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414 – Cryo-SEM Characterization of Multimodal Injectable Soft Tissue Markers: A Novel Tool for Diagnostic Imaging and Therapeutic Intervention in Cancer Treatment

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Multimodal injectable fiducial markers comprised of hydrophobic, viscoelastic ("gel-like") oligosaccharides are a novel invention for use in diagnostic imaging and therapeutic intervention in cancer treatment. These gel-like markers are based on esterified carbohydrates that form homogenous injectable liquids when dissolved in biocompatible co-solvents such as ethanol[1]. The carbohydrate scaffolds in the gel-like structures can be functionalized to enable multiple imaging modalities including computed tomography, near infrared (NIR) imaging, ultrasound, magnetic resonance imaging, and positron emission tomography, and can also be tuned to form solid markers for in situ palpation.

Once injected, the liquid marker undergoes non-solvent induced phase separation (NIPS). The co-solvent diffuses out of the hydrophobic carbohydrate and into the surrounding soft tissue or water resulting in the formation of hydrophobic, gel-like solids with a wide variety of viscoelastic properties based on the selection of functionalization on the carbohydrate, additives (e.g. polymers) and co-solvents. The microstructure of these gel-like depots are of particular interest for studying their formation as well as explaining the observed differences in viscoelastic properties between different marker compositions. To this end, scanning electron microscopy (SEM) was performed on samples injected into milliQ water.

Initially, samples were vacuumed dried after injection, snap frozen in liquid nitrogen, and fractured with a razor blade to expose the internal structure. The markers exhibited properties very similar to candy, ranging from hard candy like, shattering upon cutting, to soft taffy like, deforming upon cutting. The taffy like markers were impossible to prepare in this manner, whereas the hard candy like markers often completely shattered. Figure 1(A) shows a small piece from a hard candy like marker, which had shattered upon fracture. We can observe a porous structure but cannot accurately place this within the context of the entire marker. Figure 1(B), was acquired from a softer vacuum dried marker that had been left at ambient conditions for over a week after drying and before fracture and imaging, it shows a very large pore at the center of the marker, likely due to the coalescence of the pores present in the marker after drying. Both images highlight the limitations of vacuum drying and traditional SEM.

We then proceeded Cryo-SEM to enable imaging of the markers in a fully hydrated state, eliminating the need for vacuum drying and at the same time enabling us to fracture softer, taffy like markers. The samples were injected into milliQ water and allowed to equilibrate at 37°C for 7 days, with the water changed at day 1, 2 and 4 in order to ensure complete diffusion of the co-solvent to form solid markers. After 7 days, the markers were removed from the water, attached to an SEM stub with carbon paste and plunge frozen in liquid nitrogen. The markers were attached to a Leica VCT100 cryo transfer arm and loaded into a Leica MED020 freeze fracture and coating system. The markers were fractured, allowed to sublimate at -90°C for 1 minute to increase and coated with 6 nm of a C/Pt alloy. The markers were then transferred to a FEI Quanta 3D FEG SEM operating at 2 kV at -140°C for imaging.

Figure 2(A) shows a marker prepared from a fully hydrated state. The bottom of the image corresponds to a surface moving towards the center of the marker at the top of the image. The density of pores is much higher near the surface and eventually decreases to zero near the center. As the marker forms, a small

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quantity of water diffuses into the marker during the efflux of ethanol (ethanol-water exchange). As the ethanol continues to efflux and water in exchange flows in, the local water concentration increases to a point where it separates from the carbohydrate phase, forming pores. This results in a higher concentration of water pores near the surface and very little to no water pores at the center of the marker. Figure 2(B) shows small channels that appear to connect a number of pores. These channels are of great interest as they may provide a mechanism for the controlled release of water-soluble compounds from the marker into the surrounding tissue. These channels would not be observable in a sample prepared and imaged at room temperature.

Cryo-SEM is a powerful tool for imaging hydrophobic viscoelastic carbohydrate markers in their native hydrated state. This technique has enabled us to identify a pore density gradient within the markers as well as the presence of channels connecting the pores. Cryo-SEM is a versatile technique for imaging a wide variety of carbohydrate markers in a hydrated state [2,3].

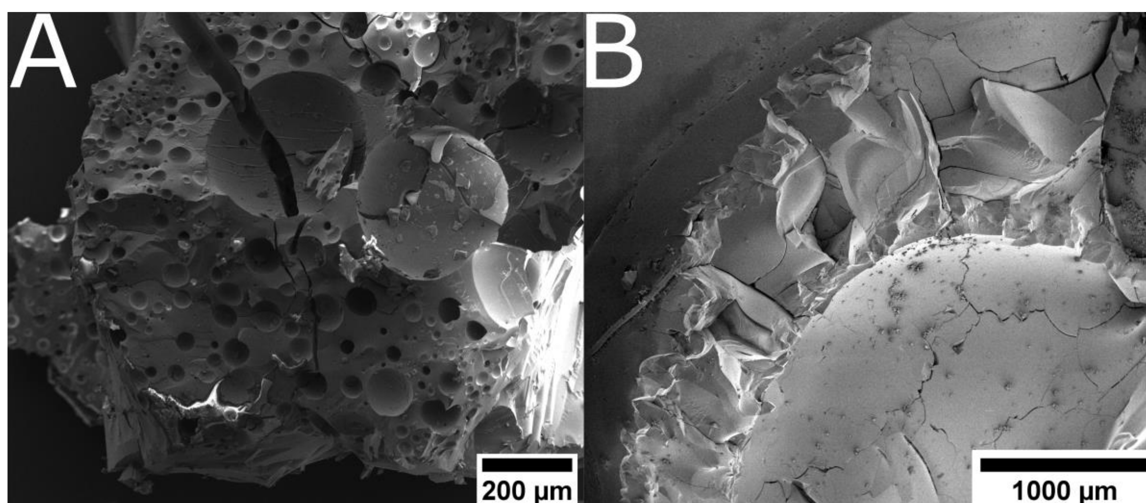

Figure 1. (A) SEM micrograph showing the presence of a numerous pores of different sizes from a shattered sample. There is no information about the location of these pores within the overall marker structure. (B) SEM micrograph of a vacuum dried then aged marker showing one large pore, that could be the result of the coalescence of a number of smaller pores within the material.

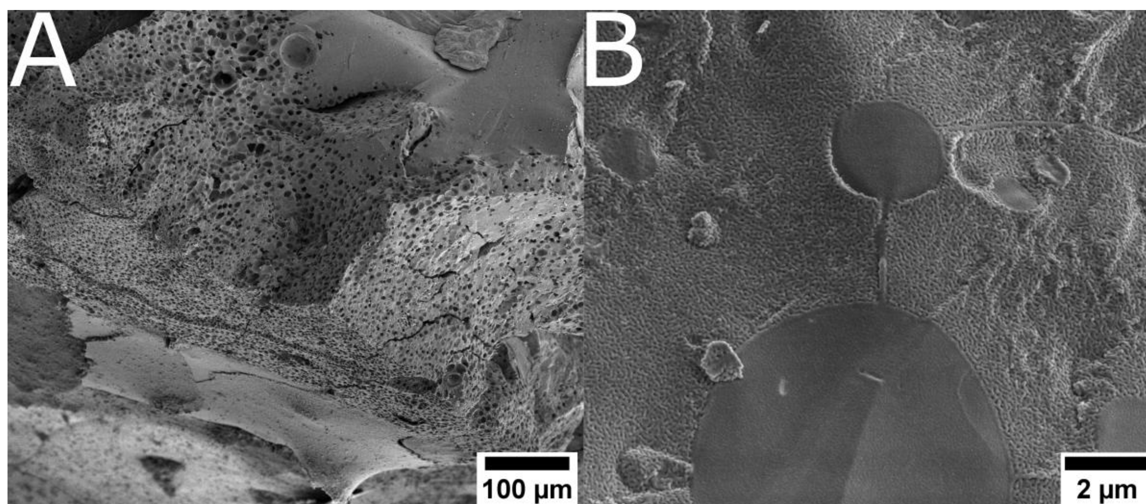

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Figure 2. Cryo-SEM micrographs showing (A) the presence of a pore-density gradient with the density decreasing as you move further into the marker material and (B) channels connecting pores, a possible mechanism for controlled release of water-soluble compounds from the marker.

References

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[2] We acknowledge the Core Facility for Integrated Microscopy, Faculty of Health and Medical Sciences, University of Copenhagen
[3] We acknowledge the Center for Electron Nanoscopy, Technical University of Denmark

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