Retention and sequential release of gold nanoparticles from ultrasonically responsive polymeric capsules

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Statement of Purpose: The ability to retain and trigger the release of biologics from implantable systems is desired in many biomedical situations. This is particularly relevant in regenerative engineering where multiple signaling factors must be sequentially delivered in order to coordinate the complex sequences of biological events required to regenerate complex, functional tissues. However, most drug delivery biomaterials are limited in that they release drugs in a predetermined, diffusive manner. We hypothesized that polymeric capsules could be engineered to rupture, releasing encapsulated cargo, when remotely stimulated with ultrasonic signals. We thought in particular that capsules could be designed to rupture at specific ultrasonic doses, allowing for sequentially triggered rupture and release.

Methods: Gold-nanoparticle-containing capsules were created by dropping a solution of divalent cation, sucrose, and gold nanoparticles into a 0.25% wt alginate bath (Fig. 1A, i). Once surrounded by un-crosslinked alginate, divalent cations begin leaking out of the droplet, interacting with the un-crosslinked alginate (Fig. 1A, ii), eventually forming a crosslinked hydrogel shell that encapsulates the gold nanoparticles (Fig. 1A, iii). These capsules were designed to have different susceptibilities to ultrasonic rupture by varying the amount and type of divalent crosslinker: 50 mM $CaCl_2$ for weak capsules and 100 mM $BaCl_2$ for strong capsules. Capsules release studies were conducted by exposing capsules to ultrasonic signals of varying amplitude and duration, and measuring gold nanoparticle release via optical absorbance at 540 nm. In each experiment, a total of 62.5 μg of gold nanoparticles were loaded in roughly 15 capsules. Plotted values represent means and standard deviations (N = 4).

Results: Strong and weak alginate capsules ruptured at distinctively different ultrasonic doses. At 20% amplitude (~25 mW/cm^2 at 20 kHz), 10 s to 100 s of stimulation was required for weak capsules to transition from 0% to 100% release (Fig. 1B, i, red curve). Strong capsules required 100 s to 500 s to yield the same release (Fig. 1B, i, blue curve). Critically, 100% could be released from weak capsule at ultrasonic doses resulting in 0% release from strong capsules (Fig. 1B, i, vertical dashed line at 100 s). At 80% amplitude (~100 mW/cm^2), only 1 s was required for 50% release from the weak capsules and 10 s for 100% release (Fig. 1B, ii, red curve). Strong capsules required 10 s to 50 s to transition from 0% to 100% release (Fig. 1B, ii, blue curve). Again, 100% release from the weak capsules and 0% release from the strong capsules could be achieved by stimulating for 10 s (Fig. 1B, ii, vertical dashed line). Capsules exhibited excellent nanoparticle retention for prolonged periods. When unexposed to ultrasound for 7 days, capsules released nanoparticles in amounts under our detection threshold (< 3 μg per day). These capsules were also capable of being ultrasonically ruptured when integrated into larger tissue engineering scaffolds. We demonstrated that capsules ruptured when encased in a bulk alginate hydrogel at ultrasonic doses low enough to preserve the structural integrity of the bulk gel (Fig. 1C). Finally, these ultrasonically responsive capsules were capable of being triggered to release their payloads in a sequential manner. Strong (blue) and weak (red) capsules were exposed to 100 mW/cm^2 of ultrasound for 15 s and then for 50 s (Fig. 1D). The 15 second exposure ruptured all the weak capsules without rupturing the strong capsules (Fig. 1D, middle). A second ultrasonic signal lasting 50 s was then used to rupture the strong capsules (Fig. 1D, right).

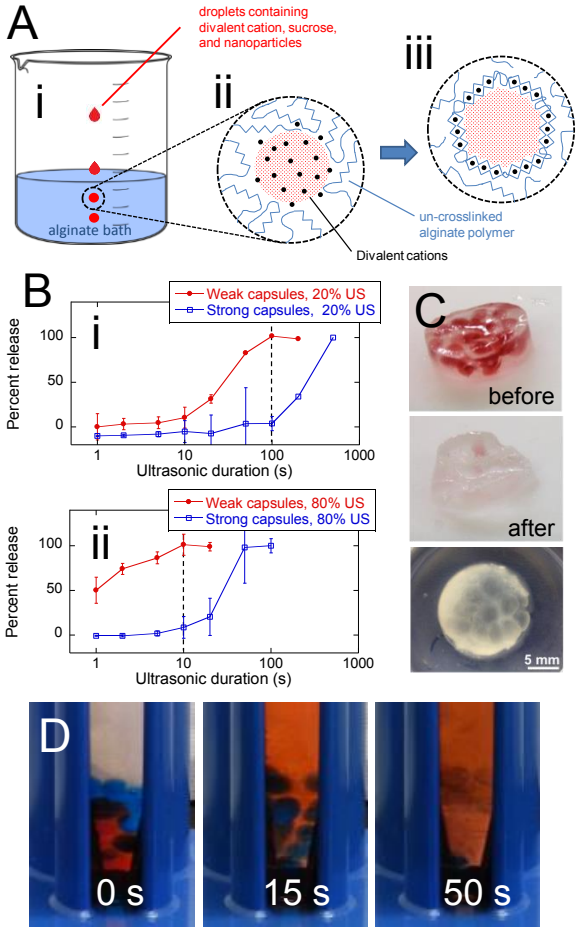

Figure 1.

Conclusions: Alginate capsules exhibited excellent payload retention capability and released their payloads when stimulated at ultrasonic doses low enough to preserve the structural integrity of a larger, encapsulating alginate scaffold. Capsules could be tailored to release their cargo at different ultrasonic thresholds, providing a means to sequentially trigger the release of multiple payloads. We believe that this system, when combined with nanoparticles that have been functionalized to present bioactive peptide, will be a powerful tool in fundamental biology research and regenerative engineering in scenarios where the timing, dose, and directionality of multiple signaling factors must be precisely coordinate.