The Phosphoproteomics Landscape of the Interaction between Biomaterials and Cells.

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Statement of Purpose:

The complexity of the interactions that occur between cells and materials make understanding and controlling biological responses to biomaterials challenging. Unlike many biological model systems that have simple single ligand-receptor interactions, materials have multiple inherent properties that lead to complex multicomponent interactions with cells. The effect of biomaterial on cells *in vitro* and *in vivo* is often studied from a material point of view by determining the effects of physical and chemical properties, such as charge, stiffness or porosity, on cell morphology and function by observing changes in cell survival, proliferation or differentiation. This has led to the development of many useful materials; however, many devices still fail *in vivo* or in the clinic. An understanding of the mechanism of interaction between cells and materials at the molecular level will give crucial information to allow materials to be designed to succeed *in vivo*. The current study focuses on developing an unbiased method to understand cell-material interactions by investigating the molecular changes within cells minutes after they are exposed to a material. Using phosphoproteomics a profile of signal transduction changes within the cells was determined after treatment with the biomaterial.

Methods:

Macrophage-like cells (dTHP1) were grown on porous Transwell inserts and exposed to copolymer films[1] of isodecyl acrylate and 40% methacrylic acid (MAA), or methylmethacrylate (MM) by placing polymer-coated glass coverslips on top of a monolayer of cells for 10, 20, or 30 minutes. The cells were grown on the well inserts to allow for nutrient and oxygen exchange between the cells and the medium below the cells; there were no obvious changes in cell morphology for the short incubation periods, as expected. After incubation, the cells were lysed in 8M urea buffer and the proteins digested with sequencing-grade trypsin (Promega) in solution using a protein to trypsin ratio of 50:1. The phosphopeptides were then enriched from the total peptide mixture by Ti column separation and identified using an Orbitrap Velos mass spectrometer[2]. Peptide lists were generated from the mass spectrometry data using MaxQuant.

Results:

Approximately 1000 unique peptides from 600 proteins were identified depending on the type of polymer and incubation time. At each time point, there were a greater number of peptides and proteins that were uniquely phosphorylated in cells exposed to 40% MAA than MM. While phosphorylation does not necessary correlate to protein or cell activity, it suggests that MAA generates more global changes of dTHP1 cell signalling cascades. To visualize the data and to identify global trends, enrichment maps were made using Cytoscape with the enrichment map plugin (p-value<0.05, FDR<0.1). Figure 1 illustrates the cellular processes that had an increase in protein phosphorylation at 10 min after exposure to 40% MAA or 40% MM. There are three large clusters of nodes indicating that MAA increased the phosphorylation of proteins involved in cell death and apoptosis, RNA splicing and chromosome rearrangement. In addition, there are individual nodes that show MAA is also involved in cytoskeleton rearrangement, endocytosis and signalling pathways. The MM material had fewer unique nodes in the enrichment map and these nodes were involved in cell migration and signalling events.

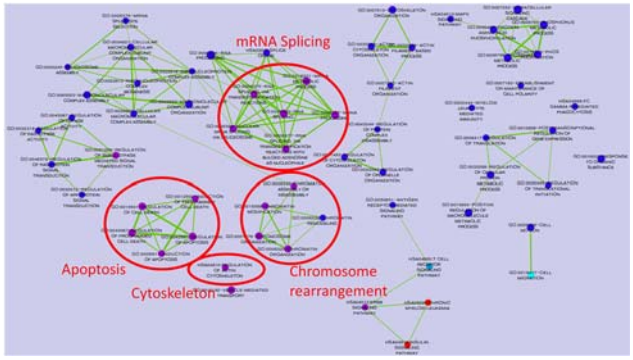

Figure 1. An enrichment map of the phosphoproteomics data from the 10 minute data point. Each node represents proteins with a common gene ontology (GO) term classification. Interacting nodes are linked by green lines. Blue nodes contain proteins that were phosphorylated with treatment by both materials. Purple and red nodes contain proteins that were phosphorylated with MAA treatment. Cyan nodes contain proteins that were phosphorylated with MM treatment.

Conclusions:

This method identified expected (cytoskeleton rearrangement) and novel (apoptosis) pathways that are regulated by material interaction with the cells in this proof-of-principle experiment. We propose that identification and evaluation, by phosphoproteomics, of actively phosphorylated proteins in cells exposed to different materials will identify important and novel interactions for future follow-up studies, and perhaps eventually allowing for the development of "rules of interaction" between cells and biomaterials.

References:

(1) Wells, L.A., et. al. *Biomaterials Science* **2014**, 10.1039/C4BM00159A.
(2) Guo, H., et. al. *Proteomics* **2013**, *13*, 1325-33.