

Engineering liver tissue with nanofilm biomaterials

Jennifer Phelps of the Department of Chemical Engineering at Yale University discusses her work that could one day lead to the development of cell-based therapies for patients with compromised liver function. She has been carrying out studies under the guidance of Prof. Paul Van Tassel.

Work with such an explicit biomedical application is actually quite new to the group, which historically has focused on problems of macromolecular adsorption, where electrostatic interactions are prominent. One current project involves molecular simulation of charged macromolecules under confinement, and the colloidal forces that result, while another project deals with the experimental measurement of polyelectrolyte adsorption under an applied electric potential. An emerging third area, and the one relating to Jennifer's studies, involves polyelectrolyte nanofilms formed via Layer-by-Layer (LbL) assembly.

The interest is in developing nanofilm biomaterials for tissue engineering applications, with current efforts being directed toward human liver cells. "We seek to understand the cellular response in terms of the nanofilm properties. My project within this larger framework involves assembling films and characterizing their properties," says Jennifer. Her work is well motivated by the following simple yet sobering fact: each year, end-stage liver disease claims thousands of lives. Orthotopic liver transplant is currently the only treatment for a poorly or non-functioning liver, but is far from ideal owing to its high relative cost, the adverse effects associated with surgery and immuno-suppression, and the severe lack of donor organs. Liver tissue engineering, where one seeks to augment liver function by implanting functional liver cells, offers an attractive alternative.

Jennifer's approach to liver tissue engineering involves polyelectrolyte nanofilm biomaterials formed via electrostatically driven LbL assembly. Hepatocytes are known to be very sensitive to substrate properties, such as rigidity and biofunctionality, and owing to the "tunability"

of multilayer film properties -- via processing variables such as polyelectrolyte chemistry, ionic strength, pH, and cross-linking extent -- one can engineer films promoting hepatocyte proliferation and function.

A key challenge is to understand the relation between film properties and the cell response. The QCM-D apparatus has been very useful in this regard, yielding film compositional and viscoelastic properties. It must be stressed that these films are very thin (thickness ca. 50 nm, or 5×10^{-6} cm), so conventional material characterization methods are not applicable here. Another challenge lies with the liver cells themselves. Hepatocytes are notoriously difficult cells with which to work, and only progenitor cells show significant regenerative potential (and they tend to be poorly characterized). Liver tissue engineering offers high impact and may benefit significantly from nanofilm biomaterials.

Liver cells under investigation include human hepatocellular liver carcinoma (HepG2) and human fetal liver cells, both chosen for their high proliferative capacity. Films are constructed via batch LbL assembly of biological polyelectrolytes, such as the polypeptides poly(L-lysine) and poly(L-glutamic acid) and the polysaccharide alginate, and the synthetic polyelectrolytes poly(allyl amine) and poly(styrene sulfonate). In certain cases, chemical cross-linking is employed to influence film rigidity.

As a result of this work, a particular question arose. "What is the relative importance of the chemical versus the mechanical properties of the film and how do they relate in the best performing films?" This is where the QCM-D came into the picture. The best performing films -- in terms of promoting cell proliferation and function -- tend to be rigid, as induced by chemical cross-linking and as quantified via QCM-D shear and viscosity data.

"We are using our QCM-D to provide clues as to the optimal mechanical properties for nanofilm biomaterials," says Jennifer Phelps. Optical Waveguide Lightmode Spectroscopy (OWLS) is also used to measure film

growth in situ, and AFM and fluorescence microscopy are used to obtain topographical information – however, only QCM-D is capable of providing in situ measures of film mechanical properties.

“We have relied heavily on our QCM-D data to interpret the response of contacting liver cells. For example, we showed that for certain films, rigidity is the dominant factor in determining cell response, while for other films, chemical effects dominate”, explains Jennifer Phelps.

“We will continue to extensively use the QCM-D as these studies progress.” The ultimate goal of the work is to optimize best performing films and to investigate the placement of biofunctional ligands within multilayer films

to control liver specific functions. The next step in this group’s research is to extend the cell culture to three dimensions using a nanofilm-coated, porous polymer scaffold. Ultimately, in vivo studies (mouse model) are planned. The long-term aim of the work is to achieve nanofilm-based engineering of human liver tissue for cell-based therapies.

The liver cell work was done in collaboration with Prof. Martha Harding’s laboratory in the Section of Comparative Medicine at the Yale Medical School, as well as with Prof. Mark Saltzman’s laboratory in Biomedical Engineering and numerous helpful discussions with Prof. Erin Lavik’s laboratory in Biomedical Engineering.