# Supplemental Information
# for "Determining Tension-Compression Nonlinear Mechanical Properties of Articular Cartilage from Indentation Testing"

This supplemental study investigates the predictive capability of the two tension-compression nonlinear models employed in the manuscript, *i.e.*, biphasic CLE and biphasic CFD models. The hypothesis tested here is that the mechanical parameters obtained by curve-fitting the indentation creep curve can be used to predict the indentation testing at a different level of load, as proposed by a reviewer.

Cartilage-bone blocks were harvested from the tibia plateau of calf (3-6 months old) knee joints. Two indentation tests were performed on the cartilage using the same protocol as described in the paper. The two tests employed 50 mN and 150 mN step loadings, respectively. In between the two creep tests, the sample was left intact on the device to ensure that the two tests are performed at the identical location. The cartilage was allowed to recover after the first test for a three-hour period. Protease inhibitors were supplemented in the PBS to help preserve the mechanical integrity of cartilage.

The creep curve generated with the 150 mN loading was fitted using the CLE and CFD models as described in the manuscript, and then the cartilage response under 50 mN loading was predicted by the determined mechanical parameters and plotted together with the actual experimental data. Comparison of the results from three different samples is shown in Fig. S1. Since the two constitutive models demonstrated similar results, only those from biphasic CLE model are presented.

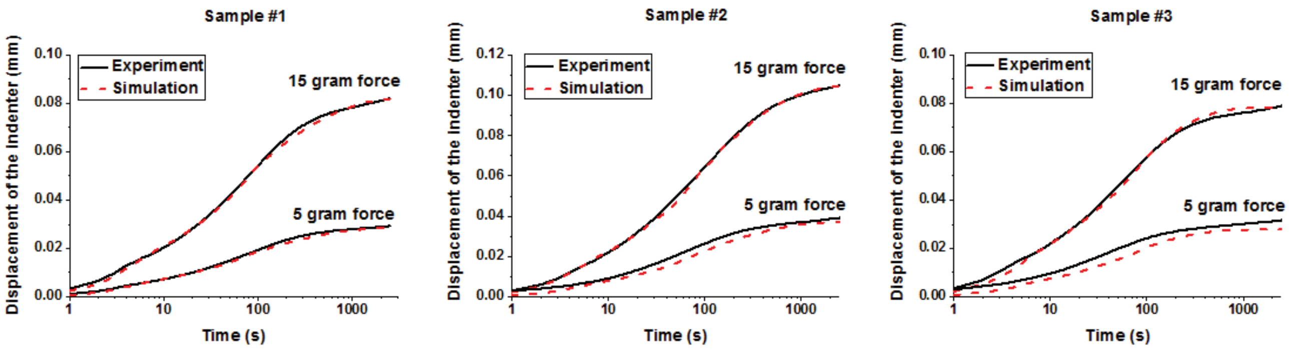

Fig. S1: Indentation creep curve generated by a 150 mN loading was fitted using the method described in the manuscript to determine the mechanical properties. Then, the response from a 50 mN loading was predicted using the parameters and plotted together with the actual experimental measurements. Results from three samples are presented here with each sample in a separate plot.

In general, the theoretical prediction matches the experimental data well ($R^2$ value: 0.96, 0.99 and 0.91 for samples # 1, 2 and 3), while the predicted curves tend to have lower equilibrium deformations and take a longer time to reach equilibrium (curves shift to the right along the logarithm time axis). This discrepancy could be mainly due to the strain-dependent permeability and modulus of the actual solid matrix, which are not considered in the current two models. Under small deformation, both the tensile and compressive moduli of the solid matrix should be lower due to the existence of a toe region in the stress-strain curve at small strain. Therefore, the mechanical modulus determined at 15 grams over-estimates the actual tissue modulus at 5 grams of force. The over-estimation results in a smaller predicted deformation compared to the experimental data. In contrast, the permeability of the solid matrix decreases when the matrix is compressed with negative dilatation. Because the permeability of a solid matrix under a 15 gram force is smaller than that under a 5 gram force, the predicted curve reaches equilibrium slower than the experimental data under a 5 gram force. Therefore, incorporation of a high-order nonlinear stress-strain curve and strain-dependent permeability may increase the accuracy of the models but at the cost of increased complexity.

We also note that the two constitutive models studied do not consider the heterogeneous structure of cartilage layers, the osmotic pressure induced by proteoglycans, or the intrinsic viscosity of the solid matrix. These simplifications usually have a nonlinear effect on the mechanical behaviors of cartilage, making predication using the constitutive models more difficult. Unfortunately, the inhomogeneous nature of the strain fields across the tissue under indentation tests could further complicate the effects of these factors, as the mechanical response of cartilage under smaller loading is regulated more by the superficial zone of the cartilage. Nevertheless, the comparison results from this supplemental study demonstrate the predictive abilities of these two widely used constitutive models for articular cartilage. Whether such accuracy is acceptable could be highly dependent on the requirement of the particular application. Indeed the prediction shown in Fig S1 has a higher $R^2$ value than the curve-fitting using linear biphasic theory.