

# Observation of Microvilli in Intestinal Epithelial Cells Utilizing a Scanning Electron Microscope

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## Background

A functional three-dimensional (3D) Intestinal System, made up of silk scaffold and human intestinal cells, has now been previously reported (1). This system has been used to test the effects of Titanium Dioxide Nanoparticles ( $TiO_2$  NPs), in intestinal epithelium.  $TiO_2$  NPs are widely found in food-related consumer products. Understanding the effects of  $TiO_2$  on the intestinal barrier and absorption is essential for the safety assessment of these food-related products that will be in contact with the cells.

## Motivation

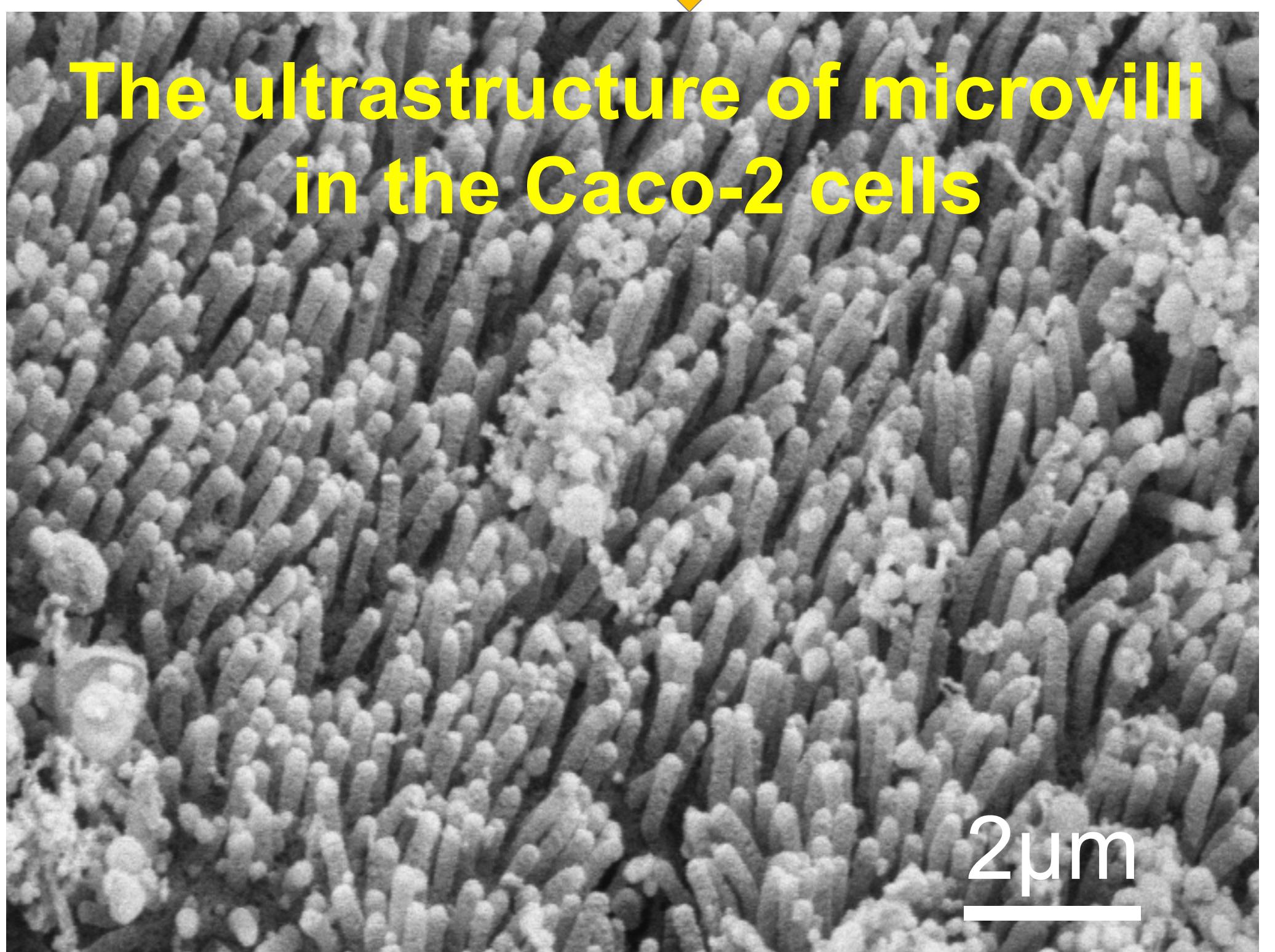
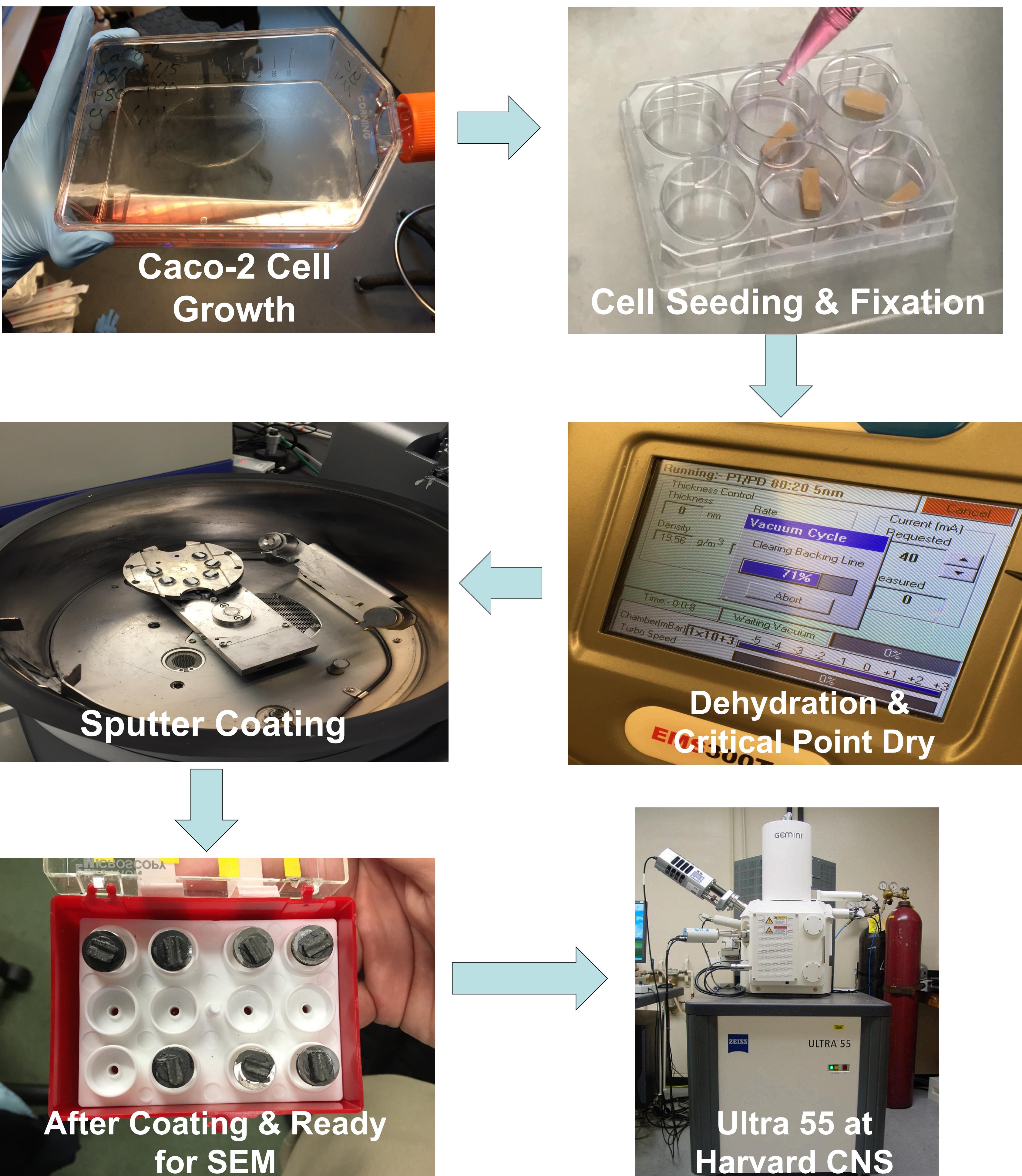
The use of the Scanning Electron Microscope (SEM) is essential to assess the microstructure of cells, such as, the microvilli. SEM can also be used to observe the entire structure of nanoparticles. Thus far, SEM has been considered as a simple and efficient characterization method of the interaction between the nanoparticles and biological systems,  $TiO_2$  NPs and intestinal epithelial cells. By SEM, we can clearly visualize where and when the  $TiO_2$  NPs bind to the intestinal epithelium.

## Summary, Conclusion & References

Through the Scanning Electron Microscope, we have successfully captured a high density of microvilli in epithelial cells. Our next step is to apply this method to visualize the interaction between  $TiO_2$  and intestinal epithelial cells.

**References:** (1) Chen Y. et al. Robust bioengineered 3D functional human intestinal epithelium. *Scientific Reports*. 2015 Sep 16; 5:13708. doi: 10.1038/srep13708.

## Sample Preparation for Scanning Electron Microscope (SEM)



## Silk Scaffold Preparation and Cell Seeding Methods & Materials

Silk fibroin was extracted from *Bombyx mori* silk worm cocoons and boiled in 2L of 0.02M  $Na_2CO_3$  solution. The degummed fibers were then rinsed thoroughly with distilled water and air dried overnight. The dried silk fibers were dissolved in 9.3M LiBr and dialyzed to yield fibroin water solution. The supernatants are removed and set in scaffold molds. The Caco-2 cells were cultured on the membrane of transwell cell culture inserts at a density of  $2 \times 10^5/cm^2$  and allowed to attach for 2 hours. The hollow Lumen of the 3D scaffolds was used to accommodate human intestinal epithelial cells. Collagen gels containing  $2 \times 10^5$  H-InMyoFib/ml were prepared and delivered into the silk scaffolds with a Teflon-coated stainless steel wire to leave the hollow channel open for the seeding of Caco-2 and HT29-MTX. After, the hollow channels were loaded with the Caco-2/HT29-MTX cells at a density of  $4 \times 10^6$  cells/mL.

## Scanning Electron Microscopy (SEM) Methods & Materials

3D intestinal tissues were cross-linked with 2.5% GA, followed by progressive dehydration in a graded series of ethanols (30%, 50%, 75%, 95% and 100% for 30 min. each). The samples were dried by critical point drying with a liquid CO<sub>2</sub> dryer (AutoSamdri-815, Tousimis Research Corp.). Then, they were imaged using a scanning electron microscope (Zeiss UltraPlus SEM or Zeiss Supra 55 VP SEM, CaPrior rl Zeiss SMT Inc.) at a voltage of 2 ~ 3 kV. The samples were coated with a 10 nm. thick layer of Pt/Pd using a sputter coater (208HR, Cressington Scientific Instruments Inc., Cranberry Twp).

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