Positive effects of acute N-acetylcysteine treatment simulated by an in silico model with overloading induced cell damage and recovery mechanisms

Joonas P. Kosonen[1], Atte S.A. Eskelinen[1], Gustavo A. Orozco[1], Jessica E. Goetz[2], Mitchell C. Coleman[2], Alan J. Grodzinsky[3], Donald D. Anderson[2], Petri Tanska[1], Rami K. Korhonen[1]

[1]University of Eastern Finland, Kuopio, Finland; [2]University of Iowa, Iowa City, IA; [3]Massachusetts Institute of Technology, Cambridge, MA
joonas.kosonen@uef.fi

DISCLOSURES: Joonas P. Kosonen (N), Atte S.A. Eskelinen (N), Gustavo A. Orozco (N), Jessica E. Goetz (N), Mitchell C. Coleman (N), Alan J. Grodzinsky (8: Osteoarthritis and Cartilage), Donald D. Anderson (N), Petri Tanska (N), Rami K. Korhonen (N).

INTRODUCTION: Injuries can disrupt cartilage homeostasis and trigger degeneration ultimately leading to post-traumatic osteoarthritis[1]. Joint injuries cause excessive strains in cartilage leading to cell damage and oxidative stress followed by apoptosis, increased activity of aggrecanases, and proteoglycan (PG) depletion[2,3,4]. Antioxidants such as N-acetylcysteine (NAC) have shown the potential to mitigate injury-related cell damage and slow down PG degradation[2,4]. However, many effects of NAC on cartilage are not fully understood, especially in terms of longitudinal responses to injury and treatment. Thus, we constructed a computational framework to simulate impact-induced cell damage and antioxidant treatment-induced reduction in cell damage (oxidative stress). We analyze cell viability and PG content after injury and compare our numerical predictions to previous experiments[5].

METHODS: *Prior in vitro experiments:* In prior experiments[5], mature bovine cartilage osteochondral plugs were subjected to drop-tower impact with 2 kg mass dropped from 7 cm height onto an indenter resting on the cartilage surface. After impact, cell viability was imaged with confocal microscopy over 3 days at the impacted region. In another group of impacted samples, cell viability was measured after a 2-day NAC culture (2 mM). Finally, after a 14-day culture, starting with 1-day NAC treatment (culture medium changed to non-NAC at day 1), 4-mm-diameter plugs from the original samples were removed from the impacted site and intact sites for comparison. Removed plugs were analyzed with dimethyl methylene blue assay for glycosaminoglycan content. *Simulated impact-induced cell damage:* The drop-tower impact was simulated with an axisymmetric finite element model (Fig. 1A) in ABAQUS (v.6.23-1 Dassault Systèmes). The impact was estimated with force (2350 N in 1 ms, sinusoidal waveform) applied to a rigid indenter in contact with cartilage. Cartilage was modeled as a fibril-reinforced poroviscoelastic material with swelling and depth-dependent constitutive properties[6,7,8]. At the peak impact force (t = 0.5 ms), the maximum shear strain $\varepsilon_{\max}$ was calculated to estimate cell damage. We assumed a non-linear relationship between the maximum shear strain and cell damage (Fig. 1A) when $40\% < \varepsilon_{\max} < 95\%$, no cell damage ($D$ = 0) when $\varepsilon_{\max} < 40\%$[9] and full damage ($D$ = 1) when $\varepsilon_{\max} > 95\%$[5]. *Simulated cell death and PG loss with/without treatment:* We added the biomechanics-based estimate of cell damage to an axisymmetric reaction–diffusion model[10,11] with NAC in Comsol Multiphysics (v5.6 Comsol Group). We included healthy[12] and damaged cell states ($C_{d,c}$ in Fig. 1A) after impact as an initial condition. Damaged cells were hypothesized to release proteolytic aggrecanases which degrade the PGs[4]. Post-impact PG degeneration rate parameter, cell apoptosis rate ($k_{apoptosis}$), and NAC-induced temporal cell transition rate from damaged to healthy cell ($k_{d\rightarrow h,c}$) were based on the prior *in vitro* experiments[5]. Lastly, we simulated the evolution of cartilage PG content during 1-day NAC treatment followed by a 13-day period without NAC. We calculated a ratio of the bulk PG contents in the 4-mm-wide impacted region (Fig. 1A) and intact region for comparison against experimental data[5].

RESULTS: The mechanobiological model predicted the greatest loss of cell viability and PG content in the impacted region (21 % PG loss at day 14), whereas the intact region showed only minor PG loss compared to day 0 (5 % loss at day 14). Cell damage was observed through the depth of the cartilage, while PG loss was mainly localized at the superficial zone of the tissue in the impacted region (Fig. 1A). Conversely, the model also showed that NAC treatment was able to substantially prevent PG loss in the same site (Fig. 1B). The model with NAC treatment predicted 11%-point higher relative PG content (impact region divided by intact region), compared to the model without treatment, whereas the experiments showed 16%-point higher values (Fig. 1C).

DISCUSSION: We developed a mechanobiological approach to estimate the effects of NAC treatment after injurious loading of cartilage. Interestingly, our modeling approach showed cell damage both in the superficial and deep zones of cartilage, but through the depth of the tissue the PG degeneration from injury was hindered by NAC. The computational modeling provided results consistent with experimentally observed treatment effects by implementing potential mechanisms behind the treatment. Our model suggests that by reducing overload-triggered cell damage (oxidative stress), NAC treatment can inhibit cell death, proteolytic activity, and PG loss after injury. However, additional experimental data at several time-points are needed to better calibrate parameters related to cell damage (oxidative stress) induced PG degeneration and NAC induced reduction in PG degeneration.

SIGNIFICANCE: Our computational approach simulates subsequent oxidative stress-mediated cell damage, as well as degenerative and regenerative mechanisms in injured cartilage. After careful calibration, this approach could be used to optimize treatment timing/dosage to enhance the development of effective treatment strategies for PTOA.

REFERENCES: [1]Anderson DD+2011 *J Orthop Res* 29(6):802-9; [2]Coleman MC+2018 *Sci Transl Med* 10:eaan5372; [3]Brouillette MJ+2014 *Biomech Model Mechanobiol* 13(3):565-72; [4]Riegger J+2016 *Osteoarth Cartil* 24(12):2171-80; [5]Martin JA+2009 *J Bone Joint Surg* 91(8):1890-97; [6]Wilson W+2005 *J Biomech* 38(6):1195-204; [7]Wilson W+2004 *J Biomech* 37(3):257-66; [8]Julkunen P+2007 *J Biomech* 40(8) 1862-70; [9]Orozco GA+2018 *Sci Rep* 8(1):1-16; [10]Kosonen J+2023 *PLOS Comp Biol* 19: e1010337; [11]Kar S+2016 *Arch Biochem Biophys* 594:37-53; [12]Jadin K+2005 *J Histochem Cytochem* 53(9):1109-19;

ACKNOWLEDGEMENTS: Doctoral Programme in Science, Forestry and Technology of the University of Eastern Finland, Novo Nordisk Foundation (grant no. NNF21OC0065373), the Research Council of Finland (#354916, #324529), and Sigrid Jusélius Foundation.

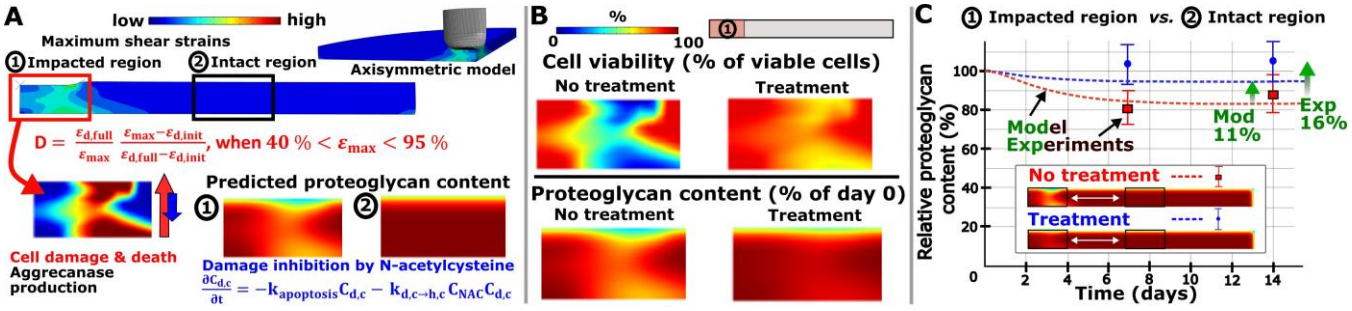

Fig 1. A) Axisymmetric finite element model to estimate impact loading, cell damage and proteoglycan content with and without N-acetylcysteine treatment in impacted region 1 and intact region 2. **B)** Cell viability and proteoglycan content in the impacted region 1 at day 14. **C)** Relative proteoglycan content between the impacted region 1 and intact region 2 with and without N-acetylcysteine treatment.