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REGARDING THE TEMPERATURE-DEPENDENT MECHANICS OF CARTILAGE AND COLLAGEN UNDER LARGE-STRAIN SHEAR

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INTRODUCTION

Joints of extremities experience temperature fluctuations depending on external environments [1]. Joints, and the cartilage inside them, may also be exposed to supraphysiological temperatures during treatments such as thermal chondroplasty [2] and *ex vivo* tissue engineering [3]. Despite varying thermal conditions, researchers commonly idealize and model cartilage assuming isothermal conditions. Thus, little is known about thermal effects on the mechanical responses of cartilage and if any permanent damage occurs after high-temperature treatments. Furthermore, little information exists that links a temperature-dependent bulk response of cartilage to the underlying molecular mechanisms that may cause potential changes.

In this study we aimed to determine whether and how the bulk mechanical responses of cartilage change during large-strain shear (1) in the *in vivo* temperature range, and (2) during and after high-temperature treatments.

METHODS

Mechanical Test. We harvested 3×3 mm^2 full-thickness cuboids of healthy human knee cartilage (Musculoskeletal Transplant Foundation) from five healthy donors. We performed large-strain shear tests using our mechanical testing apparatus (Messphysik, Furstenfeld, AT). In these tests, we applied shear strains parallel to the split-line direction (SLD) at ±10% and ±15% of the specimen's thickness (excluding any subchondral bone). To probe both *in vivo* and supraphysiological temperature ranges, we tested each specimen at both strain levels and at three temperatures: 24°C, 40°C, and 70°C. Then, one specimen from each donor was cooled back to 24°C after the 70°C treatment and sheared again at both strain levels.

Images and Analyses. To visualize any molecular, structural changes within the tissue, we performed Second Harmonic Generation (SHG) microscopy, Transmission Electron Microscopy (TEM), and histology (Picrosirius Red and Safranin-O). In the TEM images we measured collagen fibril diameters as indication of thermal damage [4].

Mechanical Analyses. Using our mechanical data we calculated shear strain and shear stress as $\gamma = \Delta l/L$ and $\tau = f/A$, respectively, where Δl is applied displacement, L is the original specimen thickness, f is the measured force parallel to the SLD, and A is the cross-sectional area of the specimen. We then used these values to calculate the strain-energy dissipation density (E_{DI}), peak-to-peak shear stress (τ_{PP}), and peak-effective shear modulus (G_{PE}) as

$$E_{\mathrm{DI}} = \oint_{\gamma_{\min}}^{\gamma_{\max}} \tau \mathrm{d}\gamma \,, \tag{1}$$

$$\tau_{\mathrm{PP}} = \tau(\gamma_{\max}) - \tau(\gamma_{\min}) \,, \tag{2}$$

$$G_{\mathrm{PE}} = \frac{1}{2}\left(\left(\frac{\tau_{\mathrm{avg}}}{\gamma_{\mathrm{avg}}}\right)_{+} + \left(\frac{\tau_{\mathrm{avg}}}{\gamma_{\mathrm{avg}}}\right)_{-}\right), \tag{3}$$

where $\gamma_{\max}$ and $\gamma_{\min}$ are the maximum and minimum shear strains, respectively, τ_{avg} is the vertical projection of the best-fit line, and γ_{avg} is the complementary horizontal projection. We calculate these last two values at the location of the maximum slopes of both the positive and negative loading curves [5].

Statistical Analyses. We compared medians of mechanical and morphological results using the Wilcox Rank-Sum test to determine significance ($p < 0.05$). We also probed inter- and intra-donor variability with the Kruskal-Wallis test by ranks with donor (D1-5), anatomical position (medial or lateral condyle), and joint location (L1-7) as categorical variables.

Molecular Simulations. We performed Steered Molecular Dynamics (SMD) simulations to determine the effects of varying temperature on the unfolding of type II collagen molecules at 24°C, 40°C, and 70°C. In total we performed 18 molecular pulling simulations

(six at each temperature) using a pulling rate of 1 m/s. We compared ratios of peak molecular forces to median peak-to-peak shear stresses to correlate the bulk mechanical response to molecular changes.

RESULTS

In total we performed 216 shear tests on 36 healthy human cartilage specimens from five donors. We present representative shear stress-strain plots in Fig. 1.

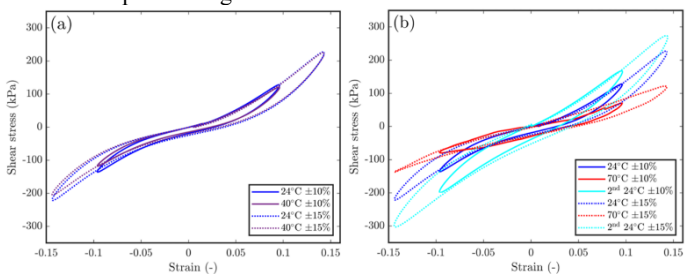

Figure 1: Representative shear stress-strain plots show nonlinearity with distinct hysteresis at both shear strain magnitudes (a) spanning the *in vivo* range (24-40°C), and (b) upon cooling from 70°C to 24°C where the tissue recovers stiffness.

We observed significant decreases in strain-energy dissipation densities, peak-to-peak shear stresses, and peak-effective shear moduli at all three temperatures and at both strain magnitudes. In the repeated 24°C tests (after 70°C heating) we observed no significant differences between peak-to-peak shear stresses and peak-effective shear moduli, but the strain-energy dissipation density increased significantly.

The TEM images showed swelling of collagen fibrils as temperature increased. We calculated median collagen fibril diameters of 72 nm at 24°C, 91 nm at 40°C (22.8% increase), and 106 nm at 70°C (38.2% increase). SHG and histology showed little changes among the three temperatures.

Using our molecular models, we characterized two deformation regimes in our force-strain profiles: unwinding in tensile stretch as strands rotated, and slipping in shear (strand 3 against strands 1 and 2) as shown in Fig. 2. Fewer protein-water hydrogen bonds remained at higher temperatures.

DISCUSSION

We demonstrated the temperature dependence of human articular cartilage under large shear strains in the *in vivo* and supraphysiological temperature ranges and linked molecular changes of the recruited type II collagen fibers to the bulk responses at these temperatures.

Although the overall shear response agrees with previous studies [6, 7], our results show decreasing peak-to-peak shear stresses and peak-effective shear moduli in the *in vivo* range, which contradicts one study that found increased temperatures produce a stiffer response [8]. However, we used quasi-static, fluid-independent loading rates, and June and Fyhrie [8] used dynamic mechanical tests which tend to reduce collagen recruitment [9].

We observed swelling of the collagen fibrils through TEM, indicating thermal damage to the fibrils at 70°C [4]. However, the temperatures of testing did not fully thermally denature the fibril bundles since tissue sheared at 70°C possessed some shear stiffness, and the SHG signal was consistent among the three temperatures [10].

Our molecular model suggests the temperature-dependent bulk response may be the product of the disruption of hydrogen bonds within the collagen molecule at higher temperatures. At lower temperatures the triple helical structure preserved intramolecular hydrogen bonds, but higher temperatures disrupted these bonds more quickly causing strands

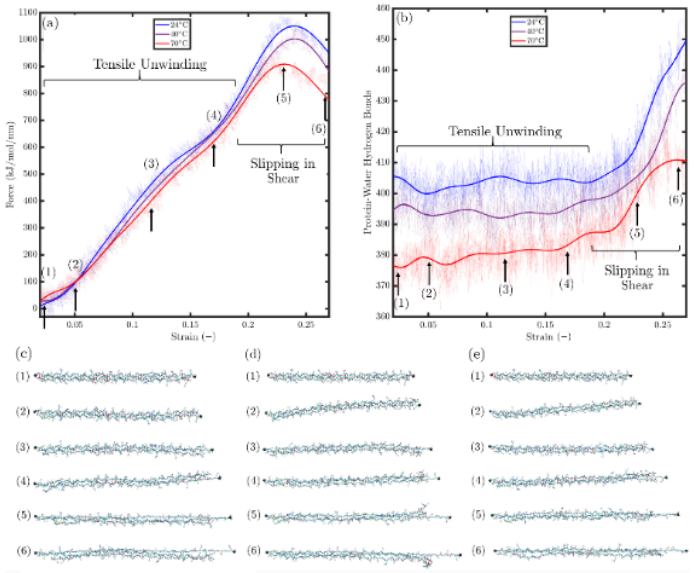

Figure 2: Collagen type II deformation at three temperatures of interest shows (a) average force-strain relationship, (b), average hydrogen bonds using polynomial best fits of averaged response from six simulations, and deformation states of the collagen type II molecule at (c) 24°C, (d) 40°C, and (e) 70°C at six strains: (1) 0%, (2) 5%, (3) 12%, (4) 17%, (5) 23%, and (6) 27%.

to slip more readily. These molecular effects are likely compounded by the complex hierarchy of collagen fiber bundles within cartilage and possibly by cross-linking among fiber bundles.

Our results highlight that a full understanding of the mechanical response of cartilage must consider the effects of *in vivo* and, depending on the application, supraphysiological temperatures. Abdel-Sayed et al. showed that osteoarthritic tissue does not dissipate as much energy as healthy cartilage [11]. Coupled with our results, temperature regulation in the knee may have implications for cell homeostasis through disease states. Further understanding of thermally regulated mechanisms may illuminate new treatment targets for osteoarthritis and other rheumatic diseases. Temperature may play an important role in long-term cartilage performance and health and may present implications for disease onset and progression in people in extreme climates.

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