**In vitro and In vivo Evaluation of Controlled Payload Release using Acoustically-Responsive Scaffolds**

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**Purpose:** Fibrin hydrogels are protein-based scaffolds frequently used in tissue engineering for delivery of bioactive payloads (e.g., growth factors). Conventional fibrin scaffolds enable very limited spatial and temporal control of release, especially after implantation; this is potentially problematic since tissue regeneration is tightly regulated by biomolecules in spatiotemporal manner. Acoustically responsive scaffolds (ARSs) - which are fibrin scaffolds doped with perfluorocarbon (PFC) emulsion - have been developed, thus enabling on-demand, spatiotemporally-controlled release of encapsulated payload using ultrasound (US). US is ideally suited to modulate release since it can be applied non-invasively and focused with sub-millimeter precision. This work investigates the US-modulated release of a surrogate payload from ARSs using *in vitro* and *in vivo* models.

**Methods:** Sonosensitive perfluorohexane (PFH, C\(_6\)F\(_{14}\)) double emulsions (W\(_1\)/PFC/W\(_2\), mean diameter: 2.1 ± 0.1 µm) were prepared as described previously by our group with AlexaFluor 680 dextran (10 kDa) in the W\(_1\) phase. For *in vitro* release studies, 0.5 mL ARSs were cast in 24 well plates by doping 5 mg/mL fibrin gels with 1% (v/v) emulsion and then covered with 0.5 mL of overlying medium. ARSs were exposed to focused US (2.5 MHz, P\(_r\) = 8 MPa, 13 cycles, 100 Hz pulse repetition frequency) for 2 min daily (starting on day 1). Aliquots of the overlying media were sampled throughout the experiment and analyzed with a plate reader. For *in vivo* studies, ARSs (0.25 mL volume per ARS) were prepared as described previously and injected subcutaneously in the lower back of BALB/c mice. After polymerization, a subset of the implanted ARSs were exposed to US (as previously described). Animals were imaged periodically using a preclinical, fluorescence imaging system to quantify the dextran signal remaining in the ARSs.

**Results:** *In vitro:* Over 6 days, US caused an 8.2-fold increase in dextran release compared to the –US condition (–US: 2.7 ± 0.6%; +US: 22.2 ± 3.0%). *In vivo:* US caused an increase in dextran release compared to the –US condition on day 3 (–US: 1.43 ± 6.47%; +US: 30.43 ± 5.33%) and day 7 (–US: 51.7 ± 9.6%; +US: 85.9 ± 7.1%), respectively.

![Figure 1](image-url)  
**Figure 1:** Focused US can control the release of dextran from ARSs in both *in vitro* (a) and *in vivo* (b) settings. US exposure began 1 day after polymerization/implantation. N = 5 for *in vitro* studies and N = 7 for *in vivo* studies. (β): p < 0.05.

**Conclusions:** Controlled release from an ARS can be achieved using focused US. Greater payload release was observed *in vivo*, for both –US and +US conditions; this is likely due to scaffold degradation mechanisms (e.g., cellular infiltration) that are present *in vivo* but not *in vitro*. Future work will look at optimizing ARS formulations for *in vivo* use to minimize payload release in the absence of US.