Mechanoresponsiveness is Rescued by Loading Despite Wnt Activation in an Explant Model of Osteoarthritis

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INTRODUCTION: Mechanical signals are key factors that contribute to joint homeostasis but they are also implicated in the development of osteoarthritis (OA), the most common chronic joint disease[1]. A better understanding of the chondrocytes' mechanoresponse in homeostasis and disease may not only help to better position drug interventions in the disease course but could also be used to develop mechanical loading strategies that impact the effect of drugs on the joint. This requires untangling the interactions of mechanical loading with different intracellular pathways that either promote or dysregulate cartilage homeostasis. Particularly, increased activation of canonical Wnt signaling has been implicated in the development of OA[2]. However, how Wnt signaling pathway activation affects the chondrocytes' mechanoresponse in health and disease remains largely unexplored. Therefore, we aimed to study the role of Wnt signaling in the mechanoresponsiveness of healthy and osteoarthritic human cartilage, upon short-term physiologic mechanical loading.

METHODS: Informed consent and ethical approval was obtained for this study (S56271, Leuven, Belgium). Non-OA human cartilage explants were harvested from the hips of 11 donors with no history of OA. OA human cartilage explants were obtained from the hips of 12 patients undergoing hip replacement surgery due to OA. In a first experiment, the physiological loading protocol for use in the following experiments was determined. To this end, four different protocols were tested: (**1**) 10% compressive strain (CS) at 1Hz for 1 h + 1 h of free swelling (FS), (**2**) 10% CS at 1Hz for 1 h + 1 h of FS + 10% CS at 1Hz for 1 h + 1 h of FS, (**3**) 20% CS at 1Hz for 1 h + 1 h of FS, (**4**) 20% CS at 1Hz for 1 h + 1 h of FS + 20% CS at 1Hz for 1 h + 1 h of FS. Upon selection of the most relevant protocol, in a second experiment, mechanical loading was applied to non-OA explants in the presence and absence of Wnt activation with CHIR99021. Molecular read-outs of anabolic chondrogenic markers, pericellular matrix markers and matrix remodeling markers were used to investigate the effect of Wnt activation on cartilage mechanoresponse. In a final experiment, the same *ex vivo* compression set-up was used to study the effect of loading in cartilage from patients with established OA. Mechanical characterization of the non-OA and OA explants was performed using an in-house developed code in Matlab 2021b based on the recorded force and displacement data. For comparisons between two groups, 2-tailed Student's t test was used. A linear mixed model was used for the experiments with CHIR99021-treated samples with individual donor as random factor. Tukey correction was used for multiple comparisons.

RESULTS SECTION: Based on the increased expression levels of the well-established mechanosensitive genes *cFOS* and *cJUN*, the protocol consisting in 2 cycles of 10% CS for 1 h was selected for all subsequent experiments (fig 1a). Next, we investigated to what extent excessive Wnt activation induced prior to loading could affect cartilage mechanoresponsiveness (fig 1b). We observed that increased Wnt signaling compromises the chondrogenic mechanoresponse of non-OA cartilage: in the absence of Wnt hyperactivation, mechanical loading significantly upregulated *SOX9*, while its expression levels appeared lower when mechanical loading was applied in the presence of CHIR99021. *COL2A1* and *ACAN* did not show consistent modifications after loading. However, CHIR99021 treatment significantly reduced their expression levels and this decrease was not compensated by mechanical stimulation, with expression levels remaining significantly lower than the loading condition. No changes were observed in the expression levels of *COL6* but loading significantly upregulated Perlecan. This loading-induced increase in Perlecan levels was lost in the presence of CHIR99021. Surprisingly, loading upregulated *MMP1* and *MMP13*, but less consistently *MMP3*. When mechanical loading was applied in combination with the CHIR99021 treatment, there was a downward trend in the levels of *MMP1* and *MMP13*. Next, we confirmed increased Wnt signaling in cartilage explants with established OA prior to loading. In particular, expression levels of Wnt target genes *TCF-1* and *LEF-1* were upregulated in OA compared to non-OA cartilage, whereas the levels of the Wnt antagonist *DKK1* were significantly downregulated. No significant differences in terms of overall tissue stiffness between non-OA and OA cartilage were observed. Finally, despite Wnt activation in OA cartilage, we observed that explants from OA patients positively responded to dynamic cyclic compression, showing preserved mechanoresponsiveness and rescued chondrogenic phenotype (fig 1c).

DISCUSSION: Our results confirm that physiological loading maintains expression of anabolic genes in non-OA human articular cartilage, which contributes to cartilage homeostasis, but suggest a possible deleterious effect of Wnt signaling activation in the chondrogenic mechanoresponsiveness. Targeted inhibition of Wnt signaling in OA could therefore boost the beneficial effect of compressive loading to promote cartilage anabolism. Interestingly, our study highlighted obvious differences in mechanoresponse in the model of Wnt activation as compared to the established OA samples. These discrepancies could be explained by the existence of OA-related compensatory mechanisms that antagonize with the canonical Wnt pathway[4]. Indeed, it is well known that OA has a complex nature, with overlapping of multiple and simultaneous cellular processes[3]. These observed differences need to be further explored in the context of research questions on the differential impact of Wnt signaling on cartilage mechanoresponsiveness changes in early *vs* established OA.

SIGNIFICANCE: This study sheds light into human cartilage mechanoresponsiveness both in non-OA and OA conditions. This knowledge has the potential to contribute to the development of strategies that optimize the therapeutic effect of dynamic compression by optimizing OA pathological cell signaling.

REFERENCES: [1]GBD results (2020). [2]Lories RJ et al. Rheumatol Ther (2020); [3]Martel P et al. Nat Rev Dis Prim (2016); [4]Grumolato et al. Genes Dev (2010).

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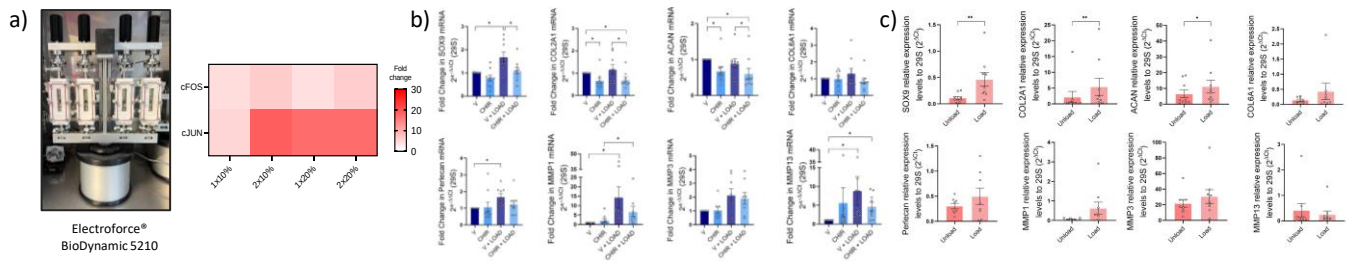

Fig.1: a) Electroforce® BioDynamic 5210 Bioreactor set-up and heatmap showing cFOS and cJUN fold change upon the four tested loading protocols. **b)** Chondrogenic mechanoresponse in CHIR99021-treated non-OA (V = vehicle DMSO) and in **c)** established OA human cartilage explants.