Fate of Mechano-Sensitive Microcapsules after Intra-Articular Onjection in a Large Animal

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Introduction

Intra-articular injections are commonly administered to relieve joint pain, dampen inflammation, and ultimately slow the progression of joint disease. However, within the synovial joint environment most drugs and drug delivery systems are rapidly cleared, limiting their efficacy. To extend the joint residence time of injected agents, a number of drug delivery vehicles are under development, including nanoparticles[1] and microcapsule-based carriers.[2] Microcapsules are particularly appealing for their efficient encapsulation of biologics that are contained within an aqueous core. The physical dimensions of theses microcapsules, which we term mechanically activated microcapsules (MAMCs), can be tuned to release their contents based on hydrolytic degradation of the (lactic-co-glycolic-acid) (PLGA) shell or via direct mechanical rupture.[3] In this study, we sought to assess the fate of MAMCs and determine their retention, localization, and integrity after intra-articular injection into a large animal knee. We hypothesized that the MAMCs would be retained in the joint space and progressively rupture over time *in vivo*.

Methods

MAMC fabrication

Microcapsules were fabricated using a glass capillary device as previously described (Figure 1A, B).[4]

Animal model

Six 1-year old castrated male Yucatan minipigs were used for this study. Each animal received a single intra-articular knee injection (1mL, 500,000 microcapsules/mL) in the left hind limb, either 2 weeks (N = 3) or 1 day (N = 3) before euthanasia (Figure 1C). For each knee injection, an anterolateral approach was taken whereby a 21 gauge 1 1/2 inch needle was positioned perpendicular to the skin with the tip of the needle directed at a 45-degree angle into the center of the knee (Figure 1D). Sterile saline was first injected to ensure proper placement of the needle. Post-injection, the animals were weight-bearing and walking within an hour.

Limb preparation & In Vivo Imaging System (IVIS®) (microcapsule retention).

After sacrifice, both the left (injected) and right (control) limbs were removed, skinned, and segmented to remove the proximal femur and distal tibia. The hind limbs were then imaged (Perkin Elmer IVIS Spectrum) to assess the retention of injected microcapsules. The excitation and emission wavelengths were set in accordance with Nile Red (Ex. 549/Em. 628), marking the microcapsule shells. Each knee was imaged in four positions—anterior, posterior, lateral, and medial, to determine the viewpoint with the maximum radiant efficiency. Images were analyzed using the Living Image Software and FIJI.

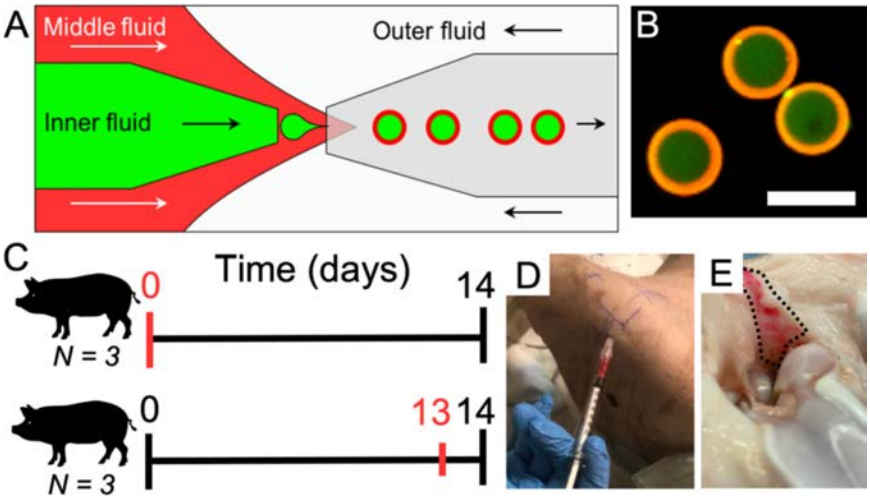

Figure 1. *Microcapsule fabrication and injection.* **(A)** Capillary device forms the microcapsules. **(B)** Injected capsules. Scale bar = 40 μm. **(C)** Study timeline. Red lines depict injection time point. **(D)** Anterolateral injection. **(E)** Microcapsules adhered to and within the adipose tissue (outlined).

Microcapsule localization & integrity

Following IVIS imaging, the left (injected) limbs were finely dissected in order to assess microcapsule localization (Figure 1E) and integrity (% intact). A Zeiss Axio Zoom.V16 was used to acquire images, with n = 100 + microcapsules/animal quantified using the FIJI cell counter.

Statistics.

Figure 2B, C: n.s. Fig. 3: One-tailed Mann-Whitney test. Significance $p \leq 0.05$. Data shown are the mean ± standard deviation.

Results

IVIS imaging showed the presence of the MAMCs within the knee joint both 1 and 14 days post-injection (Figure 2). There was no signal from the control limbs. On average, both the maximum radiant efficiency and the total signal area decreased with increasing time *in vivo*, as expected. After opening joint capsule, MAMCs were observed to be dispersed throughout the synovial and adipose tissues (Figure 3A, B). Most excitingly, intact MAMCs were identified after both 1 (average: 76.1% intact, std dev: 17.1) and 14 days (average: 26.6% intact, std dev: 9.2) (Figure 3C-E). There was a significant

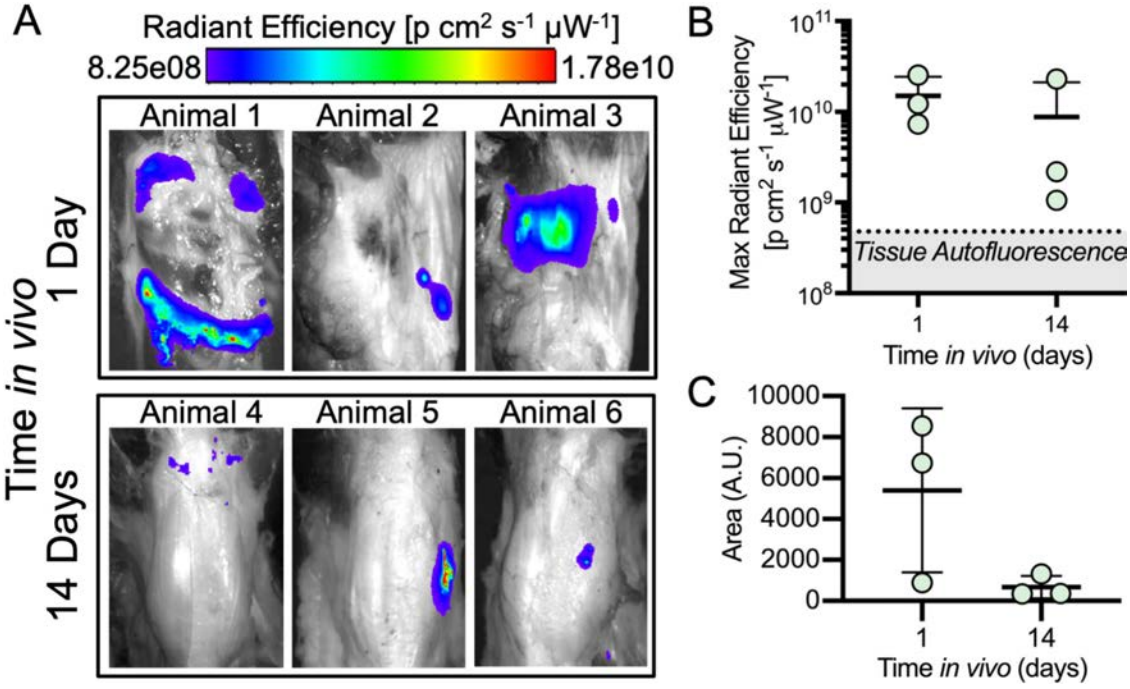

Figure 2. Contact pressure maps (top) and quantification of peak contact pressure. *Microcapsule retention in knee joint.* **(A)** IVIS images at the viewpoint of the maximum signal. **(B)** Quantification of the maximum radiant efficiency. **(C)** Quantification of the total signal area, in arbitrary units (A.U.).

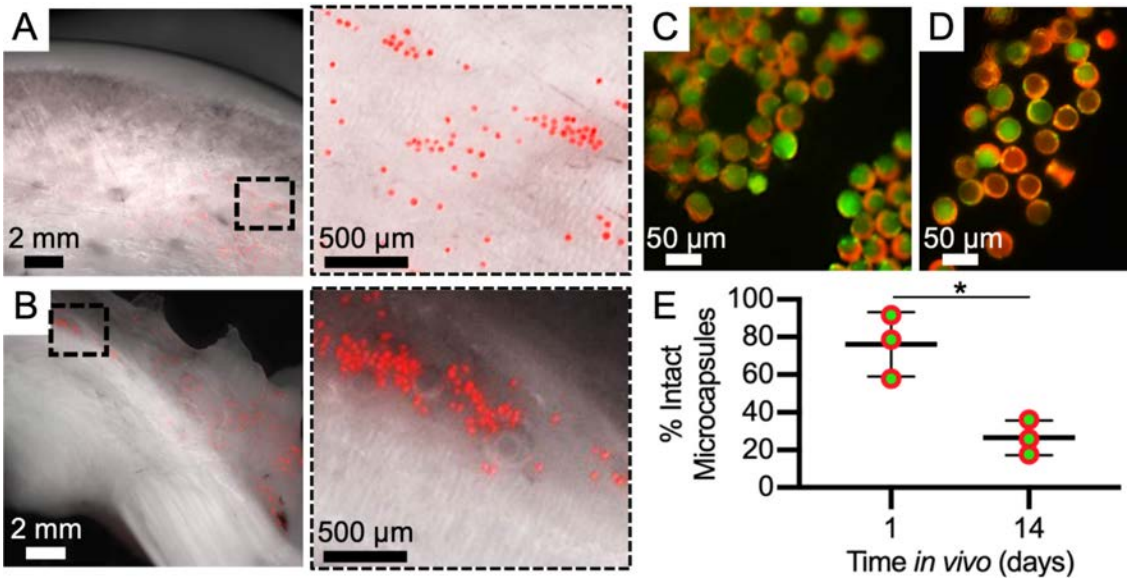

Figure 3. *Microcapsule localization and integrity in knee joint.* **(A, B)** Microcapsules were adherent to the boundaries of the trochlea, and meniscus, respectively. **(C, D)** Microcapsules after 1 and 14 days *in vivo*, respectively. **(E)** Quantification of % of microcapsules that remained intact (with green interior).

decrease in the percentage of intact MAMCs from 1 to 14 days ($p = 0.05$).

Discussion

The variability in the IVIS signal area and location motivates local, directed delivery of the MAMCs for the treatment of specific tissues. However, a simple injection may be advantageous for non-specific delivery of anti-inflammatory molecules, disease modifying agents, or corticosteroids throughout the entire joint. To further modulate the sustained release of an aqueous therapeutic using these microcapsules, a mixture of microcapsule sizes (diameter and shell thickness) with unique rupture profiles and degradation rates could be injected. Future work will also tune the adhesivity of the microcapsules to improve retention at specific locations (e.g., to direct mechano-activation), as well as explore the incorporation of MAMCs within regenerating tissues.

Significance

This work establishes that MAMCS can be injected into the joint space as a potential delivery vehicle for therapeutics and demonstrates a pipeline for assessing their retention, localization, and integrity in a large animal model. Additionally, these results show promise for hydrolytically and mechanically activated sustained delivery of therapeutics to the knee joint.

Acknowledgments

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