Cartilage-bound large multilamellar liposomes show potential cartilage protection from inflammatory stimulus by attenuating aggrecan degradation

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Introduction

- Cartilage wear during osteoarthritis (OA), involves degradation of 2 of its structural components, aggrecan, and collagen type II by aggrecanases and matrix metalloproteases (MMPs), respectively
- It is well established that during the progression of OA, degradation of aggrecan is an early event that precedes collagen type II degradation[1]
- Degraded aggrecan has been shown to activate proinflammatory signaling pathways, which in turn can induce further cartilage damage
- MM-II, a suspension of large empty multilamellar liposomes for intra-articular injection, was clinically demonstrated to reduce pain. Results from a randomized, double-blind study are presented at the current OARSI congress
- MM-II was previously shown in vivo to slow down cartilage degeneration in a surgical OA rat model, and shown ex vivo to coat cartilage and reduce friction and wear

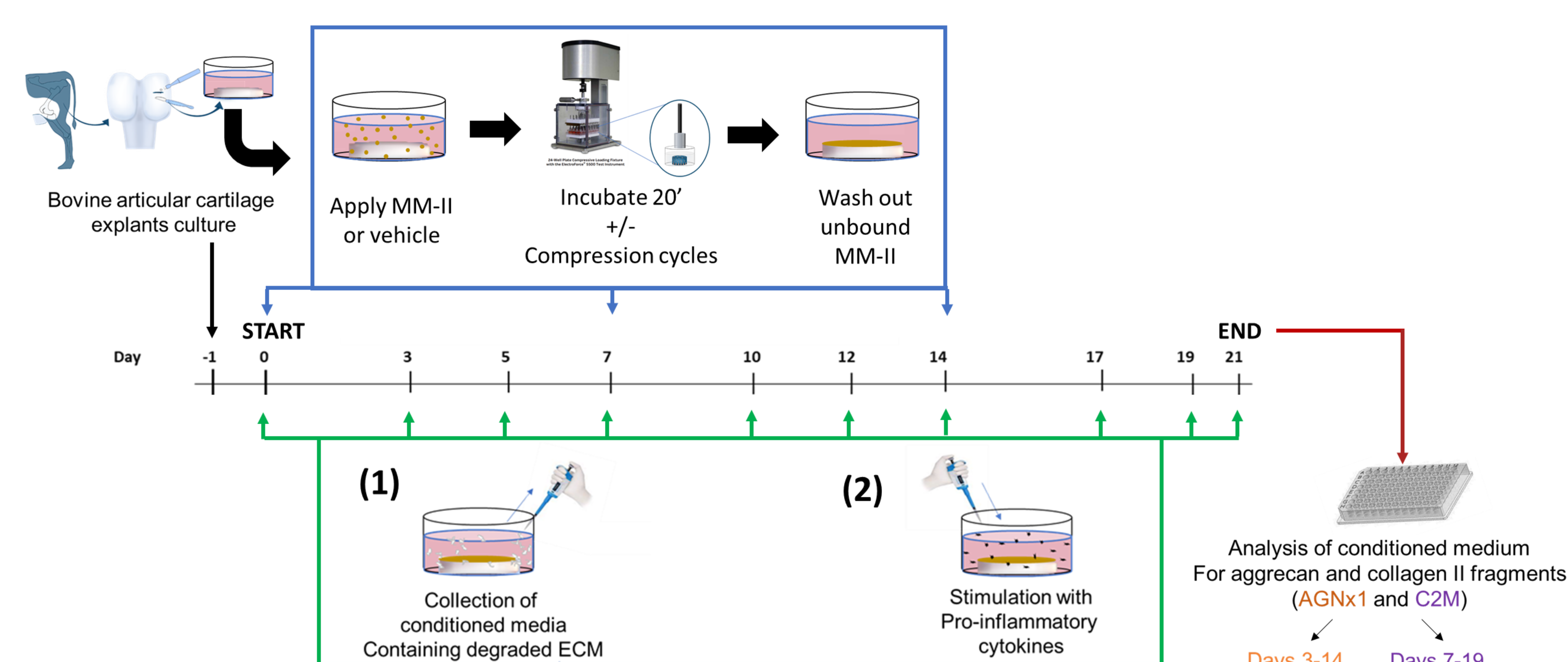
Objective

- To assess the ability of MM-II liposomal coating to protect cartilage explants from inflammatory cytokine-induced degradation using an established explant model[2,3]

Methods

Study setup: Cartilage explant culture, treatments, and analysis

Figure 1. Experimental setup



AGNx1, aggrecanase-generated aggrecan fragment; C2M, matrix metalloprotease-generated type II collagen fragment.

- Bovine cartilage explants (ø = 6mm) were cultured in growth medium
- At days 0, 7, and 14, MM-II liposomes or MM-II carrier buffer ("vehicle") were applied onto the explants with or without cyclic compression load for 20 minutes followed by thorough washing to remove unbound liposomes
- The explants were exposed to stimulation with inflammatory cytokines (Oncostatin M and tumor necrosis factor-α; O+T) from day 0 throughout the study
- Conditioned medium was collected at 2–3-day intervals and levels of extracellular matrix fragments, aggrecanase-generated aggrecan fragment (AGNx1), and MMP-generated type II collagen fragment (C2M) were measured by proprietary ELISA kits for days 3–14 (AGNx1) and days 7–19 (C2M)
- Metabolism of the tissue over the entire duration of the study was assessed by Alamar Blue at days 0, 7, 14, and 21

Table 1. Experimental groups

Tested material	Cytokine (O+T) stimulation	Compression
Vehicle	–	(+)
MM-II	–	(+)
Vehicle	+	(+) or (–)
MM-II	+	(+) or (–)
Vehicle + inhibitor control	+	(+) or (–)

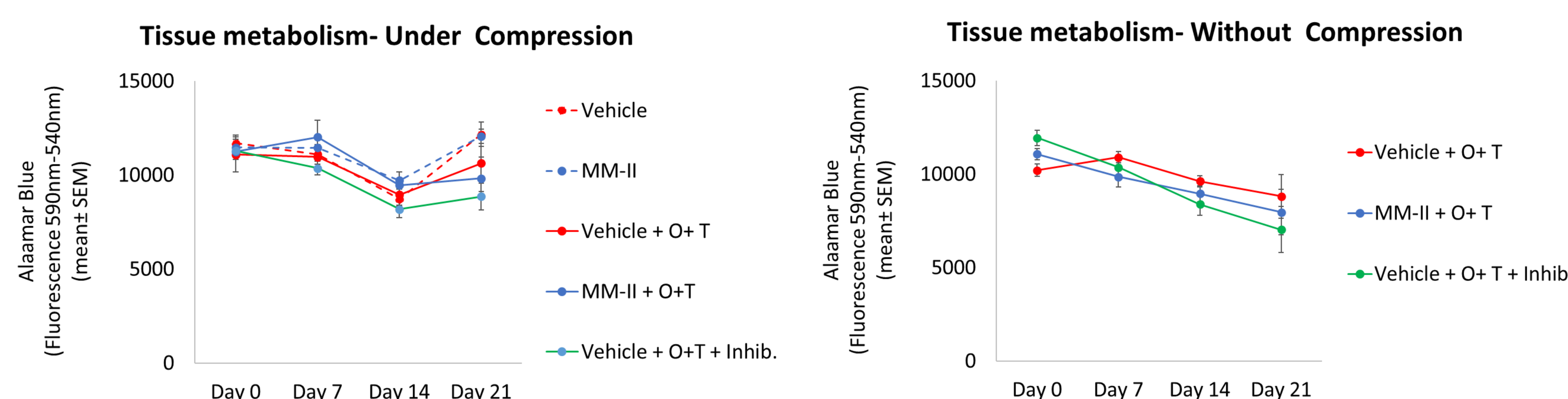
Vehicle: MM-II carrier buffer (histidine mannitol, pH 6.5); MM-II: Large, multilamellar DMPC/DPPC liposomes (150 mM total lipids); Inhibitor control: MMP (GM6001; 10 μM) + ADAMTS (PCI; 25 μM) inhibitors.
ADAMTS, A disintegrin and metalloprotease with thrombospondin motifs; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; MMP, matrix metalloprotease; O+T, Oncostatin M + tumor necrosis factor-α.

Results

Metabolism of cartilage tissue (Figure 2):

- No apparent toxic effects of MM-II were detected
- Stimulation with O+T did not significantly impact tissue metabolism
- Some deterioration was noted in the "no compression" group at day 21

Figure 2. Metabolism of cartilage explants during the study



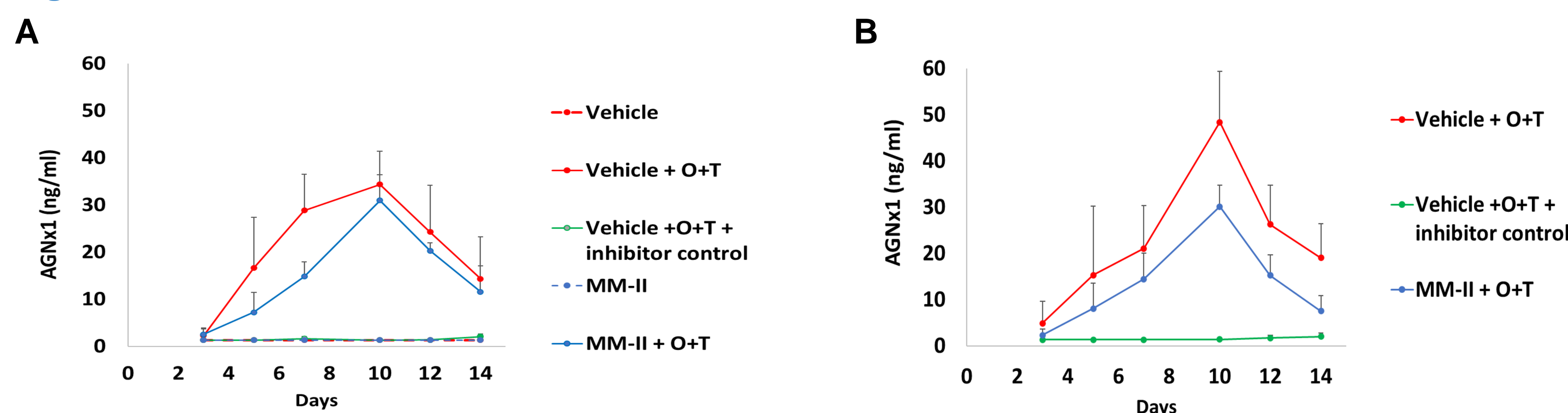
AB reagent was used on a weekly basis to quantify the metabolic activity of the explants in order to verify that the explants remain viable throughout the culture period and, thus, that any observed changes to biomarkers released into conditioned media were not due to tissue death. Briefly, conditioned media were collected for biomarkers analysis followed by addition of 10% AB in culture medium to the explants and to empty control wells. After 3 hours of incubation at 37°C under 5% CO_2, the oxidized AB was transferred to black microtiter plates and the fluorescence read at 540 nm excitation and 590 nm emission wavelengths. Background from control wells was subtracted from the readings.

AB, Alamar Blue; CO_2, carbon dioxide; Inhib, inhibitor; O+T, Oncostatin M + tumor necrosis factor-α; SEM, standard error of the mean.

MM-II reduces aggrecan degradation but not collagen type II degradation

- Stimulation of vehicle-treated (control) cartilage explants with inflammatory cytokines O+T increase the release of aggrecan fragment, AGNx1; this effect was fully reversed by aggrecanase and MMP inhibitors control (**Figure 3** and **Figure 4**)
- Application of MM-II combined with cyclic compression, reduced the release of AGNx1 in response to O+T in explants at days 5 & 7 as compared with application of vehicle control under identical conditions (**Figure 3A**)
- Application of MM-II without cyclic compression, reduced the release of AGNx1 in response to O+T in explants at days 10, 12, and 14 as compared with application of vehicle control under identical conditions (**Figure 3B**)
- Calculation of area under the curve shows modest but statistically significant effect of MM-II in attenuating aggrecanase-mediated degradation of aggrecan (**Figure 4**)
- The effect of MM-II was seen regardless of whether it was applied with or without compression (1.4- and 1.7-folds reduction in AGNx1 release, respectively; **Figure 4A** and **4B**, respectively)
- Application of MM-II with or without compression, had no effect on the release of C2M (**Figure 4C** and **4D**, respectively)

Figure 3. Levels of released AGNx1 over time

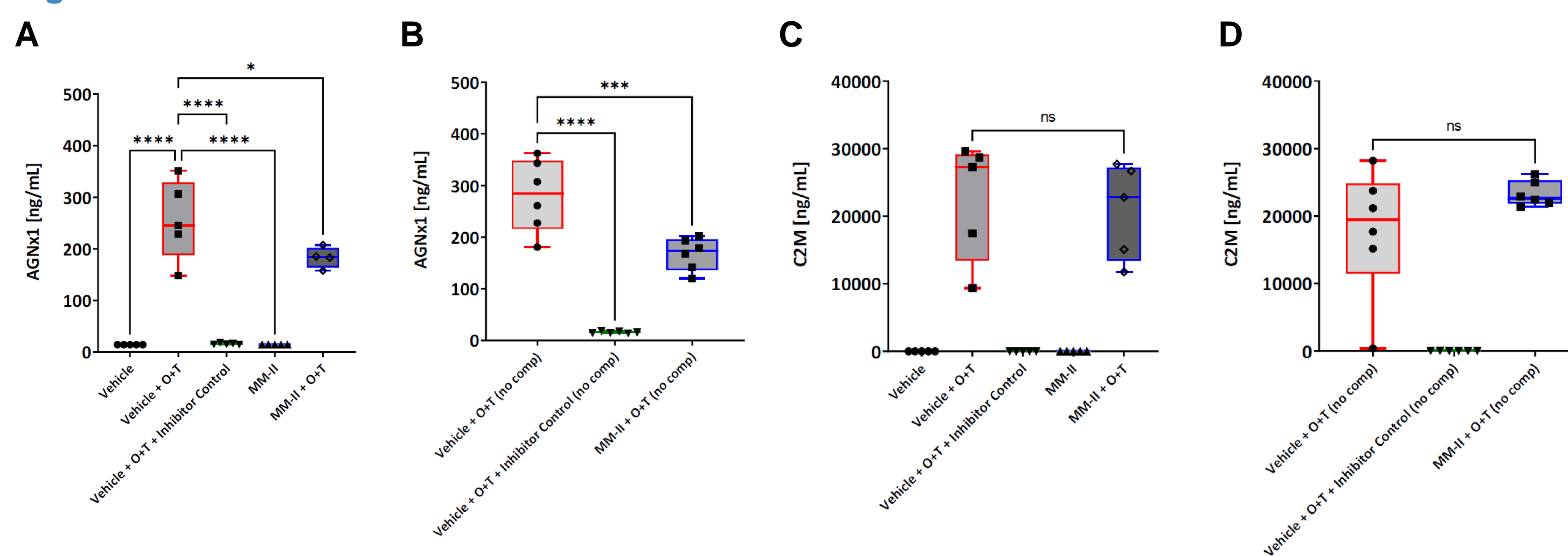


(A) Treatments with MM-II or vehicle were done under compression; (B) Treatments with MM-II or vehicle were done without compression.

In both conditions, a treatment with aggrecanase and MMP inhibitors was used as positive control to demonstrate full inhibition of AGNx1 release.

AGNx1, aggrecanase-generated aggrecan fragment; MMP, matrix metalloprotease; O+T, Oncostatin M + tumor necrosis factor-α.

Figure 4. Accumulated levels of released AGNx1 and C2M



(A, B) Accumulated levels of AGNx1; (C, D) Accumulated levels of C2M. (A, C) Treatments with MM-II or vehicle were done under compression; (B, D) Treatments with MM-II or vehicle were done without compression.

In both conditions, a treatment with aggrecanase and MMP inhibitors was used as positive control to demonstrate full inhibition of AGNx1 and C2M release.

*p <0.05; **p <0.01; ***p <0.001; ****p <0.0001

AGNx1, aggrecanase-generated aggrecan fragment; C2M, MMP-generated type II collagen fragment; MMP, matrix metalloprotease; O+T, Oncostatin M + tumor necrosis factor-α.

Conclusions

- Cartilage-bound MM-II liposomes were able to reduce aggrecanase-mediated aggrecan degradation, that occurs early during cartilage degradation, as reflected by reduced release of AGNx1
  - In contrast, MM-II had no effect on MMP-mediated collagen type II degradation
- **These findings suggest that MM-II liposomes provide some protection from inflammatory cytokine-induced early cartilage degradation, possibly through formation of a physical barrier layer on the cartilage surface**

References

1) Pratta MA, et al. *J Biol Chem.* 2003;278:45539–45. 2) Thudium CS, et. al. *J Vis Exp.* 2019;(153): doi:10.3791/59467. 3) Kjelgaard-Petersen CF, et al. *Biochem Pharmacol.* 2019;165:91–8.

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