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 different pervasive pathogenic states 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 clearance5,6. 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 factors4. However, the relatively short shelflife of therapeutic “window of opportunity” following an acute knee injury necessitates an “off-the-shelf” solution5,6. 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 environments7. 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 were fabricated as previously described and stored in PBS at 4°C until use. Before testing, MAMCs were suspended in a microtub in either 50% v/v PBS + 10% w/v trehalose or 50% v/v HA. MAMC Analysis: MAMC percent (%) full was determined by confocal microscopy (Fig 1). ImageJ was used to count the total number of MAMCs per field of view. MAMC % full was quantified on day 1, 3, and 7. Percent full 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°C to allow complete diffusion of the inner contents. MAMCs were frozen at -20°C for 30 min and thawed at 20°C for 30 min over 1-5 freeze-thaw cycles. Temperature Stability: MAMCs were stored at -20, 4, 20, 37, and 50°C for 7 days. % full was quantified on day 1, 3, and 7. Percent full was normalized to the 4°C 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<0.05.

RESULTS: MAMCs suspended in HA were more resilient to physical agitation over longer durations compared to those suspended in PBS (Fig 2A). MAMCs passed through 18-, 23-, and 27-gauge needles. Freeze-Thaw: MAMCs were frozen at -20°C for 30 min and thawed at 20°C for 30 min over 1-5 freeze-thaw cycles. Temperature Stability: MAMCs were stored at -20, 4, 20, 37, and 50°C for 7 days (Fig 2B). In HA, MAMCs experienced no loss of inner contents at 4, 20, and 37°C over 7 days (Fig 3C). In both carriers, the duration of storage at -20°C with a single freeze-thaw cycle had no impact on MAMC payload retention (Fig 3B-C).

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 withheld 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 also maintained MAMC payload retention at 4, 20, and 37°C over 7 days, which allows for 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/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.


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