Unraveling the mechanical topography driving cartilage mechano-adaptation in vitro

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INTRODUCTION: Osteoarthritis (OA) is a whole joint disease with characteristic cartilage degeneration, often linked to mechanical cues disrupting cartilage homeostasis. Prior studies have merged experiments and computational models, suggesting mechanical factors – such as maximum shear strain [1], fluid velocity [2], and energy dissipation [3] – as potential drivers of changes in cartilage biological constitution (mechano-adaptation). Yet, distinct contribution of the individual mechanical factors in maintaining or perturbing cartilage homeostasis remains unknown. Our study employs a combination of in silico modeling and longitudinal in vitro loading experiments on human cartilage to bridge this gap. Using an innovative combination of long-term bioreactor tests on chondral explants, histological analyses and finite element (FE) modeling, we elucidate how mechanical triggers contribute to cartilage glycosaminoglycan (GAG) content changes during loading. We craft a novel mechano-regulatory mathematical model predicting GAG content evolution over time across cartilage thickness in intact and damaged cartilage. After calibrating and validating the model, we conduct a sensitivity analysis to unravel the distinct contributions of individual mechanical triggers to the changes in GAG content.

METHODS: Ethical approval (S56271) was secured from the University Hospitals Leuven Ethics Committee. Sixteen human cartilage explants (80-year-old female, no OA, fractured hip replacement) were harvested. Intact and defect explants, the latter with a half-thickness surface cut (Fig. 1), were loaded in a bioreactor (10% compression at 1Hz, 1hr on-1hr off-1hr on) for 7 days, while control samples were kept in free swelling. On days 1, 3, 5 and 7, one explant per group was sectioned and stained for fibril orientation and GAG content measurements using digital densitometry (DD) and polarized light microscopy (PLM). Sample-specific FE models were made using a fibril-reinforced poro-elastic (FRPE) model to obtain dissipated energy (DE), maximum shear strain (MSS) and fluid velocity (FV). A normalized factor was defined using each FE model output (curves of NF_{DE}, NF_{MSS} and NF_{FV} in Fig. 1) defining the effect of each parameter on GAG content. The NF_i values were used to predict the total change factor representing the mechanics-driven GAG content changes over cartilage thickness in one day of loading:

$$CF_{total_j} = \left(\sum CF_i\right) \times GAG_{w_j} \text{ with } CF_i = k_i NF_i \quad (1)$$

where, CF_i and k_i are the change and weighting factors related to each of the FE model outputs and i can be DE, MSS and FV. GAG_{w_j} is the depth-dependent weight of change in GAG content where j can be the superficial, middle or deep zone. Finally, the GAG content in each day of loading was obtained:

$$GAG_d = GAG_{d-1} + CF_{total_{jd}} \times GAG_{d-1} + \Delta GAG_{Bd} \quad (2)$$

where, d is the loading day in the bioreactor and ΔGAG_{Bd} is the basal change in the GAG content due to free swelling from DD of control explants. To obtain the model parameters, the three k_i and the three GAG_{w_j} in Eq. (1) were identified by fitting the predicted GAG content by Eq. (2) to the DD of intact samples. To validate the model, it was used to predict the GAG content change in defect samples and then compare to their DD. The sensitivity of mechanical-driven GAG content change to each of the FE model outputs was studied by comparing the CF_i values for each of the FE output parameters at different loading days.

RESULTS: In vitro loading of the defect explants caused different GAG content changes compared to the intact explants (Figs. 2A and 2B), showing the different mechanical states and mechano-adaptation in both groups. Model fit to the DD results of intact explants (R^2=0.96, Fig. 2A) yielded these parameters: $k_{DE} = 0.63$ mm^3/mJ, $k_{MSS} = 2.83$, $k_{FV} = 90.48$ s/mm, $GAG_{w_{sup}} = 1.97$, $GAG_{w_{mid}} = 1.38$ and $GAG_{w_{deep}} = 0.98$. Using them to predict the defect explants GAG content yielded a mean error of 16.1% versus DD (Fig. 2B). In intact explants (Fig. 2A) minimal effect of DE, negative effect of MSS and positive effect of FV on GAG content were obtained. For the defect explants, similar effects were obtained, except for a larger positive effect from DE (Fig. 2B).

DISCUSSION: The unique insights obtained through a blend of in silico and in vitro methods uncover the varied contribution of specific mechanical triggers on GAG content changes across cartilage thickness. Identified GAG_{w_i} values decreased from superficial (1.97) to deep zones (0.98), indicating more significance of mechanics-driven GAG changes in the superficial zone. Validation against defect explants demonstrates the model's broad applicability beyond the calibration's mechanical conditions (intact). The model-based insights uncover the distinctive role of each FE output on mechanics-driven GAG changes by quantifying the different contributions of DE, MSS and FV in the observed changes in intact and defect explants (Figs. 2A and 2B). Specifically, DE showed a larger effect on GAG production in the defect than the intact explants, highlighting the role of altered mechanical environment in the defect explants on maintaining homeostasis. In perspective, the defined mechano-regulatory model facilitates deeper comprehension of cartilage mechano-adaptation, simulating diverse mechanical conditions (e.g., increased/decreased compression, multi-axial loading, other defect types, or disorganized fibers).

SIGNIFICANCE/CLINICAL RELEVANCE: We created, calibrated and validated an in silico model to predict mechanics-driven changes in cartilage GAG content. This model enables us to evaluate the effect of individual mechanical triggers on GAG changes, a task that is currently impossible using experiments.

REFERENCES: [1] Elahi et al., Front Bioeng Biotech, 2021. [2] Orozco et al., Sci Rep, 2018. [3] Nasrollahzadeh et al., ACS Appl Mater Interfaces 2019.

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IMAGES:

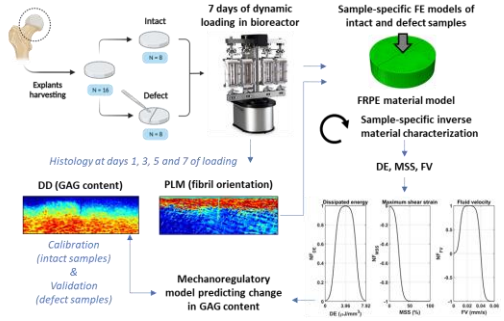

Fig. 1: Overview of the study workflow. DE: dissipated energy, MSS: maximum shear strain and FV: fluid velocity.

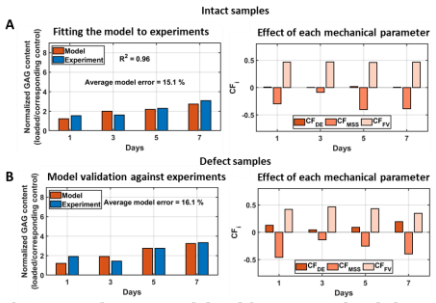

Fig. 2: Mechano-regulatory model calibration and validation. A) model fitted to DD in intact samples and sensitivity analysis results. B) predicted GAG content versus DD in defect samples and sensitivity analysis results.