of OA. In this study, ASU+GLU+CS attenuated gene expression of these inflammatory molecules. Our finding suggests that the ASU+GLU+CS combination would be potentially beneficial for reducing chronic joint inflammation in both humans and animals, such as HERDA horses. In addition, utilization of the ASU+GLU+CS combination may help reduce the risk associated with chronic NSAID administration.

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MECHANOBIOLOGICAL EFFECTS IN A PLOWING MODEL OF DIARTHRODIAL JOINTS

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Purpose: Human diarthrodial joints (e.g. the temporomandibular joint, TMJ) undergo complex rolling/plowing loading patterns scarcely investigated in live tissue. Yet, joint breakdown could be partially explained by mechanobiological effects also due to plowing, i.e. pressing a round indenter – such as a condyle – against the cartilage surface and displacing it tangentially. Objective of this study was to investigate the response of cartilage tissue models to plowing. In particular, we analyzed the effect of plowing on cell viability, gene expression and glycosaminoglycans (GAGs) release. For this purpose, we used loading data collected in vivo by means of dynamic stereometry of the TMJ, i.e. a software reconstruction of real anatomies animated by their real kinematics, that combined with numerical modeling can provide in vivo data on strains, forces, stresses, and work density.
Methods: Cartilage plowing was performed by means of a rolling/plowing explant test system previously developed and validated. It consists of an arm moving horizontally by means of linear stages holding a custom-milled indenter pushed vertically by a linear actuator against the cartilage specimen. This is kept in a tank filled with DMEM solution. Adequate sensors measure force and displacement in relevant directions. Live cartilage strips (60×17×2 mm) from bovine nasal septum of one year old calves were submitted to plowing for 2 hours at 37°C in DMEM solution by using a cylindrical aluminum indenter (diameter 25 mm) moving tangentially at 10 mm/sec and simultaneously applying normal forces of 25, 50 or 100 N respectively. Control explants (20×30×2 mm) were kept unloaded in the same medium during the whole experiments. Analyses were performed 0, 2, 4 and 24 hours post loading. Cell viability was assessed by means of calcein acetoxymethylester & ethidium homodimer assays. Gene expression was assessed after mRNA extraction by quantitative real-time polymerase chain reactions (qRT-PCR). GAGs measurements were performed by means of 1–9-dimethyl-methylene blue (DMMB) assays. To determine whether GAGs release was mechanically or enzymatically induced, supplementary tests with MMPs activity inhibition before plowing were performed. Statistical differences were analyzed using two-way ANOVAs and Student's t tests at alpha = 0.05.
Results: Overall cell viability exceeded 95% for all applied forces, although superficial zones of dead chondrocytes were observed, increasing with the applied normal force. qRT-PCR showed that plowing induces MMP-3 upregulation dependent on the applied force (peaks of 2.3×, 4.7× and 6.3× for 25, 50 and 100 N respectively). Transcription of other genes involved in cartilage turnover such as TIMP-1, aggrecan, collagen type I and type II and fibronectin were shown not to be significantly affected by plowing. Furthermore, DMMB assays revealed that cartilage strips plowed at 100 N exhibited significantly enhanced GAGs release compared to the control explants at all time points observed.
Conclusions: This study shows that (1) bovine nasal septum can be a convenient model to study chondrocyte mechanobiological response to plowing, (2) plowing of cartilage appears to induce significant dose-dependent mechanobiological effects, and (3) the loading parameters studied would promote the production of catabolic enzymes tending to degrade the extracellular matrix.

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LOW-INTENSITY PULSED ULTRASOUND DOWNREGULATES THE MESSENGER RNA EXPRESSION OF THE MATRIX METALLOPROTEINASES ON AN ARTICULAR CARTILAGE EXPLANTS MODEL

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Purpose: Low-intensity pulsed ultrasound (LIPUS) has been utilized for the enhancement of fracture healing because of its bone reconstructive ability. In addition, LIPUS has been reported to be able to increase an anabolic reaction on chondrocytes. However, the effect of LIPUS about a catabolic reaction has not been elucidated, especially on an articular cartilage explants model. The purpose of this study was to investigate the immediate effect of LIPUS to the homeostasis of articular cartilage explants by mRNA analysis. We hypothesized that LIPUS acts as a moderate (physiological) mechanical stress, and downregulates catabolic reactions.
Methods: Osteochondral plugs (6 mm in diameter) were isolated from the distal femurs of pigs, and cultured for 1 day in the CO2 incubator. We divided these samples into 3 groups, control group applied sham LIPUS, the 27mW/cm2 group applied LIPUS at 27mW/cm2 intensity (spatial-average temporal-average, SATA) and the 67mW/cm2 group applied LIPUS at 67mW/cm2 intensity (SATA) (n = 5 pigs/group). Samples of the plugs were treated for 60min each intensity. Total RNA was extracted from these samples, and then the concentration and purity were assayed by spectrophotometer. To investigate the effect of LIPUS about both catabolic and anabolic reaction on articular cartilage explants, we analyzed the mRNA expression of MMP-13 (matrix metalloproteinase-13), MMP-1 (matrix metalloproteinase-1), Col2a1 (type 2a1 collagen), ACAN (aggrecan) and CITED2 (CBP/p300-interacting transactivator with ED-rich tail 2) by Real-time PCR. The final expression value was calculated by dividing the expression level of these target genes by the expression level of beta-actin, and each value at control group was set as 1. All data were expressed as mean ± standard error. Statistical analysis was carried out using Kruskal-Wallis test and Bonferroni test for post hoc analysis. A p value <0.05 was considered to be significant.
Results: The expression of MMP-13 was significantly downregulated in both the 27mW/cm2 group and the 67mW/cm2 group in comparison with control group (0.68±0.08, 0.38±0.08, respectively). The significant downregulation of MMP-1 was found only in the 67mW/cm2 group (0.44±0.02). But the expression of Col2a1 was also downregulated significantly in comparison with control group (0.61±0.13, 0.54±0.10, respectively). There was no significant change in mRNA expression of ACAN. The expression of CITED2 was significantly upregulated in the 67mW/cm2 group (1.83±0.48).
Conclusions: Our results suggest that LIPUS has an anti-inflammatory effect. This effect may be induced partly through CITED2 upregulation, which plays a negative regulatory role in MMP transcription to compete with MMP transactivator Ets-1 for limiting amounts of co-factor p300, especially at the 67mW/cm2 intensity. However, since the mRNA expression of Col2a1 was downregulated by LIPUS treatment in our experimental condition, further study should be needed for clinical using.

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CONNEXIN 43 IS DOWNREGULATED IN SCLEROTIC HUMAN SUBCHONDRAL OSTEOBLASTS: A POSSIBLE EXPLANATION FOR DECREASE OF MECHANOSENSITIVITY IN THESE CELLS

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Purpose: In osteoarthritis, subchondral bone remodeling is increased leading to bone sclerosis. In a previous work, we have reported that osteoblasts coming from the sclerotic subchondral bone express a particular phenotype characterized by an increased production of IGF-1, IL-6, IL-8, PGE2, RANKL, MMP-3 and a decreased of OPG. We have also reported that all these genes are mechanosensitive and that osteoblast coming from the sclerotic zone were less sensitive to compression than osteoblasts of the non-sclerotic zone. In this study, we have compared the effects of compression on the expression of membrane receptors by osteoblasts coming from sclerotic and not sclerotic area in response to compression