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● *Original Contribution*

SEQUENTIAL PAYLOAD RELEASE FROM ACOUSTICALLY-RESPONSIVE SCAFFOLDS USING FOCUSED ULTRASOUND

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Abstract—Regenerative processes, such as angiogenesis and osteogenesis, often require multiple growth factors with distinct spatiotemporal patterns and expression sequences. Within tissue engineering, hydrogel scaffolds are commonly used for exogenous growth factor delivery. However, direct incorporation of growth factors within conventional hydrogels does not afford spatiotemporally controlled delivery because release is governed by passive mechanisms that cannot be actively controlled after the scaffold is implanted. We have developed acoustically-responsive scaffolds (ARSs), which are fibrin scaffolds doped with payload-containing, sonosensitive emulsions. Payload release from ARSs can be controlled non-invasively and on demand using focused, megahertz-range ultrasound. In the *in vitro* study described here, we developed and characterized ARSs that enable sequential release of two surrogate, fluorescent payloads using consecutive ultrasound exposures at different acoustic pressures. ARSs were generated with various combinations and volume fractions of perfluoropentane, perfluorohexane, and perfluoroheptane emulsions. Acoustic droplet vaporization and inertial cavitation thresholds correlated with the boiling point/molecular weight of the perfluorocarbon while payload release correlated inversely. Payload release was longitudinally measured and observed to follow a sigmoidal trend versus acoustic pressure. Perfluoropentane and perfluorohexane emulsions were stabilized when incorporated into ARSs with perfluoroheptane emulsion. These results highlight the potential of using ARSs for sequential, dual-payload release for tissue regeneration. (E-mail: ambaez@umich.edu) **© 2018 World Federation for Ultrasound in Medicine & Biology. All rights reserved.**

Key Words: **Ultrasound, Controlled release, Perfluorocarbon, Fibrin, Acoustic droplet vaporization.**

INTRODUCTION

Tissue regeneration is driven by the spatiotemporally controlled expression and regulation of multiple growth factors (GFs). For example, during blood vessel growth, pro-angiogenic GFs, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), stimulate endothelial cell migration, mitogenesis, and sprouting (Abe et al. 2013; Akeson et al. 2003; Cao et al. 2003; Li et al. 2010; Ruhrberg et al. 2002; Saik et al. 2011). The newly sprouting vessels are then stabilized by pericytes, which are recruited by platelet-derived growth factor (PDGF). During the healing of a bone fracture, bone morphogenetic protein 2 (BMP2) initially stimulates callus formation during the inflammation stage. Later, VEGF causes vascular ingrowth from the periosteum. Reviews more fully highlight the complex, temporal orchestration of GF signaling involved in the multiple stages of angiogenesis and osteogenesis (Ai-Aql et al. 2008; Carmeliet and Jain 2011).

Hydrogel scaffold-based delivery systems can provide sequential delivery of multiple GFs to enhance tissue regeneration. The motivation for generating these scaffolds is that sequential delivery of GFs can mimic critical aspects of endogenous GF signaling more closely. Additionally, certain GFs (*e.g.,* bFGF and PDGF) are mutually antagonistic when present simultaneously (Tengood et al. 2011), thus further highlighting the need for sequential delivery. Sequential delivery of two GFs (*e.g.,* GF1, GF2) has been achieved by designing a composite scaffold using one of two general

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strategies. First, GF1 is incorporated into a scaffold while GF2 is pre-encapsulated into particles, which are then incorporated into the scaffold (Richardson et al. 2001). This therapeutic approach typically results in GF1 being released at a faster rate than GF2. Second, GF1 and GF2 are pre-encapsulated into separate particles, which are then incorporated into a scaffold (Basmanav et al. 2008). In both strategies, the release kinetics of the GFs are dependent on the material properties of both the scaffold and particles, such as crosslinking density, pore size, GF affinity, and charge. Sequential delivery of VEGF/PDGF (Awada et al. 2015), bFGF/BMP-2 (Lee and Koh 2014), BMP-2/BMP-7 (Basmanav et al. 2008), VEGF/BMP-2 (Kempen et al. 2009), and BMP-2/insulin-like growth factor 1 (IGF-1) (Kim et al. 2012) has been done using the previously mentioned techniques.

A critical limitation of the previous sequential delivery strategies is that the release kinetics of GF1 and GF2 are designed *a priori*. Therefore, after the scaffold is implanted at, or adjacent to, the site of intended tissue regeneration, the release kinetics, including the initial timing of release of GF1 and GF2, cannot be altered. From a clinical perspective, this is potentially problematic because the release kinetics of the multiple encapsulated GFs cannot be adjusted based on the actual progress of tissue regeneration within a patient. Thus, scaffold-based delivery systems that enable active modulation of sequential release would be beneficial.

We have developed a scaffold-based delivery system in which release of a bioactive payload is controlled non-invasively and in an on-demand manner using focused, megahertz-range ultrasound (US). These acoustically-responsive scaffolds (ARSs) consist of a fibrin hydrogel doped with a micron-sized, perfluorocarbon (PFC) double emulsion (Moncion et al. 2016a, 2016b, 2017). Fibrin was chosen as the hydrogel because of its biocompatibility, ability to degrade with minimal inflammatory response, potential for autologous sourcing, and low viscoelastic properties that help facilitate cell migration (Lee and Mooney 2001; Markert et al. 2013). A water-soluble payload is encapsulated within the PFC double emulsion having a water-in-PFC-in-water (W_1/PFC/W_2) structure. When exposed to pulsed (*i.e.,* non-thermal) US above a certain pressure, the PFC phase within the emulsion vaporizes in a process known as acoustic droplet vaporization (ADV), which causes

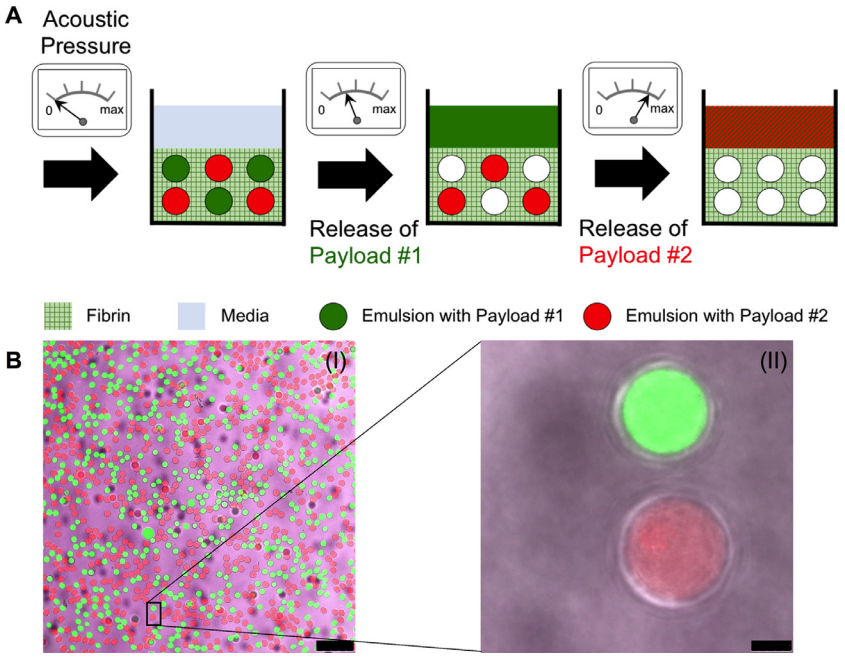

Fig. 1. (A) Sequential release of two payloads using an acoustically-responsive scaffold (ARS). Two similarly sized emulsions, each containing a different PFC and payload, are incorporated into an ARS. For release of payload 1, the ARS is exposed to ultrasound at an acoustic pressure above the acoustic droplet vaporization threshold of the emulsion with payload 1, but below the threshold of the emulsion containing payload 2. At a later time, the same ARS is exposed to ultrasound at an acoustic pressure above the acoustic droplet vaporization threshold of the emulsion with payload 2, thus releasing payload 2. (B) Confocal microscopy images of an ARS with two payloads at 25 × (I) and 100 × (II) magnification. The ARS contained 0.67% (v/v) perfluorohexane emulsion with Alexa Fluor 488-labeled dextran in the W_1 phase (*green*), 0.33% (v/v) perfluoroheptane emulsion with Alexa Fluor 594-labeled dextran in the W_1 phase (*red*), and Alexa Fluor 647-labeled fibrinogen (*magenta*) in the fibrin matrix. Bar = 75 and 5 μm for B(I) and B(II), respectively.