**The Effects of Molecular Weight on Viability within PEGDA Hydrogel Microspheres**

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**Statement of Purpose**: Poly(ethylene glycol) diacrylate (PEGDA) is often used as a hydrogel scaffold in tissue engineering, but there is disagreement over which molecular weight provides optimal viability for encapsulated cells[1-3]. We aim to determine cell viability of a variety of cells in PEGDA hydrogels of various molecular weights, and to explore possible causes for any differences. To avoid hypoxic conditions common towards the center of hydrogel scaffolds, we encapsulate cells within microspheres in order to remain within diffusion limits of oxygen. Concurrent diffusion studies were performed to assess the size molecules permitted access by the hydrogels. Results demonstrated higher cell viability in higher molecular weight PEGDA microspheres.

**Methods:** Hydrogel precursor solution was prepared as previously described.[4] Briefly, 10 % (w/v) PEGDA (3.4, 5, 10, or 20 kDa, or 5% 10 and 5 % 20 kDa) 1.5% (v/v) triethanolamine, 1.0% (v/v) pluronic F68 in HBS with 1.0 µM Eosin Y, and 37.5 mM 1-vinyl-2-pyrrolidinone. Mouse osteoblasts (MC3T3-E1) were combined with precursor solution at a concentration of $10^7$ cells/mL. Mineral oil was combined with 3 µl/ml of 2,2-dimethioxy-2-phenylacetophenone in1-vinyl-2-pyrrolidinone (300 mg/ml). Microspheres were formed while photopolymerizing a vortex-induced emulsion of 200 µl of hydrogel precursor solution in 1 mL of oil under white light. Microencapsulated cells were incubated for 7 days in DMEM in 6-well plates with 0.4 µm pore polycarbonate membrane transwell inserts. Cell viability was measured at days 1, 4, and 7 using a calcein AM/ethidium homodimer 1 LIVE/DEAD assay. Hydrogel permeability was determined by forming hydrogel disks (absent the surfactant pluronic F68). Disks measuring 0.8 mm in height and 1cm in diameter were placed into a 24-well plate and first incubated in 2 mL of 50 µM sodium azide solution for 24 h. Then, .50 µg/mL fluorescein isothiocyanate–dextran (ranging between 10 kDa and 140 kDa) was added to each well, and hydrogels were incubated for another 24 h. Hydrogel disks were then placed in fresh buffer for 24 h and dextran release was quantified.

**Results:** Cells encapsulated in PEGDA microspheres showed similar cell viability for the first day of culture. After seven days, cells in lower molecular weight microspheres showed significantly decreased viability (Fig 1). Low molecular weight dextran was released similarly by all molecular weight hydrogel disks. Higher molecular weight hydrogel disks showed increased retention of high molecular weight dextran (Fig 2).

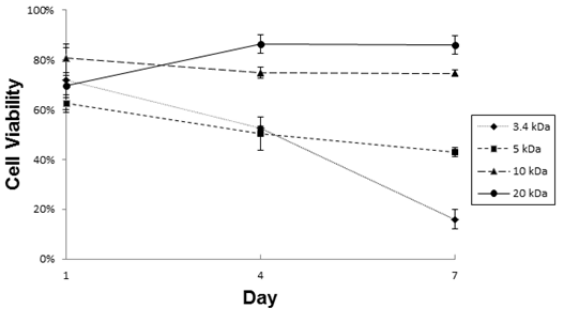


**Figure 1:** Viability of encapsulated mouse osteoblasts over time based on PEGDA molecular weight

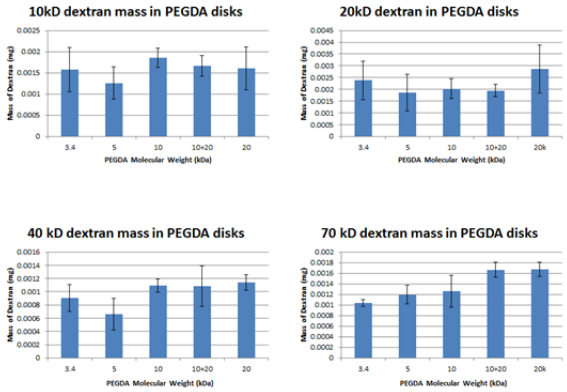


**Figure 2:** Mass of dextran retained in PEGDA disks after 24 h

**Conclusions:** Low molecular weight PEGDA microspheres had the lowest cell viability. Since osteoblasts are shown to apoptose without sufficient levels of serum[5], we hypothesized that large serum proteins could not pass through the smaller mesh size that comprises low molecular weight PEGDA, resulting in the increased cell death observed. To confirm this hypothesis, future studies will examine cells that thrive in serum-free media.

**References:**

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