

Biphasic Finite Element Modeling Reconciles Mechanical Properties of Engineered Cartilage Constructs Derived from Different Testing Modalities

¹Gregory R. Meloni
¹Brendan D. Stoeckl
^{1,2}Matthew B. Fisher
^{1,2}George R. Dodge
^{1,2}Robert L. Mauck

¹Department of Orthopaedic Surgery,
University of Pennsylvania,
Philadelphia, Pennsylvania

²Translational Musculoskeletal Research
Center, Department of Veteran Affairs,
Philadelphia, Pennsylvania

Introduction

Cartilage is a hydrated, load bearing and specialized tissue with unique biomechanical properties. Given its poor healing capacity, a number of tissue engineering and regenerative medicine strategies have emerged to address the repair of large cartilage defects. There has been significant progress in this field, with various scaffolds, preconditioning bioreactors, and cell types generating cartilage-like tissue *in vitro* whose bulk properties approach that of native cartilage.¹ Attention has now turned towards fabrication of engineered cartilage with anatomic curvature to reconstitute complex joint geometries, as well as evaluation of biophysical properties of engineered constructs *in vivo*. While a number of standard mechanical assays (e.g., confined and unconfined compression) are used to assess mechanical function of native tissue and engineered constructs, these assays generally require samples of regular geometry and assume homogenous material properties across the test specimen. These are generally not compatible with complex anatomic constructs maturing in an *in vivo* setting. As a consequence, indentation testing, using either spherical or plane-ended indenters, is the standard analysis tool for *in vivo* analysis. Given the dissimilarity in testing profiles and boundary conditions across these testing configurations, however, reported 'moduli' can differ by as much as an order of magnitude. To address this disconnect, we developed a biphasic finite element model using the freeware FEBio representing two common testing configurations (indentation and unconfined compression). The goal of this study was to develop this methodology and evaluate the maturation of tissue engineered constructs using these testing configurations side-by-side, and to determine whether the FE models and curve fitting procedures could provide a reconciled set of mechanical properties across testing platforms.

Methods

To carry out this study, juvenile bovine mesenchymal stem cells (MSCs) were seeded at a density of 2×10^7 cells/ml in 1% weight/volume methacrylated hyaluronic acid hydrogels.²

Constructs were cultured in a chemically defined chondrogenic medium for 3, 6, and 9 weeks.³ At each time point, constructs were subjected to mechanical testing (in indentation and unconfined compression, $n = 4$ per time point). Indentation testing consisted of three consecutive stress-relaxation tests (compressive ramps spanning 0-5, 5-10, and 10-15% strain applied at a rate of 0.1%/second, with 600 second relaxation periods between each ramp). Unconfined compression testing consisted of creep, stress-relaxation, and dynamic tests as previously described.³ Quasi-axisymmetric finite element models of both mechanical tests were created in FEBio by modeling a 5° wedge and scaling loads accordingly (Figure 1 A,D).⁴ The engineered cartilage constructs were modeled as a biphasic material consisting of an isotropic neo-Hookean solid phase with an isotropic fiber distribution. Both the indenter and loading platen were modeled as rigid bodies. The parameter optimization algorithm in FEBio was utilized to curve-fit the material parameters Young's Modulus, ksi (a measure of fiber modulus), and permeability for both the unconfined compression stress-relaxation tests and the second step of the indentation test. Calculations of traditional outcome measures were also performed; the equilibrium and dynamic moduli from unconfined compression tests were calculated along with an estimation of modulus using the Hertz model for indentation testing.^{3,5} Statistical analysis consisted of 1-way and 2-way ANOVA with Bonferroni post hoc test.

Results

Biphasic finite element models were successfully constructed in FEBio. Fitting of stress relaxation data from indentation and unconfined compression was readily accomplished at each time point (Figure 1C,F). Traditional mechanical property outcomes from unconfined compression showed an increase in equilibrium modulus (Figure 2A) from week 3 (39.6 ± 19.7 kPa) to week 6 (341.4 ± 86.7 kPa) and week 9 (325.9 ± 56.0 kPa), and increase in dynamic modulus (Figure 2B) from week 3 (576.3 ± 65.6 kPa) to week 6 (2248 ± 533 kPa) and week 9 (2301 ± 231 kPa). The indentation

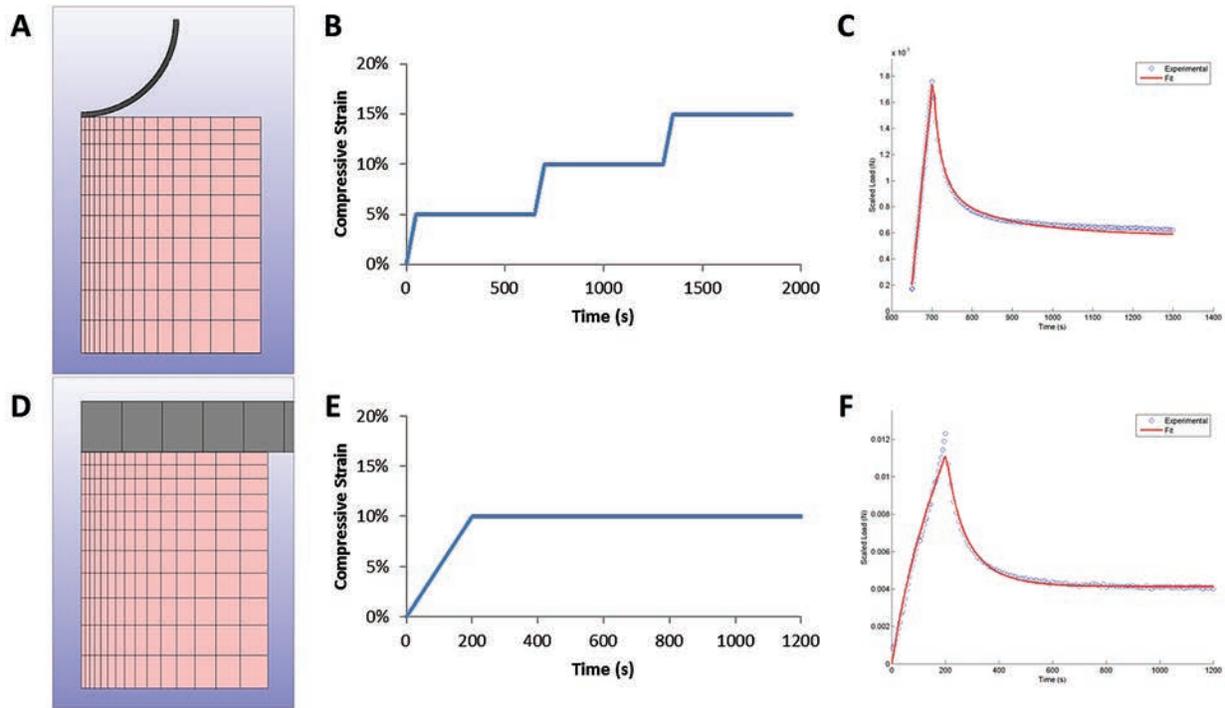


Figure 1. Axisymmetric FEBio model of (A-C) indentation and (D-F) unconfined compression testing showing the mesh configuration and loading platens (A & D), applied deformation profile (B & E), and a representative curve fit of experimental stress relaxation data (C & F).

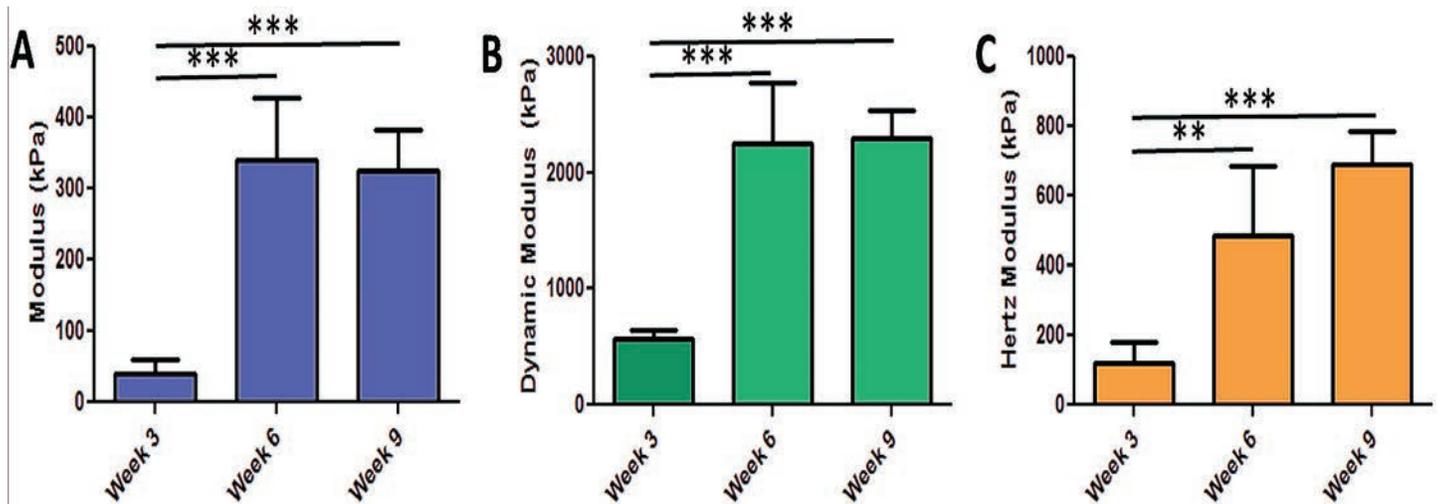


Figure 2. Mechanical properties of engineered cartilage after 3, 6, and 9 weeks of culture determined from unconfined compression testing (equilibrium (A) and dynamic (B) modulus) and indentation testing (Hertz modulus (C) approximation derived from indentation testing). (** $p < 0.01$, *** $p < 0.001$)

modulus derived from a Hertzian followed a similar trend (Figure 2C), increasing from week 3 (119.9 ± 59.7 kPa) to week 6 (483.6 ± 200.9 kPa) and week 9 (689.3 ± 96.2 kPa). Curve fitting the time dependent stress relaxation testing using the FEBio parameter optimization built into FEBio yielded values for the Young's modulus (E_V), the fiber modulus (ksi), and the permeability (k) at each time point and for each testing modality. In general, there was an increase in the Young's modulus (Figure 3A) and fiber modulus (Figure 3B) from week 3 to weeks 6 and 9 for both the indentation and unconfined compression models, while the permeability parameter decreased with time. Importantly, when comparing across

these two different testing modalities, the FEBio extracted parameters were consistent across platforms, aside from one parameter at one time point (week 6 Young's modulus).

Discussion

As the field of cartilage tissue engineering expands, and transitions into non-traditional shapes and *in vivo* settings, it is essential to develop testing modalities that can be employed across these more clinically relevant platforms. Moreover, given the large body of literature using traditional tests of uniform samples (e.g., unconfined compression), it was essential to develop analysis methods that enable comparison across

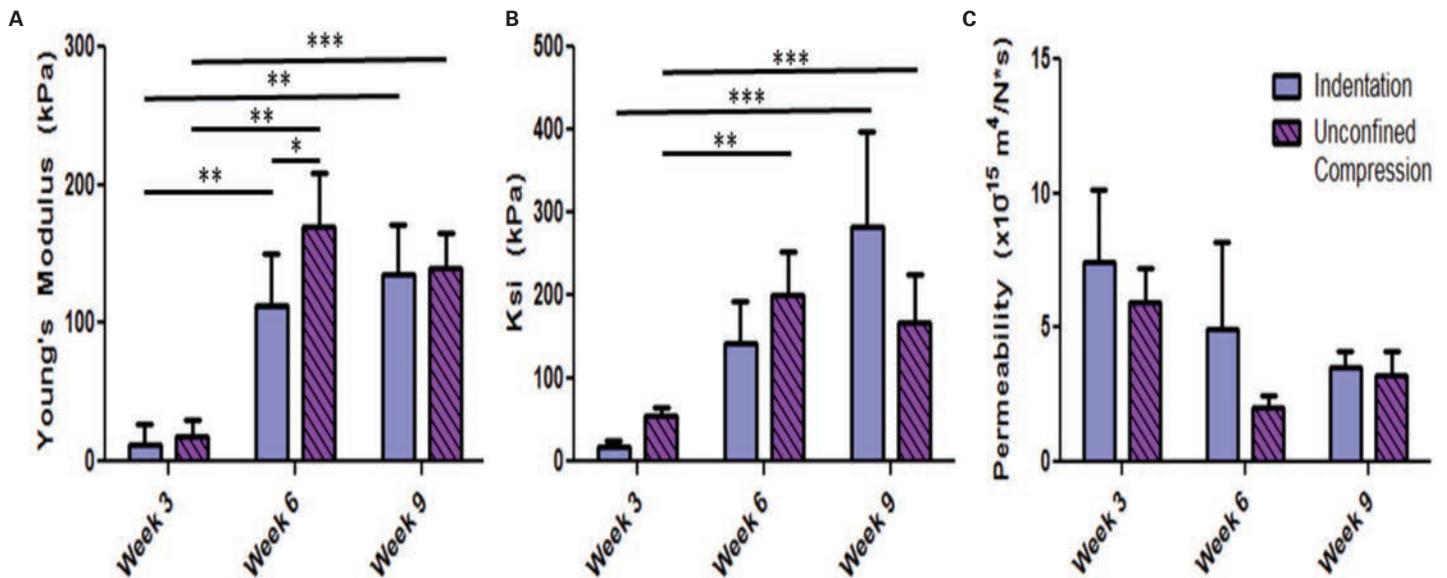


Figure 3. Parameter optimization results from FEBio for the (A) Young's modulus, (B) fiber modulus (ksi), and (C) permeability obtained by fitting indentation and unconfined compression tests. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

data sets in the same context. Here, we developed a FEBio model for indentation testing and unconfined compression. Our data showed increases in unconfined compressive Young's modulus, dynamic modulus, and indentation modulus, consistent with previously reports.⁶ While all three outcome measures show significant changes in modulus with time, it is not possible to make direct comparisons between them. While both the Hertz model and unconfined compression modulus are essentially reporting the same mechanical attribute, values derived from the Hertz model are almost two times greater than those from unconfined compression testing. Finite element modeling and parameter optimization of both of these test allowed for unification of these analyses and direct comparisons between the same set of material parameters from two very different mechanical tests. Taken together, this work demonstrates the reconciliation of multiple testing modalities, through finite element modeling, in order to achieve comparable mechanical parameters.

Significance

This versatile mechanical analysis platform enables comparisons between newly emerging *in vivo* and historical data sets obtained using different test configurations.

Reconciliation of this data in one material model allows for comparison of findings across groups, and tests of multiple samples or locations, which may speed the development of functional cartilage replacements.

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