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The Application of Cryogenic Focused Ion Beam Scanning Electron Microscopy to Hydrogel Characterization.

Christopher Parmenter[1], Abdulraman Baki[2], and Kevin M Shakesheff[2]

[1.] Nottingham Nanoscale and Microscale Research Centre, University of Nottingham, Nottingham, UK
[2.] Wolfson Centre for Stem Cells, Tissue Engineering and Modelling (STEM), School of Pharmacy, University of Nottingham, Nottingham, UK

Hydrogels are an important material as support matrices for cells to promote growth. These systems have been characterized by electron microscopy, through the application of fixation or sucrose embedding followed by ultramicrotomy. In this way the porosity of the gels can be assessed by transmission electron microscopy (TEM) and/or scanning electron microscopy (SEM). While this approach does give a guide to porosity of samples the absence of water in a hydrogel will have a detrimental effect and its absence distorts the dimensions of the remaining gel. Focused ion beam scanning electron microscopy (FIB-SEM) has been used on dried hydrogels [1], however, we propose a new method of gel porosity characterization by the use of cryogenic-FIB-SEM (cryo-FIB-SEM).

Cryo-SEM is a long established technique to preserve the water content of a sample and more recently it has been demonstrated that cryo- FIB-SEM can be used for biological and soft matter materials [2]. In this work, the authors have used cryo-FIB-SEM to investigate the porosity and structure of gels whilst in the presence of water. Gels were plunge frozen in slush nitrogen or using a metal mirror freezer and transferred under liquid nitrogen to the sample shuttle of a Cryo-SEM system (Quorum PPT 2000, Quorum Technologies). In the prep-chamber, the sample was coated for 60 seconds using a Pt sputter target. The samples were then loaded into the FIB-SEM (FEI Quanta 3D, FEI). Once in the SEM chamber, the gels were prepared for FIB by deposition (3-4 seconds) of a platinum precursor from the gas injector (set to 27 °C) of the microscope.

The hydrogel samples were milled using an initial current of 1-3 nA to make a rough cut and then by further cuts at lower milling currents (0.3 nA-50 pA), to remove the common milling artefact known as curtaining. SEM micrographs of the visible milled face showed dark patches with largely white areas in between. It was initially postulated that the darker areas were the pores of the gel. In order to test this, the temperature in the SEM chamber was raised to -90 °C, leading to slow sublimation of the water at the FIB milled face (Figure 1). Over approximately 20 minutes images of the slowly subliming gel were acquired. The resulting images show a transition from the black features amongst the majority of lighter contrast through to images with inverse contrast. The final sublimed gel images are clearly interpretable as a porous gel where now the lighter contrast features are identified as the gel strands and the pores are now darker and devoid of water. With a better understanding of the location of these components the original non-sublimed images can be re-examined and the black contrast correlated directly to the polymer and the white to the water.

By inverting the contrast of the original milled face image, it is possible to give an image equivalent to the dehydrated image, but which has all the water bound and is therefore a truer representation of the gel's morphology (Figure 2). The major advantage to this is that the sample does not undergo shrinkage, and that the process of imaging the milled face hydrated saves time. Additional slices of the freshly milled face may then be acquired to yield a series of slices suitable for 3D rendering (figure 3).

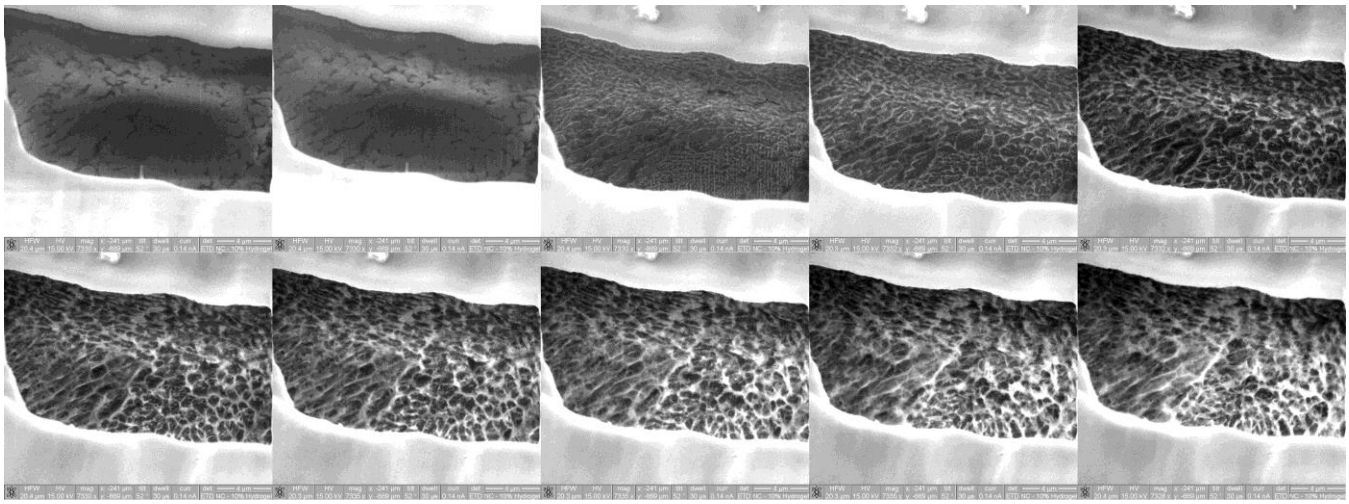

Figure 1. A montage of the localized sublimation from the milled face of a hydrogel, elapsed time ~20 minutes. Scale bars 4 µm.

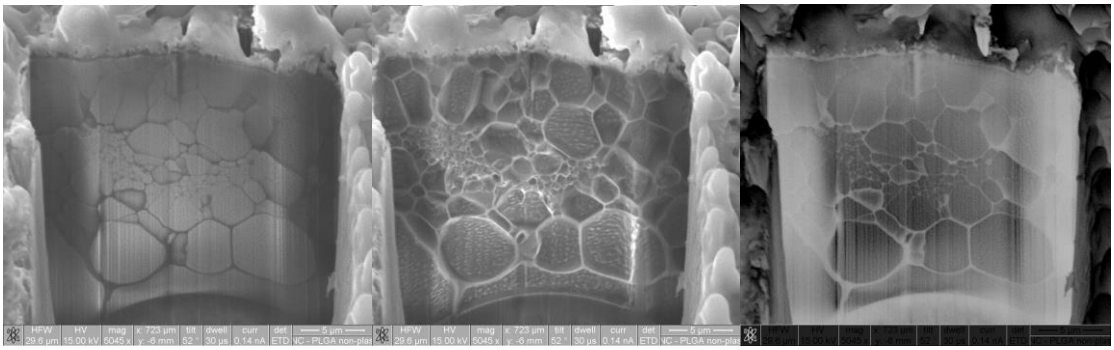

Figure 2. Images of the non-sublimed (left) and post-sublimation (center) of the hydrogel structure. Inverted contrast image of non-sublimed yields an analogous image to the post-sublimed sample (right). Scale bar 5 µm

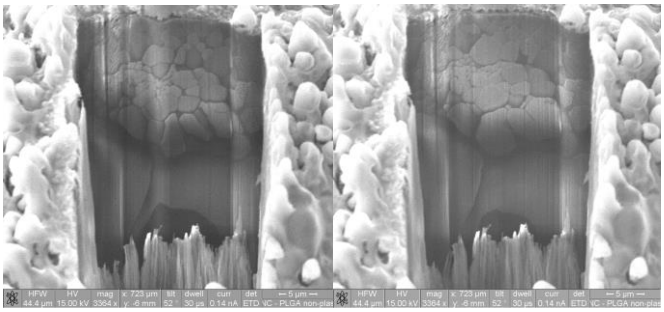

Figure 3. Two sequential images of FIB-milled faces of a hydrogel, which can be used to build-up a 3D data set of the porosity in the presence of the water. Scale bar 5 µm

References:

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