Mechano-activatable Microcapsules for Tunable Drug Delivery

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Introduction

A particularly desirable feature of controlled drug delivery is self-regulation, wherein physiological feedback actively regulates release kinetics. Extant systems in this domain commonly employ stimuli-responsive vehicles that rely on internal triggers to precipitate release, such as temperature, pH, or chemical reactions.[1,2] The sensitivity of these triggers can be tuned by biomaterial composition or structure to regulate release in a given physiologic or pathophysiologic state. However, to date, such delivery vehicles have not been specifically tuned for mechanically activated release.

Tissues within the body experience mechanical perturbations across multiple force magnitudes and length scales, from mechanotransduction at the cell level[3] to the dynamics of load-bearing joints.These forces not only maintain tissue homeostasis, but can also initiate degenerative processes when applied at supra-physiologic levels.[4,5] Given the centrality of mechanical loading in normal tissue function, our objective was to develop a mechanically activated drug delivery system to stimulate regeneration and repair in mechanically loaded musculoskeletal tissues (e.g. cartilage, muscle, bone). Towards this goal, we developed a novel class of mechano-activatable microcapsules (MAMCs, Ø~50-100μm), and illustrate here the tuning of design parameters to enable differential responses to varying applied mechanical stimuli.

Methods

MAMC Fabrication: Mechanically activated microcapsules (MAMCs) were produced with a poly(lactic-co-glycolic) acid (PLGA) copolymer shell (doped with Nile Red) and an aqueous core containing FITC-dextran, 2 MDa, (Figure 1C). Microcapsules were fabricated using a glass-capillary microfluidic system to produce a highly monodisperse water-in-oil-in-water (W/O/W) emulsion with ~100% encapsulation efficiency[6] (Figure 1A, B). Using osmotic annealing, the wall thickness to radius ratio (t/D) was tuned by modulating the concentration of PLGA (50:50) in the middle phase, as well as the solute (NaCl) concentration in the inner and outer phases.[6] Three batches of microcapsules were fabricated,

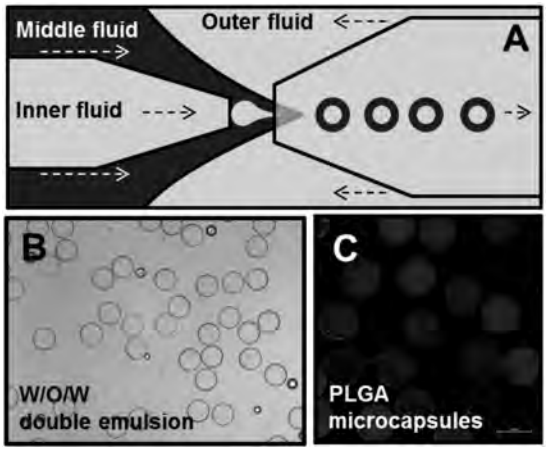

Figure 1. (A) Schematic and (B) microscopy image of W/O/W double emulsion generated from a capillary microfluidic device for fabrication of (C) PLGA microcapsules.

with a t/D ratio of (1) 0.008 (t~400nm, D~50μm), (2) 0.012 (t~600nm, D~50μm), and (3) 0.015 (t~1500nm, D~100μm). All capsules were maintained in 0.15M NaCl at 23°C.

MAMC Compression: To demonstrate mechano-activation, a single layer of MAMCs was subjected to increasing levels of load between two glass coverslips (Figure 2). Microcapsules were loaded to ~50g, 200g, and 500g; intact, unloaded MAMCs served as a negative control while MAMCs sheared between two glass slides served as a positive control. After overnight incubation at 23°C in phosphate-buffered saline (PBS), z-stack images were collected at 20X magnification using a Nikon confocal microscope.The mid-slice was used to determine whether a single MAMC was "full" or "empty" (devoid of FITC-dextran). Fluorescence intensity of the supernatant was also assayed to measure bulk MAMC release (Ex/Em: 490/520).

MAMC Mechano-activation in 3D Hydrogels: To validate mechano-activation in a three-dimensional construct, MAMCs were embedded in a photo-crosslinked poly(ethylene glycol) diacrylate (PEGDA) hydrogel. Using a custom micromechanical compression device mounted atop a confocal microscope (Figure 3A),[7] MAMC-laden hydrogels were compressed in unconfined compression, from 0-20% strain at steps of 4%, followed by compression until hydrogel failure. Individual MAMCs (n = 2/hydrogel) were

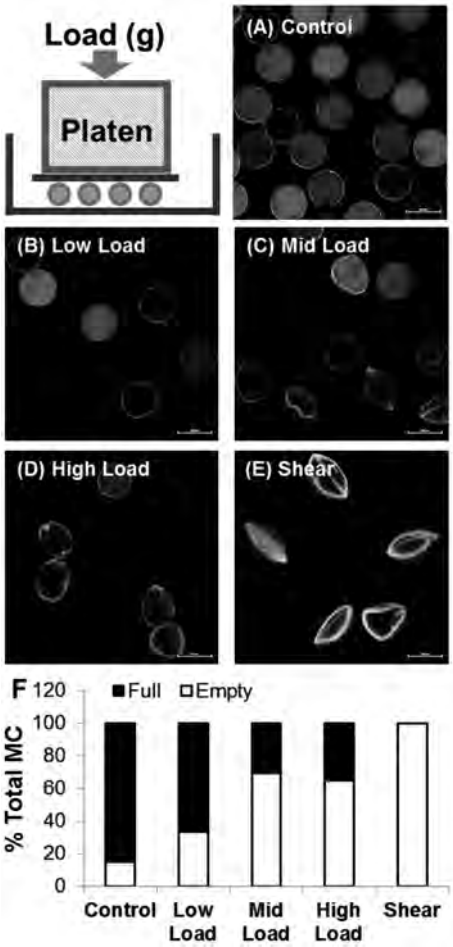

Figure 2. Mechano-activation of single MAMCs. (A-E) MAMCs containing dextran (green) with labelled shells (red) deform and fracture at higher loads. (B) Quantification of release as a function of load.

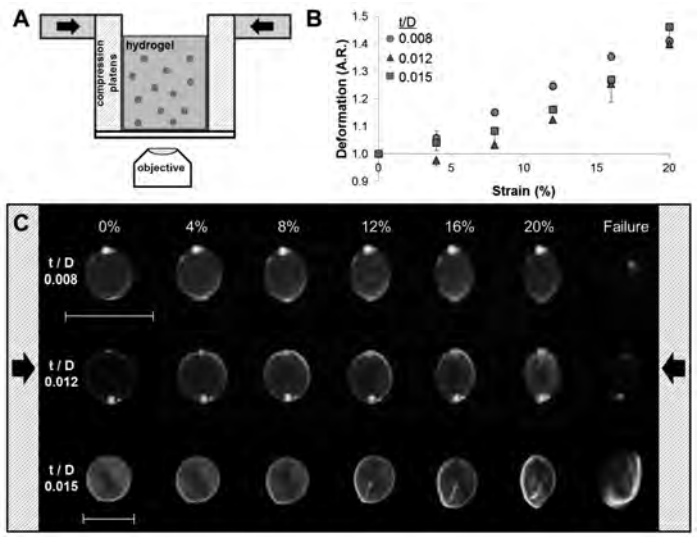

Figure 3. Mechano-activation in a 3D context. (A) Micromechanical device for imaging gel-MAMC composites with deformation. (B) Quantification of MAMC aspect ratio (A.R., n = 2) as a function of fabrication parameters. (C) MAMC deformation during progressive compression of the hydrogel.

tracked through 20% strain and z-stacks collected (at 20X) at each strain level. Microcapsule deformation was quantified by measuring the elongation of the microcapsule at the mid-point for each strain step.

Results

To assess mechano-activation, isolated MAMCs (t/D~0.015) were subjected to increasing load (Figure 2). MAMC deformation increased with applied load, resulting in a graded pattern of microcapsule rupture and release of encapsulated FITC-dextran. Sheared MAMCs served as a positive control, with ~100% of MAMCs devoid of a fluorescent signal. The fluorescence intensity of the buffer solution also correlated with applied load, confirming MAMC mechano-sensitivity (not shown).

To demonstrate mechano-activation within a 3D context, MAMCs were embedded in a 30% (v/v) PEGDA hydrogel and evaluated using a confocal-mounted compression device (Figure 3A). 30% PEGDA was chosen as an encapsulating matrix as it has a stiffness comparable to both native and mature engineered cartilage.[14,15] With compression of the hydrogel, MAMCs deformed in a dose-dependent fashion, becoming ellipsoid at 20% strain and rupturing upon hydrogel fracture (observed at ~57% strain). At t/D ratios of ~0.012 and 0.015, MAMCs underwent folding and/or sharp bending of the capsule wall, while at a t/D ratio of ~0.008, MAMCs maintained a rounded appearance with compression.

Discussion

In this study, we developed a method to generate microcapsules that are mechano-sensitive and characterized the behavior of their mechano-activatable properties towards the development of a novel platform for controlled and self-regulated drug delivery. Within this MAMC population, the amount of rupture and release was readily controlled by the magnitude of the applied load. While low loads resulted in rupture of only a small fraction of MAMCs, higher loads resulted in > 50% release. Within the context of a 3D environment, the deformation behavior and mechanism of MAMC rupture was in part dependent on the wall thickness-to-radius ratio (t/D).[8]

Previously, drug delivery mediated by mechanical rupture has primarily focused on mechano-chemical or thermal mechanisms to initiate release, wherein pH or temperature induced phase changes result in structural defects in the capsule shell.[2,9] Our MAMCs are designed in a manner analogous to that of self-healing polymers used in material science applications, where microcapsules containing a "healing agent" are embedded in a matrix and, upon fracture, initiate polymerization and 'repair'. Building from this self-healing concept, mechano-activation of MAMCs can be designed to stimulate repair or regeneration through biologic mechanisms spurred by the release of factors from the MAMCs. Future studies will focus on tuning MAMC mechanical behavior via alteration of fabrication parameters, with the goal of designing

a cohort of microcapsules with different failure thresholds in order to program sequential therapeutic delivery. Techniques such as atomic force microscopy and finite element modelling will be used to characterize MAMC strain and rate-dependent deformation, rupture, and release profiles. In addition, initial efficacy studies will measure the chondrogenic effects of growth factors (e.g. TGF- β) released from MAMCs in a dynamically loaded engineered cartilage model. This work provides an initial characterization of MAMCs, and sets the stage for the widespread application of this drug delivery system for musculoskeletal repair and regeneration.

Acknowledgements

This work was supported by the National Science Foundation, the Department of Veterans' Affairs, and the Penn Center for Musculoskeletal Disorders.

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