Temporal Control of 3D Hydrogel Stiffness

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Statement of Purpose: Hydrogels are widely used to study many aspects of cell function in more realistic 3D microenvironments. It is well recognized that microenvironmental stiffness is a key regulator of cell phenotype, and hydrogels have been developed to span the stiffness of most soft tissues. However, in many biological processes, such as embryogenesis, wound healing, and tumor development, microenvironmental stiffness is highly dynamic. Thus, to more accurately investigate these dynamic phenomena, we need to develop hydrogels with dynamic and controllable stiffness. Here, we present a strategy to temporally tune the stiffness of 3D alginate gels using light triggered release of calcium to stiffen or chelators to soften.

Methods: Temperature sensitive liposomes were fabricated from DPPC lipids using the interdigitation fusion method. Gold nanorods were synthesized and encapsulated in the liposomes along with $CaCl_2$ or DTPA (calcium chelator, Fig. 1a). These liposomes were distributed within a 3D alginate gel. An 808 nm continuous wave laser was used to irradiate the samples, which causes release from the liposomes (Fig. 1b). Rheometry was performed to determine the storage modulus (8 mm parallel plate, 1% strain, 1.8 Hz). NIH 3T3 fibroblasts were cultured within alginate gels containing 25% Matrigel for cell adhesion. After 24 hours of culture, the gels were irradiated for 0, 30, 60, or 120 s to stiffen the matrix. Two days after irradiation, phase contrast images of the cells were taken. The cell outline was traced and a best-fit ellipse was generated in Image J to quantify circularity. The cells were stained with calcein AM and 2-photon microscopy was used to obtain z-stacks.

Results: Calcium and gold nanorods could be stably loaded within the DPPC liposomes. NIR irradiation of gold nanorods induces surface plasmons that generate local heating, causing a gel-to-fluid phase change in the lipid bilayer. This allows calcium ions to diffuse out, and in the presence of alginate, increase the crosslinking density. Calcium release from liposomes in 3D alginate gels resulted in stiffening of the gels, from an initial value of less than 100 Pa to a final value of greater than 1100 Pa. (Fig. 1c). Alginate crosslinks are reversible by chelating calcium from the gels. We sought to temporally soften gels by releasing a chelator, DTPA in place of calcium from the previous experiment. Irradiation of initially stiff gels with DTPA loaded liposomes resulted in softening of the gels ($\Delta G'$=502 Pa). The change in stiffness for both experiments was dependent on irradiation time, making our system highly controllable. Further, the magnitude of stiffness change is within the range observed in fibrotic diseases.[1] 3T3 fibroblasts encapsulated in compliant gels adopted extended morphologies (Fig. 1d). In contrast, cells in gels that were stiffened by irradiation remained rounded, yet still viable, as indicated by positive calcein AM staining. We observed a consistent decrease in circularity with irradiation time (thus, stiffness), because cells were less capable of deforming the surrounding matrix[2] (Fig 1e). Overall, the induced stiffness changes generated a morphologic response from the fibroblasts, implying that the magnitude of change is sufficient to alter cell behavior.

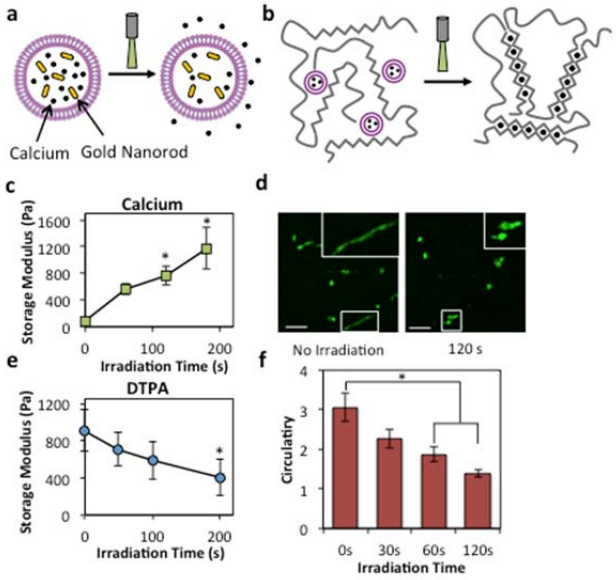

Figure 1: Scheme of light-triggered liposome release (a). $CaCl_2$ and gold nanorods are encapsulated in temperature sensitive liposomes. NIR irradiation generates local heating from the nanorods, resulting in calcium release. In the presence of alginate, released calcium will increase the crosslinking density, and thus the stiffness of the gel (b). Compliant alginate gels are stiffened in proportion to irradiation time by releasing calcium (c). Similarly, initially stiff gels are softened by releasing DTPA (e). 3T3 fibroblasts in compliant gels adopt elongated morphologies, while stiffened gels restrict elongation (d). Cells become increasingly circular with increasing irradiation time (f).

Conclusions: Here, we demonstrate the ability to temporally stiffen or soften bulk hydrogels with light. We were able to induce morphological changes in fibroblasts, indicating that stiffening occurs over a range in which cells can sense and respond. Development of hydrogel systems such as ours is essential for the investigation of dynamic phenomenon in well-controlled settings.

References: 1) Georges, PC. Am J Physiol Gastrointest Liver Physiol. 2007:293(6);G1147-54. 2) Gillette, BM. Adv. Mater. 2010:22;686-91.