doi:10.1017/S1431927615001725 Paper No. 0093 Microsc. Microanal. 21 (Suppl 3), 2015

Comparison of Sample Preparation Methods for Analysis of Mucus-Secreting Colon Cancer Cells by Scanning Electron Microscopy

Xin Wang[1,2], Reiner Bleher[1,2], Vadim Backman[3], Gajendra S. Shekhawat[1,2], Vinayak P. Dravid[1,2]

1. Northwestern University Atomic and Nanoscale Characterization Experimental Center, (NU*ANCE*), Northwestern University, Evanston, IL 60208, USA
2. Department of Materials Science and Engineering, Northwestern University, Evanston, IL 60208, USA
3. Department of Biomedical Engineering, Northwestern University, Evanston, IL 60208, USA

Scanning electron microscopy (SEM) is a well-established technique for obtaining morphological information of biological samples with high spatial resolution. However, proper sample preparation is essential to avoid or minimize sample alterations, e.g. shrinkage, molecular structure collapse, and loss of soluble components during processing. Chemical fixation is most commonly used to achieve meaningful results for analyses of initially hydrated biological samples with structural and molecular integrity under the high vacuum condition required for SEM. In this study, we compared the cellular surface structure of mucus-secreting colon cancer cells prepared by critical point drying (CPD), by turbo freeze drying (TFD), or with high-pressure freezing and cryo SEM, which is the current gold standard for the preservation of native sample morphology.

In order to investigate the differences in appearance between results obtained by CPD, TFD, and cryo SEM, we have chosen mucus-secreting human colon cancer cells (CSK, shRNA transfected HT29 cells [1]) as a model system. Cells were seeded on gelatin-coated cover slips (Φ = 12 mm) in culture medium, grown for less than 24 hours, and washed three times in sterile D-PBS. For CPD, cells were fixed in glutaraldehyde (2.5%), formaldehyde (2%) and tannic acid (0.5%) in PBS for 30 min at room temperature. The cells were briefly rinsed three times with D-PBS and then three times with DI water. Cells were dehydrated through a series of ascending ethanol concentrations of 25%, 50%, 75%, 90% and 100% for 5 min each, followed by critical point drying (Samdri-795, Tousimis). The TFD samples were chemically fixed and rinsed similarly as the CPD samples before plunge-freezing in liquid ethane (Vitrobot Mark III, FEI) and turbo freeze drying (K775X, Emitech) over a 6-hour period in a vacuum. CPD and TFD samples were coated with osmium (OPC60A, Filgen) and observed in a SEM (S4800-II, Hitachi) at an accelerating voltage of 5 kV. For cryo-SEM, cells were cultivated in a T-25 flask, trypsinized, diluted into 4 mL of culture medium and centrifuged at 100×g for 10 min to form a pellet. The supernatant was aspirated and the cells were resuspended in a small volume of PBS. 3 mm sample carriers with 150 µm recessions (Technotrade) were filled with the cell suspension, mounted, and high-pressure frozen (HPM100, Leica). The frozen samples were freeze-fractured at -120^{o} C, etched for 6 min at -105^{o} C and coated with platinum in a high vacuum cryo coater (ACE600, Leica). Samples were observed at -120^{o} C in a SEM (S4800-II, Hitachi) equipped with a cryo stage at an accelerating voltage of 2 kV.

SEM images of CPD and TFD cell surfaces showed overall comparable cell surface morphology with a dense brush of microvilli and few mucus conglomerations. The microvilli in the CPD samples appeared to be marginally smaller in diameter (d = 94.0 ± 11.7 nm, n = 10), more numerous, and clustered in bundles, compared to the individually spaced microvilli in the TFD samples (d = 103.4 ± 18 nm, n = 10). In both samples, the plasma membranes were well

preserved with no visible membrane damage, such as breakage or pitting. However, rapid freezing is used to provide superior ultrastructure and to preserve the cellular surface closer to the native state than any chemical fixation procedure [2]. In the cryo-SEM samples of high-pressure frozen cells, only the upper regions of individual microvilli were visible, and the surface of the cells appeared to be coated with an even layer of mucus. The microvilli were slightly larger in diameter as in the TFD samples (d = 115.2 ± 22 nm, n = 10). When cells were plunge-frozen and freeze dried without prior chemical fixation, microvilli were not distinguishable at all (not shown). In summary, the high-pressure frozen cryo-SEM samples presented evenly distributed mucus on the cell surface as opposed to localized mucus conglomerations on CPD and TFD samples. Also, the mean diameters of microvilli were largest in the cryo-SEM samples and smallest in the CPD samples.

References:
[1] D Damania, H Subramanian, A Tiwari, Y Stypula, D Kunte, P Pradhan, H Roy and V Backman, Biophysical Journal (2010) 99: 989-996.
[2] H Schatten, Micron (2011) 42: 175-185.
[3] L Denault *et al.*, Microscopy & Microanalysis 14 (Suppl 2), 2008.
[4] Electron Probe Microscope studies in this work made use of EPIC (Electron Probe Instrumentation Center) facilities at NUANCE (Northwestern University's Atomic and Nanoscale Characterization Experimental Center), which has received support from the MRSEC program (NSF DMR-1121262) at the Materials Research Center, and the Nanoscale Science and Engineering Center (EEC-0118025/003), both programs of the National Science Foundation, the State of Illinois and Northwestern University.

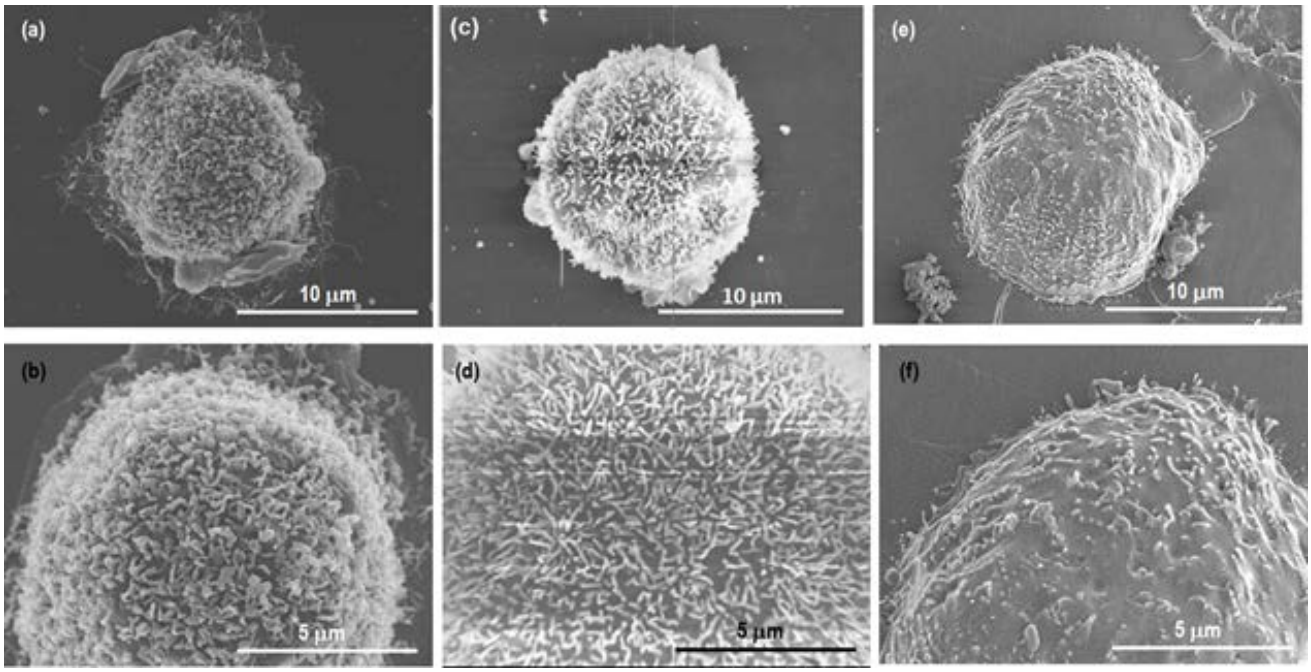

Figure 1. SEM images of human colon cancer cells (CSK) prepared by chemical fixation and CPD at magnifications of 5,000x (a) and 10,000x (b), TFD sample with pre-fixation by glutaraldehyde, formaldehyde, and tannic acid at 5,000x (c) and 10,000x (d), and a freeze-fractured cryo-SEM sample at 5,000x (e) and 10,000x (f).

https://doi.org/10.1017/S1431927615001725 Published online by Cambridge University Press