had a direct contact with a liquid nitrogen that enables a rapid freezing before the drying process. In addition, the existence of fine hairs on its surface might assist in maintaining the rigidity of the sample. Figure 3 shows an FESEM images of E-Coli bacteria prepared using air-drying and FD methods. It can be observed that the original structure of the sample prepared using FD methods has been slightly modified, which is considered due to the rapid freezing in liquid nitrogen. In addition, mucus can also be seen in both samples suggesting that the removal of solution during sample preparation process is incomplete. Same results can be observed in the sample of algae, as shown in Figure 4.

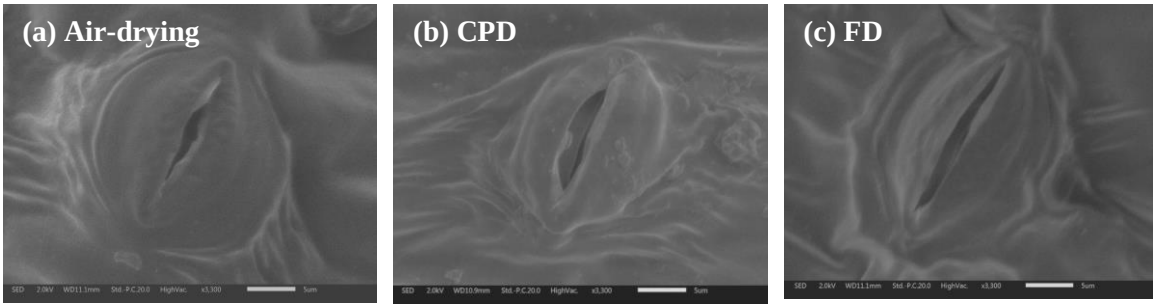

Figure 1. LVSEM image of fresh crepe jasmine leaf sample

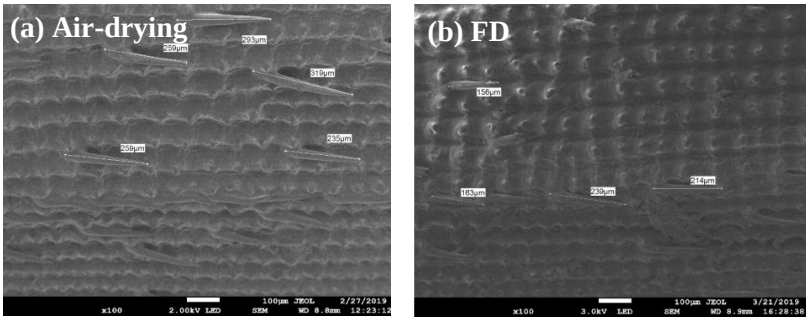

Figure 2. FESEM images of rice hulls (non-fresh/dried) sample

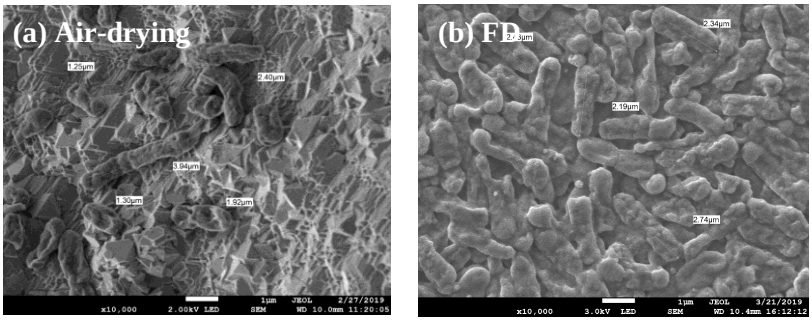

Figure 3. FESEM images of E-Coli sample

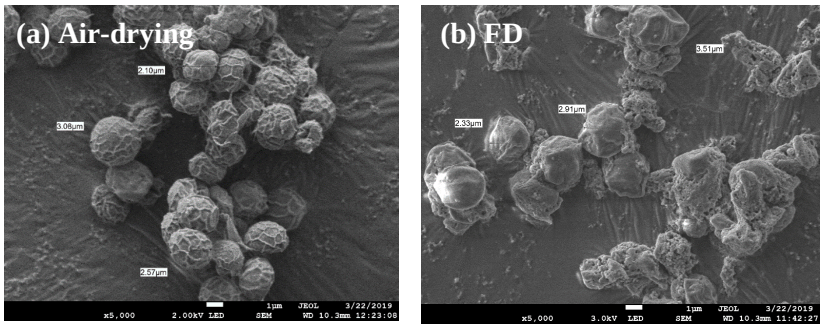

Figure 4. FESEM images of algae sample

4. Conclusion

This paper presents a results on the study of biological sample preparation for high resolution imaging using scanning electron microscope. Different approaches on sample preparations involving air-drying process using HMDS, CPD and FD methods were introduced to evaluate the quality of imaging based on respective sample preparation methods. The comparison was made to get insight on the suitable sample preparation method for the specific biological sample. It is found that air-drying or CPD methods are suitable for the liquid-rich biological sample, while FD method can be a preference for biological sample that contains less liquid.

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6. References

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