Materials at the Interface of Biology

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Characterizing materials at the interface of biology requires unique sample preparation and imaging techniques to succeed. These samples are often in a hydrated state and composed of organic matter, both of which present a number of challenges for imaging in the electron microscope. In their native state these materials are not vacuum compatible, highly sensitive to the electron beam, and suffer from very low contrast. It is thus necessary to alter the state of these materials to improve their compatibility, stability, and contrast. In this presentation I will highlight the sample preparation and characterization of three unique materials at the interface of biology.

Multimodal injectable fiducial markers are a novel invention for use in diagnostic imaging and therapeutic intervention in cancer treatment. These gel-like markers are based esterified carbohydrates and form a homogenous injectable liquid that undergoes non-solvent induced phase separation upon injection into tissue [1]. The microstructure of these marker depots are of particular interest for understanding their formation, both *in vitro* and *in vivo,* as well as their viscoelastic properties. These markers are however highly dependent on their surroundings and any change will dramatically affect the microstructure. As such, we utilized Cryo-SEM of fast frozen markers to characterize the internal structure of these markers in a fully hydrated state. Through this technique we were able to observe differences in the microstructure near the surface of markers injected in water and those injected *in vivo*. This difference is likely due a constraint on diffusion *in vivo* that is not present in water.

Characterizing the protein corona on liposomes is of great interest as it may affect the fate and as a result the effectiveness of these materials *in vivo*. Generally, liposomes are injected intravenously, allowed to circulate and then collected. The liposomes, and associated protein corona, are then isolated from the blood using size exclusion chromatography (SEC) and any protein measured in the associated elution volume is assumed to be from the protein corona. To better understand this we characterized samples with and without liposomes isolated using size exclusion chromatography [2]. We observed similar protein levels between the samples with and without liposomes indicating that protein was eluting at the same time point and thus must be of similar size to the liposomes. To confirm this we utilized negative stain TEM to observe both sample sets. Negative stain TEM enabled us to better observe the naturally occurring nanomaterials from the blood, likely lipoprotein particles and protein aggregates created during sample processing for SEC.

Liposomes are of great interest as drug delivery systems. However, they are often difficult to track and image *in-vivo* because they are very similar to the surrounding biological material, they lack contrast. My colleagues have developed a unique method for forming gold nanoparticles inside of liposomes that can then be used a means to track these particles via mass spectrometry or electron microscopy [3]. Cryo TEM enabled direct observation of these liposomes in their native hydrated state revealing gold nanoparticles on average 11 nm in diameter. Imaging these same liposomes *in vivo* however requires considerable more effort. In

a proof of concept experiment these gold nanoparticle containing liposomes were injected intravenously into mice. The livers from these mice were then fixed, stained, dehydrated, and embedded in resin to improve the tissues compatibility, stability, and contrast. Thin sections were cut using an ultramicrotome and imaged in the TEM, revealing the accumulation of these liposomes in Kupffer cells in the liver, demonstrated that it is possible to use these gold loaded liposomes as a means to directly image liposomes in tissue in the electron microscope.

The three materials discussed above represent the unique challenges that exist when imaging materials at the interface of biology. Each material is unique and as such requires a unique approach both in sample preparation as well as imaging to obtain the best possible results.

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