Statement of Purpose: In vitro studies of nanoparticle – cell interactions often involve cells grown in a monolayer on tissue-culture polystyrene and exposed to nanoparticles suspended in their culture medium. Once the medium is exchanged, uncaptured nanoparticles are removed. Even if the particles are replenished, any changes which may have occurred due to the particles' exposure to biological media, such as a protein corona, are lost. Long-term studies require an advanced tissue-like environment in which nanoparticles are encapsulated with a population of cells for real-time and end-point analysis. By using soft tissue-mimicking hydrogels, the cells are provided with an environment that promotes normal cell function during prolonged nanoparticle exposure. Rat pheochromocytoma (PC12) cells in Collagen I and poly(ethylene glycol) (PEG) 4600 hydrogels are examined here, with quantum dots (QDs) as the model nanoparticle.

Methods: QDs (Invitrogen) with a CdSe core, ZnS shell, and three different surface chemistries, QD-NH₃, QD-COOH, and QD-PEG, were purchased. Transmission electron microscopy (TEM) verified spherical particles of about 4.7 nm diameter. Collagen I (Advanced BioMatrix) was induced to gel following manufacturer instructions. Two-layer hydrogels were created; cells were suspended in the upper layer at 1 x 10⁶ cells/mL. PEG 4600 dimethacrylate was synthesized, dissolved in medium containing QDs and 0.05 wt. % Irgacure 2959 (Ciba Geigy), and photopolymerized (365 nm, 3 mW/cm²) for 15 minutes. Cells were suspended at 2 x 10⁶ cells/mL. PC12 metabolic activity was monitored by AlamarBlue reduction (Invitrogen). QD dispersion was examined by confocal microscopy.

Results: We observed that 0.4 mg/mL Collagen I hydrogels promote greater neurite outgrowth than 0.8 mg/mL to 2.0 mg/mL hydrogels. QDs were visualized near cell nuclei (Fig. 1). However, they were observed to move through the hydrogel on short time scales, e.g., during imaging, suggesting loss of nanoparticles.

Conclusions: Cross-linked 10 wt. % PEG 4600 hydrogels appear superior to 0.4 mg/mL Collagen I for in vitro nanotoxicology studies due to their smaller mesh size. Work is currently underway to quantify QD leaching from PEG hydrogels as a function of QD surface chemistry and macromer wt. % (which impacts mesh size) as well as PC12 differentiation under these conditions.

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References:
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