Effects Of Different Biological Sample Preparation Techniques On Image Quality Using Scanning Electron Microscope

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A scanning electron microscope (SEM) is a suitable technique for biological research as it provides a better understanding of surface morphology at both micro- and nanoscales. However, since the samples must be prepared before the SEM imaging, the likelihood of distortions and artifacts at any stage during the SEM characterization is a concern to obtain both qualitative and quantitative information. Thus, the full potential of SEM is not fully realized for the biological samples. In this study, lung cancer cells (A549) were prepared using four different techniques, such as 1) 2.5% glutaraldehyde and 0.4% paraformaldehyde in Na-P buffer, 2) 4% glutaraldehyde and 0.4% paraformaldehyde in Na-P buffer, 3) 3.7% formaldehyde solution in Na-P buffer, or 4) 50% ethanol in Na-P buffer, with three different post-fixation procedures applied: 1) dual treatment with osmium tetroxide (OsO_4), 2) single treatment with OsO_4, or 3) no OsO_4. Samples were coated with 1 nm of the most commonly used coating materials: 1) carbon and 2) gold-palladium with Gatan PECS 682 to ensure conductivity. The samples were then examined using a JEOL JSM 6500-F field emission SEM. The results showed that a thin carbon coating is insufficient to acquire high-quality information. A gold-palladim coating, on the other hand, preserved surface morphology while exhibiting good conductivity. Fixatives with 2.5% and 4% glutaraldehyde successfully maintained the morphology and integrity of the cells without causing any noticeable deformities. However, structural disintegration resulted from the ethanol- and formaldehyde-based fixatives. Additionally, OsO_4 treatment with glutaraldehyde fixatives improved the image quality over other treatments.

Keywords: Biological samples, scanning electron microscope, coatings, fixatives, osmium treatment.

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