

## **Investigation into the effect of cryogenic sample preparation on hydrogel for subsequent SEM examination**

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The ultrastructure of hydrogels is of ever increasing interest to researchers. Hydrogels are used in drug releasing applications, hydrogels can be difficult to image in TEM due to viscosity issues resulting in thickened electron absorbing samples. As hydrogels have an inherently high water content within their network, the rapid immobilisation of this water during freezing is critical to the prevention of ice crystal formation in order to interrogate the native structure of the hydrogel. We investigate and compare the morphology of a hydrogel system and a reference tissue after freezing by:

1. Plunge freezing into liquid nitrogen slush with applied vacuum according to the Gatan Alto2500 Cryo System
2. Plunge liquid nitrogen-cooled ethane
3. Plunge liquid nitrogen cooled Isopentane
4. Plunge liquid nitrogen
5. High Pressure freezing

The samples were transferred using a cryo-transfer arm, under vacuum to the cold stage (-188°C) then fractured and sublimed at -80°C for 1 hour within a Zeiss Merlin Field Emission Scanning Electron Microscope at an accelerating voltage of 3 kV and a current of 100 pA.

Comparative images were taken at defined distances from the sample periphery to determine the presence and degree of ice crystal formation by the different freezing methods to determine if artefacts occur related to sample preparation and physically changing the structure of the hydrogels.

This is in light of work conducted by Elizabeth Grondahl and the increasing demand for both cryo SEM and TEM analyses to determine the true structure at the macro and nano scales of hydrogel based materials. This information is crucial for researchers to understand and model the true mechanisms involved during hydrogel modification processes, such as controlled release drug therapy or the addition of bioactive molecules.

- [1] R. Aston, K. Sewell, T. Klein, G. Lawrie and L. Grondahl, "Evaluation of the impact of freezing preparation techniques on the characterisation of alginate hydrogels by cryo-SEM," *European Polymer Journal*, vol 82, pp. 1-15, September 2016. <https://doi.org/10.1016/j.eurpolymj.2016.06.025>