Dynamic Photo-Tunable Gels to Modulate Matrix Stiffness

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Statement of Purpose: Considerable effort has been devoted to controlling the mechanical properties of 3D hydrogels over the last decade. However, controlling matrix stiffness independently from other factors that influence cell behavior remains a challenge. Altering the mechanical properties of 3D hydrogels is generally performed by adjusting the concentration of a protein or crosslinker. Varying the concentration of proteins within a gel reduces the ability to resolve critical aspects of the system because the ligand density also changes. Photo-initiated crosslinking systems suffer from limited light penetration which restricts construct size, and cytotoxicity from initiator molecules remaining in the gel. Further, none of these techniques allow manipulation of the gel stiffness after the initial timepoint. In this study we demonstrate the ability to tune the stiffness of 3D alginate hydrogels in time using novel near-infrared triggered release of calcium from temperature sensitive liposomes.

Methods: Gold nanorods were synthesized by the seed-mediated growth method [1]. To fabricate gold nanorod loaded liposomes, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was dried to a thin film on a round bottom flask using a rotary evaporator. The lipid films were hydrated, resulting in multi-lamellar vesicles. Unilamellar vesicles were produced by sonication and then 4M ethanol was added to induce bilayer interdigitation [2]. The ethanol was removed by centrifugation and the interdigitated lipid sheets were incubated with the loading solution at 55°C. Calcium loading was determined using arsenazo III, a colorimetric indicator of calcium. Alginate gels were formed by mixing sodium alginate solution with $CaCO_3$ and glucono-δ-lactone (GdL) in polydimethylsiloxane molds. GdL slowly hydrolyzes, which releases calcium ions over a period of 10-20 minutes to form a compliant gel. Liposomes were included at 20% by volume to analyze *in situ* release and stiffness modulation. The gels were irradiated with 4.6 W/cm^2 for 5 minutes and swollen in DPBS overnight, followed by rheometry. Frequency sweeps were performed within the linear-viscoelastic range of the gels from 0.1 to 10 Hz with a constant strain amplitude of 1%.

Results: Calcium and gold nanorods can be loaded into interdigitation-fusion vesicles and maintained without leakage. The maximum internal calcium concentration achieved was 58 mM. Upon irradiation, 100% release was achieved compared to incubation of the liposomes with 10% Triton-x-100, demonstrating feasibility of the concept.

Calcium loaded liposomes were distributed within relatively compliant alginate gels and irradiated. Upon irradiation, calcium was released from the liposomes causing ionic crosslinking of the alginate, resulting in a stiffer gel (Fig. 1C). Citric acid, a calcium chelator, could also be loaded into liposomes and used to reversibly soften the alginate gels (Fig. 1E). Gold nanorods of different aspect ratios can also be used to allow for selective release from a subset of liposomes based on laser wavelength (Fig. 1D). This strategy was employed to generate a step-wise stiffening mode. Two different nanorods solutions were loaded into separate liposome batches. Then, both of the liposome batches were distributed within a compliant alginate gel. Irradiation of the gels with only one laser, either 808 nm or 1064 nm, resulted in calcium release from half of the liposomes and a moderate increase in stiffness (Fig. 1F). When the gels were irradiated with one laser followed by the second laser, a greater stiffness change was generated.

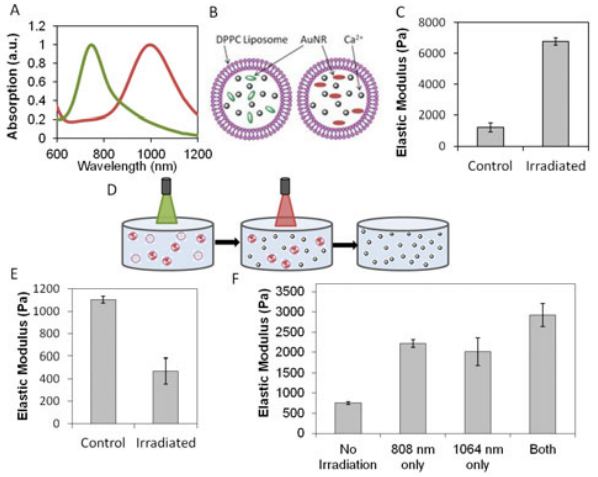

Figure 1: Absorption spectra of gold nanorods of different aspect ratios (a). Scheme of nanorod and calcium loaded liposome (b). Calcium induced gel stiffening by irradiation (c). Scheme of step-wise stiffening program (d). Chelator induced gel softening by irradiation (e). Step wise stiffening by selective calcium release based on laser wavelength (f).

Conclusions: We have demonstrated a NIR-light triggered tuning mechanism for 3D alginate hydrogels. Temperature sensitive liposomes can be loaded with calcium or citric acid and gold nanorods that undergo heating upon irradiation. The subsequent release of calcium increases the stiffness of alginate, while citric acid release softens the gel. Further, we have demonstrated the feasibility of a multistep stiffening or softening program, which could be used to model dynamic biological processes. We intend use this hydrogel system to mimic matrix stiffening that occurs in diseased tissue to develop a better understanding of disease progression as it relates to mechanotransduction.

References:

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2. Ahl, P.L. Biochimica Et Biophysica Acta-Biomembranes, 1994. **1195**(2): p. 237-244.

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