Injury-related Matrix Metalloproteinase Release Leads to Collagen Loss In A Computational Model of Injuriously Loaded Cartilage

Moustafa Hamada[1], Atte S.A. Eskelinen[1], Joonas Kosonen[1], Cristina Florea[1], Alan J. Grodzinsky[2], Rami K. Korhonen[1], Petri Tanska[1]
[1]University of Eastern Finland, Kuopio, Finland, [2]Massachusetts Institute of Technology, Cambridge, MA, USA
moustafa.hamada@uef.fi

DISCLOSURES: Moustafa Hamada (N), Atte S.A. Eskelinen (N), Joonas Kosonen (N), Cristina Florea (N). Alan J. Grodzinsky (8; Osteoarthritis and Cartilage), Rami K. Korhonen (N), Petri Tanska (N)

INTRODUCTION: Collagen fibrils play an essential role in the mechanical and structural stability of articular cartilage in the knee joint[1]. After a traumatic joint injury, cartilage function can be jeopardized not only by cell damage and proteoglycan loss but also due to collagen fibril damage and degradation [2,3], together predisposing the tissue to post-traumatic osteoarthritis (PTOA)[4]. However, the mechanisms that govern collagen fibril damage and degradation are not fully clear. It has been suggested in experimental and computational studies that excessive mechanical shear strains can lead to cell damage and an increased number of active matrix metalloproteinases (MMPs) in cartilage[5]. MMPs can in turn result in the proteolytic cleavage of collagen fibrils[3]. In this work, we develop a computational model of cartilage subjected to injurious loading and simulate cell damage and subsequent MMP-driven collagen loss. Finally, we compare the model results with our experimental observations.

METHODS: *Experimental protocol:* Cylindrical cartilage explants (n = 27, d = 3 mm, h = 1 mm) were harvested from the patellofemoral grooves of knees of 1–2-week-old calves (N = 7; Fig. 1A). Cartilage explants were subjected to injurious compression (n = 6; INJ, unconfined compression, 50% strain amplitude, 100%/s strain rate)[2] or left under free-swelling control conditions (n = 21; CTRL). The culture was terminated on day 12. *Collagen content analysis:* Collagen content was assessed from three 5-µm-thick sections per explant (Fig. 1B) using Fourier-transform infrared microspectroscopy (Agilent Cary 670)[6]. We assessed the collagen content (Amide I region in the spectrum, 1580–1720 cm^{-1}) in two full-thickness and 200 µm-wide regions of interest from intact areas of the tissue and averaged them to obtain a depth-dependent collagen content profile per explant. For statistics, we used a linear mixed effects (LME) model (level of statistical significance: 0.05). *Biomechanical model:* A 2D finite element model of articular cartilage with fibril-reinforced porohyperelastic swelling material properties[7] was generated using ABAQUS (v2023; Fig. 1C). The model was subjected to a single injurious load cycle as in the experiment. Cell damage ($C_{dam,cell}$) was triggered with a degradation coefficient ($D_{max,shear}$) at locations where the maximum shear strain value (ε) was over a threshold of 0.4 (Fig 1C)[8,9]. *Biochemical model:* The concentration of MMPs (C_{mmp}) in the model was simulated for 12 days with reaction–diffusion equations[10] (Fig. 1D&E) using COMSOL Multiphysics (v6.1). The release of MMPs was modeled with a reaction term ($R_{dam,cell}$) that considers the time delay of MMP activation following the initial cell damage (see *Biomechanical model*) and rate constant of MMP production[10]. Subsequently, the change in collagen concentration relies on the concentration of MMPs (C_{mmp}), the activity of MMPs (k_{mmp}), and aggrecan dependent catalytic activity of MMPs (k_{ag}) as it has been suggested that the aggrecan loss could potentiate the proteolytic cleavage of collagen fibrils by MMPs[10]. The used parameter values were based on previous reaction-diffusion models of Kar *et al.*[10].

RESULTS: In the experiments on day 12, the INJ group had on average ~14% smaller collagen content than the CTRL group. The LME model showed statistically significant differences at normalized depths of 0–7% and 24–100% from the cartilage surface (Fig. 1F). On the other hand, with a similar pattern as in experiment, the injured cartilage computational model showed lower collagen content on day 12 along the tissue depth with an average of ~6% smaller collagen content compared to the model representing intact, non-injured CTRL cartilage.

DISCUSSION: We developed a computational model to simulate depth-dependent loss of collagen content as observed in an *in vitro* experiment of cartilage subjected to injurious loading. As one possible degradation mechanism, we implemented the production of MMPs by damaged cells[5]. The model was successful in capturing a similar pattern of depth-dependent collagen content loss as observed in experiments, but our model predicted a slightly smaller loss on average (model ~6% *vs.* experiment ~14%). Therefore, enzymatic biochemical activity due to overloading may not explain all of the tissue damage, especially collagen loss, over short time periods[11,12]. This suggests that overload-induced rupture of collagen fibrils and subsequent release of collagen fragments could also play a role[12]. Our work opens the door for further investigation of the role of mechanical overloading to induce enzymatic and biomechanical degradation to collagen fibrils especially when physiologic cyclic loading and inflammation are also involved[2]. In the future, this modeling approach can be used to enhance joint-level models predicting PTOA progression.

SIGNIFICANCE/CLINICAL RELEVANCE: Injurious loading can result in depth-dependent collagen content loss over a relatively short time, simulated here with a novel computational model implementing cell damage and MMP activity. This disease mechanism can be incorporated into PTOA prediction models in attempts to understand disease progression.

REFERENCES: [1] Poole et al., 2001, Clin. Orthop. Relat. Res., 391:26-33; [2] Eskelinen et al., 2022, J. Biomech., 141:111181; [3] Li et al., 2015, Osteoarthr. Cartil. 23:266-74; [4] Lieberthal et al., 2015, Osteoarthr. Cartil., 23:1825-34; [5] Jin et al., 2000 J. Orthop. Res., 18:899-908. [6] Bi et al., 2006, Biochim. Biophys. Acta, 1758:934-41; [7] Orozco et al., 2018 Sci. Rep, 8:1-16; [8] Orozco et al., 2022 J. Orthop. Res., 40:1505-22; [9] Kosonen et al., 2023 PloS Comput. Biol. 19: e1010337; [10] Kar et al., 2016 Arch Biochem Biophys, 594:37-53; [11] Quinn et al., 1999, Ann. N. Y.Acad. Sci., 20:1265-73; [12] Thibault et al., 2002, J. Orthop. Res., 20:1265-73.

ACKNOWLEDGEMENTS: Research Council of Finland (grants 324529, 354916), Novo Nordisk Foundation (grant NNF21OC0065373), Sigrid Jusélius Foundation.

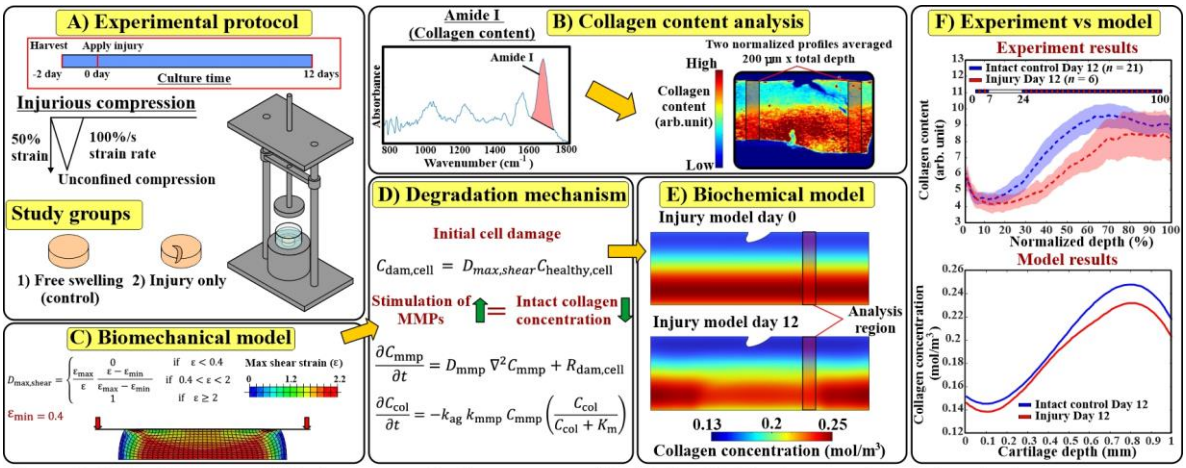

Figure 1. A) In vitro protocol. B) Assesment of collagen content (Amide I) with Fourier transform infrared microscpectroscopy. C) The biomechanical model included injurious loading capturing the experimental protocol. Maximum shear strain distribution was used to trigger the cell damage. D) Collagen degradation was driven by enzymatic cleavage after injury. E) The biochemical model showing collagen concentration on day 0 and after 12 simulation days. F) Depth-wise profiles showing decrease in collagen content after injurios loading in the experiment and computationl model.