Viscous Hyaluronic Acid Carriers Enhance the Stability of Therapeutic Mechanically-Activated Microcapsules

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Introduction

Acute knee injuries are common among accident victims, athletes, and military service members and induce diverse pathogenic cascades that often result in post-traumatic osteoarthritis (PTOA). Despite active research, there are still no FDA-approved disease modifying OA drugs that focus on early intervention to delay, attenuate, or altogether prevent PTOA. Furthermore, the efficacy of current drug delivery platforms is limited by short half-lives and rapid joint clearance.[1,2] To this end, we developed mechanically-activated microcapsules (MAMCs), which are capable of prolonging residence time within the joint while delivering an array of therapeutic factors.[3,4] However, the relatively short therapeutic "window of opportunity" following an acute knee injury necessitates an "off-the-shelf" solution.[5,6] End-users, such as first responders, emergency department providers, and military medical personnel will require ready access to these therapeutics. However, they must also follow stringent design criteria to enable use in austere environments.[7] Like any microcapsule-based drug delivery system, MAMCs are susceptible to degradation from physical agitation and temperature changes. Our objective was to determine the resilience of MAMCs under environmental stressors and identify a clinically relevant carrier capable of providing physical and thermal protection. We hypothesized that viscous, high molecular weight hyaluronic acid (HA) solutions (EUFLEXXA®) would provide both physical and thermal protection to MAMCs and increase their retention of therapeutic contents in environmental stress.

Methods

MAMCs

MAMCs containing IL-1Ra (anakinra, KINERET®) were fabricated as previously described and stored in PBS at 4° until use. Before testing, MAMCs were suspended in a microtube in either 50% v/v PBS +/− 10% w/v trehalose or 50% v/v HA. MAMC percent (%) full was determined by confocal microscopy (Figure 1). ImageJ was used to quantify the total number of MAMCs and the number of intact (full) MAMCs. The % of full MAMCs was normalized to the original % full for each group to account for differences in starting values. After each test, MAMCs were incubated overnight at 4° to allow complete diffusion of the inner contents after rupture.

Physical and Thermal Stress Tests

To identify a carrier system for MAMCs and to evaluate their clinical and commercial translatability, rigorous physical and thermal stress tests were conducted, with the primary outcome being MAMC % full. To evaluate MAMC physical stability and the protecting capabilities of the HA carrier, MAMCs were agitated using a benchtop vortex mixer on the most vigorous setting for 10-60 min. To assess injectability across a range of clinically relevant needle sizes, MAMCs were loaded

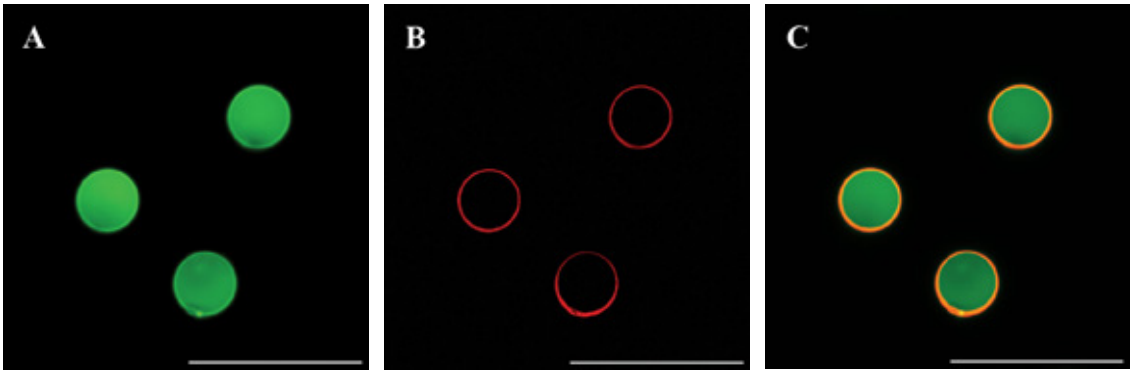

Figure 1. Mechanically-Activated Microcapsules (MAMCs). Fluorescent images of MAMCs showing their **(A)** inner therapeutic contents, **(B)** PLGA shell, and **(C)** merged composite. MAMC diameter: 38.2 μm, shell thickness: 2.3 μm, 60×, scale bar: 100 μm.

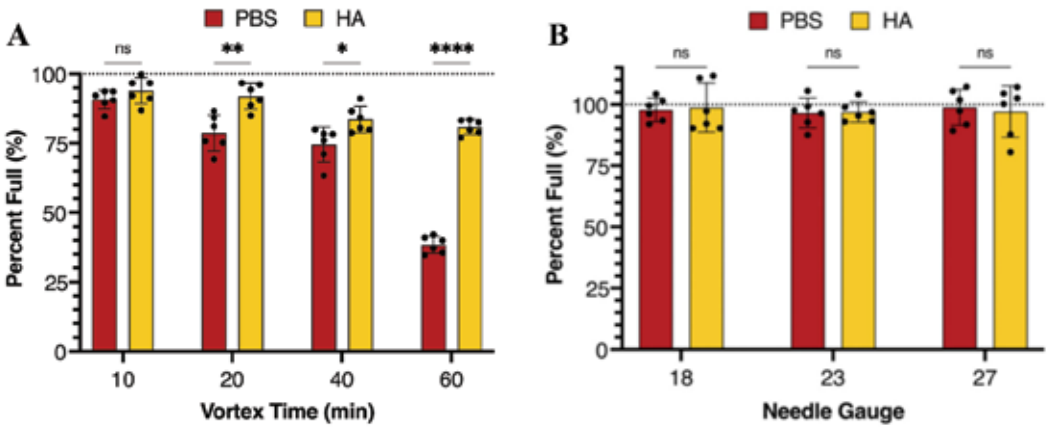

Figure 2. MAMC Physical Stability. **(A)** MAMC % full after physical agitation using a benchtop vortex mixer over 60 min; **(B)** MAMC % full after a single ejection at 10 mL/min through 18-, 23-, and 27-gauge needles. n=6, mean +/− SD, *p≤0.05, **p≤0.01, ****p≤0.0001.

into 3 mL syringes and ejected at 10 mL/min through 18-, 23-, and 27-gauge needles. To evaluate payload retention after freeze-thaw, an expected step in both the clinical and commercial cold chain, MAMCs were frozen at −20° for 30 min and thawed at 20° for 30 min over 1-5 freeze-thaw cycles. To assess the effect of temperature on payload retention, MAMCs were stored at −20, 4, 20, 37, and 50°, and % full was quantified on day 1, 3, and 7. Percent full was normalized to the 4° values on day 1.

Statistics

For all studies, n = 6/group with mean +/− SD shown. Two-way ANOVA with Fisher's LSD was used to compare carrier groups and treatment condition; $p \leq 0.05$.

Results

MAMCs suspended in HA were more resilient to physical agitation over longer durations compared to those

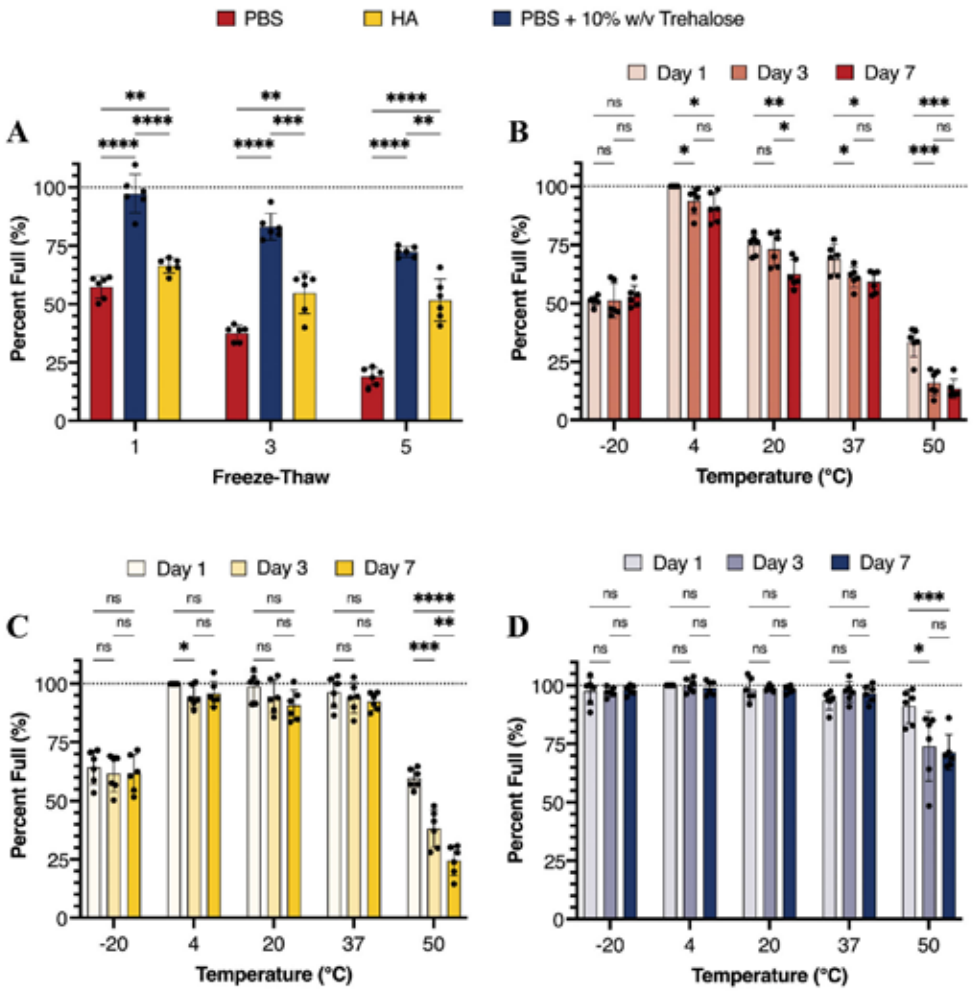

Figure 3. MAMC Thermal Stability. **(A)** MAMC % full after repetitive freeze-thaw cycles at −20° and 20°, respectively; MAMC % full after 1, 3, and 7 days of storage at −20 to 50° in **(B)** PBS, **(C)** HA, or **(D)** PBS+10% w/v trehalose. % full normalized to day 1, 4° values. n=6, mean +/− SD, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

suspended in PBS (Figure 2A). MAMCs ejected through 18-, 23-, and 27-gauge needles showed no difference in payload retention (Figure 2B). Subsequent freeze-thaw cycles ruptured MAMCs in a stepwise manner regardless of carrier, but the addition of 10% w/v trehalose or suspension in HA significantly increased cryoprotection at each cycle (Figure 3A). When suspended in PBS, MAMCs experienced significant loss of inner contents at 4, 20, 37, and 50° over 7 days (Figure 3B). In HA, MAMCs experienced no loss of inner contents at 4, 20, and 37° over 7 days (Figure 3C). In PBS+10% w/v trehalose, MAMCs did not experience any payload loss from -20 to 37° over 7 days (Figure 3D). In all carriers, the duration of storage at −20° with a single freeze-thaw cycle had no impact on MAMC payload retention (Figure 3B-D).

Discussion

Our findings show that suspension of MAMCs in HA confers significant physical and thermal protection to MAMCs and increases their payload retention when exposed to environmental stressors. HA increased MAMC physical stability after extended durations of high frequency agitation, circumstances that would be expected to be encountered in ambulances, MEDEVAC aircraft, or within the medical bags of first responders and military medical personnel. MAMCs withstood ejection from a wide range of needle gauges in both PBS or HA, facilitating use in small animal studies and patients with varying joint sizes. The use of trehalose, a common cryoprotectant, or suspension in HA, increased MAMC stability to repetitive freeze-thaw cycles. This finding diversifies and extends the shipping and storage parameters for these delivery systems. The use of HA and trehalose also maintained MAMC payload retention at 4, 20, and 37° over 7 days, which facilitates their "off-the-shelf" use where conventional refrigerated or frozen storage environments are not feasible, such as within resource-limited clinics or forward military bases. Future studies will assess the bioactivity of MAMC contents after exposure to physical and thermal stresses to ensure no loss of therapeutic efficacy and will assess how the HA carrier affects joint retention and localization of MAMCs after intra-articular injection. Overall, these data indicate the use of an HA carrier can prolong MAMC lifespan by retention of their inner contents, thus enabling the deployment of MAMCs in austere conditions and under physical and thermal environmental stress.

Significance and Clinical Relevance

This work establishes important storage and handling parameters for a novel drug delivery system and identified an FDA-approved, clinically available HA carrier that increases the physical and thermal stability of therapeutically loaded microcapsules. Our findings show that an HA carrier can be used to increase the therapeutic lifespan of microcapsules containing an anti-inflammatory factor, thus enabling their use in "off-the-shelf" applications, such as emergency or battlefield medicine.

References

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