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Cartilage Tribology: Understanding Structure-Function Relationships

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Using a central composite design of experiment, we assessed how tribological parameters affect cartilage stiffness, tissue deformation, cell viability, histopathology, and gene expression. The results show that various kinematic and kinetic factors of joint articulation can modulate cartilage responses at both the matrix and the cellular level.

Keywords: Cartilage, load, mechanotransduction

1. Introduction

Great strides have been made in understanding how cartilage can maintain a low coefficient of friction and maintain homeostasis in the absence of a blood supply. The unique tribological properties of cartilage are imperative to the tissue's health and function. Few studies, however, have robustly investigated the effects of combined motion and load on tissue properties and cell response. Thus, in this parametric study, we assessed the effects of varying combinations of contact load, migrating contact frequency, and sliding speed on cartilage mechanical and biological properties.

2. Methods

Using a central composite design (CCD) (n=2; power =99%) and a previously developed bioreactor-indenter workflow [1], we assessed how contact load, contact frequency, and sliding speed affect cartilage surface stiffness, deformation, cell viability and histopathology. In addition, we explored effects on gene expression.

2.1. Tissue Harvest and Micro-indentation

Cartilage plugs were harvested from the femoral condyles of 10 bovine stifle joints and trimmed to 3 mm thickness without the bone attached. Samples were then placed in DMEM/F12 culture media with 10% FBS and incubated at 37°C and 5% CO_2. A 3×1 indentation array was performed submerged in culture medium in the wear (center) region and non-wear (peripheral) region of explants using a 20 μm spherical indenter at an 8 μm indent depth. The reduced modulus (E*) from each indent was obtained using Oliver-Pharr and averaged per region. The values obtained in the wear region were then normalized to the non-wear region of the same explant.

2.2. Tribological Testing

Following initial characterization of surface stiffness, explants were placed into a tribological bioreactor. A 32 mm alumina hip ball was used as a counterface to apply load onto the cartilage, while migrating ±5.2 mm across its surface. Various combinations of applied load (20, 28, 40, 52, or 60 N), migrating contact frequency (0, 0.04, 0.1, 0.16, or 0.2 Hz), and ball (rotational) sliding speed (1, 1.68, 10, 59.46, or 100 mm/s) were determined based on the CCD. After 60 minutes of tribological testing, the micro-indentation array was repeated to obtain surface stiffness and tissue deformation values. Immediately after indentation, the explant was partitioned for biological readouts. Cell death was determined with a commercially available live/dead cell staining kit. Tissue damage was obtained from cartilage sections stained with Safranin-O/Fast Green. For gene analysis, 4 mm plugs were removed from both, contact and non-contact regions. RT-qPCR abundance values were obtained using the comparative threshold cycle method. All genes of interest were compared to the threshold cycle (ΔCt) value of B2M (housekeeping gene), and relative abundance (ΔΔCt) was calculated defined as $\Delta\Delta Ct=\Delta Ct_{Gene}-\Delta Ct_{B2M}$.

2.3. Data Analysis

In order to identify relationships between tribological input and cartilage mechanical biological response, an ANOVA for a reduced quadratic model was fit to the data. Backward elimination (p>0.1) was used for reduction.

3. Results & Discussion

The average normalized stiffness for pre- and post-loading were 1.3 and 6.4, respectively (p=0.0057 and F=4.15). ANOVA model terms that showed significant effects included load, all two-factor inter-action terms, and the quadratic term of 'sliding speed^2'. The average deformation of the contact region and the non-contact region were 0.39 and 0.03 mm, respectively. Upon model reduction, only the interaction of 'Load×Sliding Speed' remained significant. Load itself showed a trend. For cell death, the ANOVA was significant for the superficial zone of cartilage only. Higher loads were associated with increased cell death, particularly in the highest and lowest frequency groups. No variables contributed to differences in histological assessment, likely because the ranges defined for input articular loading were within physiological ranges, and because only short-term loading was applied. Multiple genes exhibited significant interaction and quadratic effects between kinematic motion parameters. Altogether, these results suggest that relative fold change may not be driven by single motion parameters, but the combined kinematics and kinetics of the contact leading to variations in gene expression.

4. Conclusion

This study demonstrates that motion factors do not act in isolation, but interact to affect the tissue's mechanical and biological responses. Future studies may provide insight how aberrant joint kinematics can change multiscale cartilage properties.

5. Reference: [1] Yuh C, Laurent MP, Espinosa-Marzal RM, et al. JMBBM 113:104113, 2021