Restoration of Dynamic Mechanical Properties of Cartilage by Methacrylated Hyaluronic Acid (HAMA)

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INTRODUCTION: Articular cartilage provides a unique combination of load-bearing capacity and low-friction movement, enabled by its highly specialized extracellular matrix (ECM) and hierarchical organization. However, once damaged, cartilage exhibits a very limited intrinsic healing potential, leading to progressive mechanical dysfunction and ultimately osteoarthritis (OA) [1]. Restoring the load-bearing function of damaged cartilage remains a central challenge in orthopaedic research. Hyaluronic acid (HA), a key component of the cartilage ECM, plays a critical role in lubrication, tissue viscoelasticity, and pain reduction [2]. Yet, its rapid degradation, poor mechanical stability, and poor retention in joint limit its use as a standalone strategy for cartilage repair [3]. Low molecular weight HA (≤100 kDa) functionalized with methacrylate groups (HAMA) has been shown to diffuse into cartilage tissue. Following photocrosslinking, cartilage attained improved mechanical stability, protecting the tissue from enzymatic degradation while maintaining cell viability [4–6]. However, studies on the ability of HAMA in restoring the dynamic mechanical properties of degraded cartilage are lacking. The objective of this study was to investigate the effect of HAMA treatment on the viscoelastic, dynamic properties of healthy and degraded cartilage under physiologically relevant loading conditions. We hypothesized that HAMA treatment would provide targeted mechanical restoration of degraded tissue without exerting measurable effects on healthy tissue.

METHODS: Cartilage samples ($N_{samples}$=20, N_{animal}=5) were harvested from porcine medial femoral condyle and groove. Cylindrical samples (4 mm diameter, 1.0–1.5 mm thickness) were collected along the proximal to distal axis relative to the joint center. The four experimental groups (n=5 per group) were as follows: (i) healthy control, (ii) OA tissue simulated by treatment of tissue with 30 U/mL collagenase VII, (iii) healthy cartilage treated by HAMA, and (iv) simulated OA cartilage treated by HAMA. Samples were placed in a confined chamber of matched diameter and incubated in standard culture media or media containing collagenase for 40h at 37°C in a CO_2 incubator. Then, 4% w/v HAMA hydrogel solution (~25 kDa, 40% degree of substitution) in phosphate buffer saline was mixed with photoinitiators of 0.5 mM tris(2,2-bipyridyl)dichlororuthenium(II) hexahydrate (Ru) and 5mM sodium persulfate (SPS), before being applied to the cartilage surface in the confined chamber for 10 min to allow for diffusion of HAMA into the superficial zone tissue [7]. Following that, excessive HAMA solution was removed and the intra-tissue HAMA was photocrosslinked at 400nm for 3 min at an irradiance of 20 mW/cm^2. All samples were then tested in unconfined compression setup using a Dynamic Mechanical Analyzer (ElectroForce DMA 3200), with glass as contact interface. Three steps of 5% nominal tissue strain were applied sequentially. At each loading step, tissue strain was first held for 10 min to enable stress relaxation, after which sinusoidal loading of 2% (peak-to-peak) strain was applied at decreasing frequencies of 100, 10, 1 and 0.1 Hz for 2–30s per frequency (Fig. 1A). The force-displacement data were analyzed by a custom-written python code to determine the equilibrium, instantaneous, and storage moduli [8]. Statistical analysis was performed to determine between-group difference using the Genlin procedure (SPSS, v27).

RESULTS: Following enzymatic treatment by collagenase, there was a significant decrease of 55–73% relative to the healthy control in the equilibrium, instantaneous and storage moduli at 1 Hz and 100 Hz (Fig. 1B). Treatment of simulated OA tissue by HAMA returned all tissue moduli back to the level of healthy controls. However, healthy tissue appeared to have over-stiffened instantaneous and storage moduli by 44–77% following HAMA treatment (Fig. 1B).

DISCUSSION: We successfully created an *in-vitro* simulated OA model using collagenase treatment as the enzyme-treated tissue showed substantial reduction in mechanical properties. The increase in equilibrium and dynamic moduli following HAMA treatment suggests that HA that was cross-linked *in-situ* can provide mechanical reinforcement of the degraded tissue matrix. Notably, the dynamic modulus improvement was observed across physiologically relevant frequencies (1–100 Hz), indicating that HAMA treatment could restore cartilage function under normal joint motion that includes walking, running and jumping. Given that the intrinsic stiffness of our 4% HAMA (~0.06 MPa, measured in a separate study) is much lower than that of cartilage (0.5–1 MPa) [9], the observed reinforcement likely resulted from mechanical integration of HA with the matrix rather than from the hydrogel's own stiffness. This mechanical recovery supports HAMA as a bio-agent that promotes restoration of tissue's viscoelastic properties. Nevertheless, the off-target stiffening effects, as observed in the healthy cartilage, highlight the need for further parametric optimization of this technique to achieve targeted reinforcement of degraded tissue. In conclusion, our results confirm the potential of HAMA as an integrative strategy to restore mechanical function of osteoarthritic cartilage. Further studies are required to optimize this bio-agent before the initiation of pre-clinical studies.

SIGNIFICANCE: This work provides evidence that treatment by a tissue-penetrating hydrogel is able to recover the viscoelastic properties of a degraded articular cartilage. The easy-to-use protocol and the quick reaction time (< 15min) make this treatment alternative suitable for arthroscopy procedure, and is promising for joint preservation therapy to slow OA progression, especially during the early development of OA.

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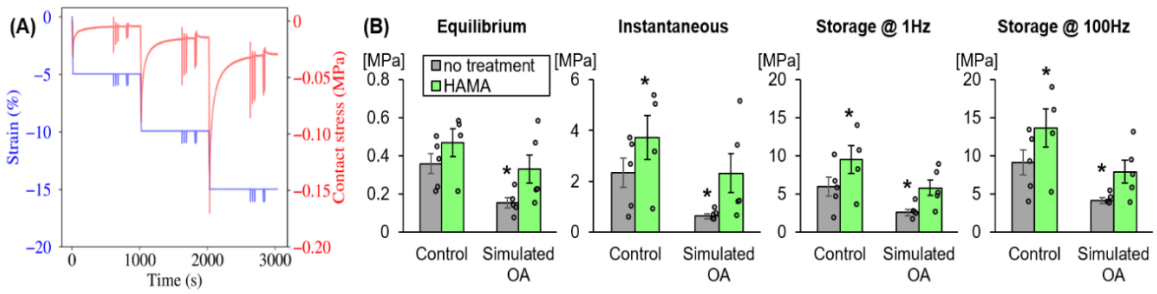

Fig 1. (A) *Stress/strain-time curves of the three-step unconfined compression,* ***(B)*** *Steady-state (equilibrium modulus) and dynamic (instantaneous & storage moduli) properties of cartilages with and without HAMA treatment. *p<0.05 compared with control, no treatment group.*