Self-assembled microgels for cell encapsulation

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Statement of Purpose: The crosslinking process of some biomaterials can be incompletely cytocompatible owing to the use of harsh physical or chemical conditions.[1] QQKFQFQFEQQ, Q11, is a self-assembling peptide that forms fibrillar hydrogels in the presence of physiological salt concentrations. These materials are non-cytotoxic, can be made non-immunogenic, and can be readily modified with a variety of peptide ligands, without compromising gelation characteristics.[2] In this work, we exploited this peptide's conditional assembly behavior to fabricate spherical microgels using water-in- oil emulsions. In this way, cell encapsulation could be achieved without temperature change, polymerization chemistry, or other harsh processing conditions. Microgel morphology and size was tunable by changing fabrication parameters. Mouse fibroblasts encapsulated in Q11 microgels demonstrated high cell viability and proliferation.

Methods: *Peptide synthesis:* Q11 (QQKFQFQFEQQ) and Nitro-benzoxadiazole (NBD)-RGD-Q11 were synthesized using standard Fmoc-based solid phase protocols. *Microgel Fabrication:* Q11 peptide solution, alone (30mM) or mixed with NIH/3T3 cell suspension (in 10% sucrose containing antibiotics/antimycotic, final cell concentration $2x10^6$ cells/mL, final peptide concentration was 30mM), was added to a beaker containing excess mineral oil and stirred with a rotor-stator-type homogenizer or a paddle stirrer at variable blade speeds (1-18kRPM for homogenizer and 1.5k for paddle stirrer) for 5 sec (cell encapsulation) or 1 min (Acellular microgels). PBS was then added to assemble the peptide in the aqueous compartment. Microgels were collected in PBS by centrifugation. Cell-laden microgels were resuspended in cell culture media and incubated in tissue culture plates. *Analysis of microgel morphology:* NBD (a green fluorophore)-RGD-Q11 was cofibrilized into microgels. Microgels were imaged with an epifluorescence microscope and the diameters were quantified with ImageJ software. *Analysis of cell encapsulation*: To study live cell yield in the encapsulation process, the same number of cells were either encapsulated in microgel or seeded on culture plate. Yields of live cells and cell proliferation were analyzed with MTS assay, and microgels were stained with Calcein-AM and ethidium homodimer to assess viability.

Results: Microgels were produced with water-in-oil emulsification with agitation from a homogenizer or paddle-stirrer. Microgels were spherical with diameters ranging from several microns to several hundred microns (figure 1 a-c). In general the paddle stirrer generated the microgels with lowest ellipticity (i.e. most spherical) and largest diameters (average diameter was 40μm). With the homogenizer, smaller microgels were produced when rotor speed was increased (average diameter ranged from 25μm to 15μm).

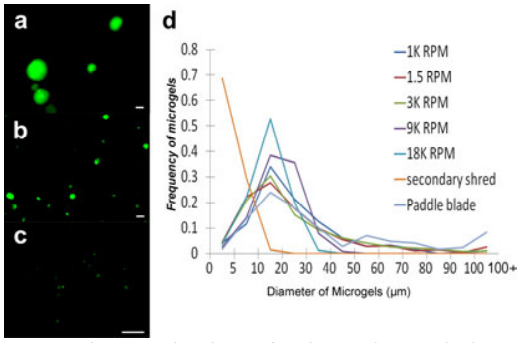

Figure 1. Characterization of Microgel morphology and size distribution. Microgels fabricated with a paddle-stirrer (a), homogenizer (b) or subjected to a secondary shear (c). They were imaged and their size distributions were quantified (d). Scale bar: 50μm

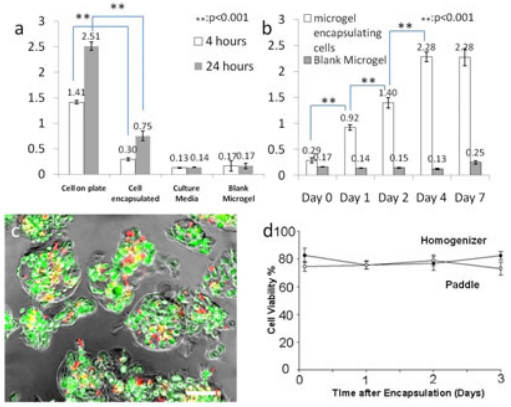

Figure 2. Analysis of NIH/3T3 fibroblast encapsulation. Yield of live cells (a), cell proliferation in microgels (b), live/dead stain at 2 days (c) and viability quantification (d). Scale bar: 100μm

Relatively small microgels were achieved when a secondary shear process was applied to microgels (with average diameter of 4.25μm) (figure 1 d).

Mixing cells with peptide solutions, followed by emulsification, produced cell-laden microgels. Encapsulation efficiencies were about 25%, compared to cells plated directly (without encapsulation), measured at 24 hours. Encapsulated cells exhibited good viability (80% up to day 7) and significant proliferation: By MTS, cell metabolic activity increased 2 fold from day 1 to day 4 (figure 2).

Conclusions: Size-tunable microgels were fabricated with a water-in oil emulsification method. Cells

encapsulated in Q11 microgels showed good viability and proliferation.

References: **1.** Koh WG, et al., *Langmuir*, 2002 Apr 2; 18(7):2459-62. **2.** Jung et al., *Biomaterials* 30, 2400–2410 (2009).