Tissue Engineered Scaffolds for Screening Nanoparticle – Cell Interactions[1]

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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[2]. 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-NH_3, QD-COOH, and QD-PEG, were purchased[3]. 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 10^6 cells/mL. PEG 4600 dimethacrylate was synthesized[4], dissolved in medium containing QDs and 0.05 wt. % Irgacure 2959 (Ciba Geigy), and photopolymerized (365 nm, 3 mW/cm^2) for 15 minutes. Cells were suspended at 2 x 10^6 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.

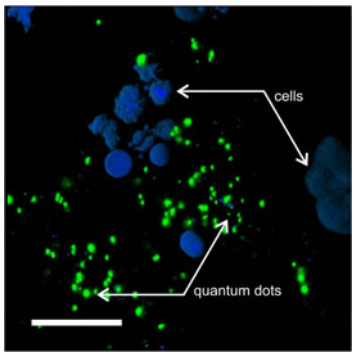

Fig 1: Confocal image of QDs dispersed with PC12 cells in a 0.4 mg/mL Collagen I hydrogel. Scale bar is 50 µm.

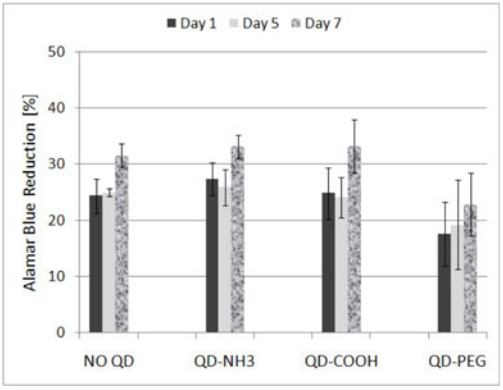

Fig 2: Metabolic activity of PC12 cells in 10 wt. % PEG 4600 hydrogels with 12 nmol/L QDs.

PC12 cells in 10 wt. % PEG hydrogels showed greater metabolic activity than those encapsulated in 20 wt. % PEG hydrogels 1, 5, and 7 days after encapsulation (data not shown). Cell metabolism was not affected by the incorporation of low concentrations of QDs (Fig. 2). Higher concentrations of QDs (32 nmol/L) were used for imaging; QD movement was not apparent.

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[5,6]. 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:

1. NIST contribution, an agency of the US government, not subject to copyright in the United States.
2. Lewinski N, Small 2008; 4: 26-49.
3. Commercial equipment, instruments, or materials are identified only in order to adequately specify certain procedures. In no case does such identification imply recommendation or endorsement by NIST, not does it imply that the products identified are necessarily the best available for the purpose.
4. Lin-Gibson S, Biomacromolecules 2004; 5: 1280-7.
5. Mahoney M, Biomaterials 2006; 27: 2265-2274.
6. Krewson C, Biotechnology and Bioengineering 1994; 43: 555-562.