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The Scaffold–Articular Cartilage Interface: A Combined In Vitro and In Silico Analysis Under Controlled Loading Conditions

The optimal method to integrate scaffolds with articular cartilage has not yet been identified, in part because of our lack of understanding about the mechanobiological conditions at the interface. Our objective was to quantify the effect of mechanical loading on integration between a scaffold and articular cartilage. We hypothesized that increased number of loading cycles would have a detrimental effect on interface integrity. The following models were developed: (i) an in vitro scaffold–cartilage explant system in which compressive sinusoidal loading cycles were applied for 14 days at 1 Hz, 5 days per week, for either 900, 1800, 3600, or 7200 cycles per day and (ii) an in silico inhomogeneous, biphasic finite element model (bFEM) of the scaffold–cartilage construct that was used to characterize interface micromotion, stress, and fluid flow under the prescribed loading conditions. In accordance with our hypothesis, mechanical loading significantly decreased scaffold–cartilage interface strength compared to unloaded controls regardless of the number of loading cycles. The decrease in interfacial strength can be attributed to abrupt changes in vertical displacement, fluid pressure, and compressive stresses along the interface, which reach steady-state after only 150 cycles of loading. The interfacial mechanical conditions are further complicated by the mismatch between the homogeneous properties of the scaffold and the depth-dependent properties of the articular cartilage. Finally, we suggest that mechanical conditions at the interface can be more readily modulated by increasing pre-incubation time before the load is applied, as opposed to varying the number of loading cycles. [DOI: 10.1115/1.4040121]

1 Introduction

Isolated, focal articular cartilage defects present one of the most challenging clinical problems for orthopedic surgeons to manage. Articular cartilage has a poor intrinsic capacity for healing that can result in significant pain, dysfunction, disability, and ultimately osteoarthritis [1]. Sixteen percent of all adults in the U.S. have a focal cartilage defect injury in the knee and there is a threefold increased incidence in athletes [2], with associated healthcare costs of up to \$185 billion annually [3]. Surgical treatments for focal cartilage injuries generally fall into one of the following four categories: (1) nontransplant salvage procedures including microfracture, subchondral drilling, and abrasion arthroplasty [4], (2) mosaicplasty—which involves autogenous transplantation of cartilage–bone plugs [5], (3) reimplantation of autogenously isolated and expanded cells [6,7], and (4) allograft transplantation. Each technique is limited in its results [1,6,8], with evidence for clinically deteriorating outcomes at longer follow-up times [9–11]. To address this shortfall, extensive efforts have been made to develop and manufacture degradable scaffolds for the purpose of either growing cartilage ex vivo for subsequent implantation [12], or for infiltration with cells immediately after in vivo implantation. In order for these scaffolds to mechanically

function in the complex environment of the knee joint, robust integration with the host tissue is a necessity [13,14].

The challenge of integrating scaffolds and articular cartilage has received much attention. Scaffolds have been preseeded with chondrocytes [15] enhanced with growth factors [16–18] augmented with adhesion proteins [19], and designed to create a press-fit into the defect site [20]. The articular cartilage surrounding the defect has also been enzymatically digested in an attempt to free the cells from their dense matrix and allow them to migrate to the interface [20,21]. But, in each in vitro model in which the effect of these treatments on integration has been assessed, the effect of mechanical load on the integrative process has largely been neglected. Mechanical stimulation is beneficial in maintaining a chondrogenic phenotype in cell-seeded scaffolds [22] and increasing the number of loading cycles can result in significant increase in type II and type IX collagens [23]. However, it is unclear if mechanical loading will have a similar beneficial effect on the scaffold–tissue interface. In joints that can experience up to five times body weight during everyday activities [24] information about the effect of mechanical load on the scaffold–cartilage interface will improve our ability to develop a clinically relevant solution toward treating these prevalent injuries.

The objective of this study was to quantify the effect of mechanical loading on integration between a scaffold and articular cartilage. We hypothesized that increased number of loading cycles would have a detrimental effect on interface integrity. To test our hypothesis, the following models were used: (i) an in vitro scaffold–cartilage explant system in which compressive sinusoidal

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Fig. 1 Schematic of the study design used to evaluate the effect of loading on scaffold–cartilage interface strength. Push-out testing was done at day 28 (before loading in the bioreactors started and used as an input to the bFEM) and at day 42 (post loading and used as an outcome metric for the experimental groups). Scaffold–cartilage interface strength was also quantified on day 0 for $n = 6$ samples (to allow for a supplementary assessment of incubation time on bFEM outcome).

loading cycles were applied for 14 days, at 1 Hz, 5 days per week, for either 900, 1800, 3600, or 7200 cycles per day and (ii) an in silico inhomogeneous, biphasic finite element model (bFEM) was used to characterize interface micromotion, shear stress, compressive stresses, and fluid flow under the prescribed loading conditions.

2 Methods

A nondegradable, macroporous poly(vinyl alcohol) scaffold (PVA) was utilized. As a nondegradable scaffold, the morphology and mechanical properties of PVA remained unchanged for the duration of the experiment, thus the temporal variability in scaffold morphology and mechanical properties associated with a degradable scaffold have been eliminated.

2.1 Manufacture of Poly(Vinyl Alcohol) Scaffolds. Surgical gelatin sponges (Ethicon-Johnson & Johnson, Somerville, NJ) were impregnated with 10% wt/vol PVA (Sigma-Aldrich, St. Louis, MO) in de-ionized water. The PVA-soaked sponges were frozen to -20°C for 20 h and then thawed at 25°C for 4 h. The freeze-thaw cycle was repeated for a total of 6 cycles. Cylindrical cores (final size: $\varnothing 5 \times 2.5$ mm) were removed from the sponges while still frozen, microtomed flat, and digested for 16 h with 400 units/mL of collagenase type II (Worthington Biochemical Corp., Lakewood, NJ) at 37°C on a shaker to completely degrade the gelatin sponge. The resulting macroporous PVA scaffolds were washed in de-ionized water three times, disinfected in 70% ethanol for 20 min and then 100% ethanol for 1 h. The scaffolds were dehydrated by placing them under laminar flow for 1 h [20].

2.2 In Vitro Model. Our study design is depicted in Fig. 1. Cartilage explants were harvested from the trochlear groove and femoral condyles of juvenile bovine knees (Max Insel Cohen, Livingston, NJ). The superficial and deep zones were removed by

placing cartilage explants into a cylindrical custom aluminum cutting jig with side slots through which microtome blades can cut the tissue (Sakura Finetek, Torrance, CA) [25,26]. The resulting cartilage explants were flat with parallel surfaces and were 10 mm diameter $\times$ 2.5 mm thick. Cylindrical cores were removed from the central aspect of each cartilage explant using a $\varnothing 3.5$ mm sterile biopsy punch. PVA scaffolds were placed into the central hole of the cartilage explants. The scaffolds were rehydrated in situ using Dulbecco's modified Eagle's medium (DMEM)/F12 culture media (Thermo Fisher, Waltham, MA). Scaffold–cartilage constructs were then cultured in 30 mL serum-free media (DMEM/F12, ITS+, L-glutamine, 1% Pen-Strep, 100 nM dexamethasone, 50 $\mu\text{g}/\text{mL}$ ascorbate-2-phosphate) with media changed every 3 days for a total of 28 days [20].

After 28 days, samples were randomly assigned to the following groups: (1) 0 cycles (unloaded), (2) 900 cycles, (3) 1800 cycles, (4) 3600 cycles, and (5) 7200 cycles of loading per day. For each of the loaded groups, six explants were loaded simultaneously using a 1 Hz sinusoidal wave applied 5 days/week for 14 days in a TGT Dynagen system (Tissue Growth Technologies Minnetonka, MN). The sinusoid ranged from 6 N (min) to 40 N (max) compressive axial force, resulting in applied contact stresses of 0.01 MPa to 0.085 MPa across the surface of each sample. Constructs were harvested for interface strength via a push-out test on day 0 ($n = 6$ samples/group), day 28 ($n = 12$ samples/group), and day 42 ($n = 12$ samples/group). Biochemical analysis ($n = 8$ samples/group), and histological analysis ($n = 4$ samples/group) were performed on the samples used for push-out testing. The experiment was run twice, each run had six explants per group.

2.3 Outcome Measures. At days 0, 28, and 42, scaffold–cartilage interface strength was determined using a custom-built mechanical testing system—the Compression Computer Automated Soft Tissue Test System [25], which was

Fig. 2 (a) A schematic of the two-dimensional axisymmetric bFEM of the macroporous PVA scaffolds-articular cartilage explant. (b) Graphic of the inhomogeneous material properties for articular cartilage: h = height of the samples, the z -axis is the direction of loading perpendicular to the nonporous platen on the base, where a value of zero is at the base of test system, r is the radius from the axis of symmetry.

modified to perform a push-out test. A 2.75 mm diameter solid, stainless steel indenter was placed on the surface of the scaffold and advanced at a rate of 10 $\mu\text{m/s}$ until an abrupt decrease in force occurred, indicating interface rupture [20,27]. Maximum stress was computed by dividing the maximum load by the surface area of the interface. Differences in push-out strength as a function of number of cycles were determined using one-way analysis of variance with Tukey posttest (significance = $p < 0.05$). After testing, scaffolds were frozen and stored at -20°C for biochemical testing for sulfated-glycosaminoglycan (s-GAG) content via dimethylmethylene blue assay, collagen via the orthohydroxyproline (OHP) assay, and deoxyribonucleic acid (DNA) content via the Picogreen assay (Thermo Fisher). Dimethylmethylene blue assay was performed as previously described by Farndale et al. [28], with a standard curve created using chondroitin 6-sulfate (Sigma-Aldrich, St. Louis, MO). OHP assay was performed as previously described by Reddy and Enwemeka [29], with standards created from trans-4-hydroxy-L-proline (Sigma-Aldrich). Concentration values obtained from the OHP assay were divided by 0.125 (the number of prolines per collagen bundle) to obtain the amount collagen within each scaffold. The Picogreen assay was performed as per manufacturer instructions, with standards created from the provided lambda DNA. The DNA content was then converted into cell number by dividing the DNA content of each scaffold by the average amount of DNA per chondrocyte (9 pg/cell).

Four of the scaffold-cartilage explants from each group were fixed in neutral-buffered formalin + 0.5% cetylpyridinium chloride for 4 h at room temperature, washed briefly in phosphate-buffered saline to remove any residual formalin, cryoprotected in a 30% sucrose solution at 4°C overnight, incubated for 2 h in 5% gelatin + 5% sucrose embedding medium, and then embedded in the gelatin-sucrose medium [30]. Gelatin was used to impregnate the PVA and increase its adherence to the slides. Blocks were cryotomed into 8 μm sections and stained using Safranin-O/Fast Green and counterstained with hematoxylin.

2.4 In Silico models. A two-dimensional axisymmetric bFEM of the PVA-articular cartilage explant was developed (Fig. 2(a)), the geometry of which matched that of the experimental test setup. The macroporous PVA scaffold was modeled as a linear biphasic material. The material properties of the PVA were input from in-house tests [31] and were as follows: Young's modulus 0.01167 MPa, Poisson's ratio 0.2, and permeability $1.56 \times 10^{-11} \text{ m}^4/\text{N s}$. Articular cartilage was modeled as an inhomogeneous material with aggregate modulus input as a third-order polynomial function from the surface to the depth of the tissue (Fig. 2(b)) [32]. Permeability was related to the strain through the function $k = k_0 J^m$, where J is the volume ratio defined as the Jacobian determinant of

the deformation gradient, m is the permeability parameter 2.2, k_0 is the initial permeability $3 \times 10^{-15} \text{ m}^4/\text{N s}$, and Poisson's ratio is 0.018. Scaffold-cartilage integration was modeled as friction contact with cohesion sliding resistance of 1.52 kPa, the value of which was measured from the physical push-out test data from day 28; as such, the model represented the conditions within the scaffold-articular cartilage explant when loading in the bioreactor commenced. The upper nonporous platen was modeled as linear elastic material with Young's modulus $E = 170 \text{ GPa}$ and Poisson's ratio $\nu = 0.28$. Contact between the upper nonporous platen and PVA-articular cartilage explant was assumed to be frictionless. The bottom nonporous platen was not explicitly modeled; instead, an impermeable roller boundary condition was applied to the bottom surface of the PVA and articular cartilage thus allowing friction-free contact between the base and the scaffold-articular cartilage construct. A sinusoidal load profile was applied on the upper nonporous platen to mimic the frequency and load profile as applied through the bioreactor. The augmented Lagrangian method developed in our previous studies [33–36] was used to detect biphasic contact in the model. The biphasic models were solved using COMSOL MULTIPHYSICS (Burlington, MA). The distributions of vertical displacement, fluid pressure, and compressive stress under peak load were computed.

3 Results

3.1 Interface Strength. Scaffold-cartilage interfacial strength was significantly decreased at day 42 compared to the unloaded controls regardless of number of loading cycles ($p < 0.001$; Fig. 3). There were no differences in push-out strength between

Fig. 3 Scaffold-cartilage interfacial strength on days 0, 28, and 42. The interfacial strength of the scaffold-cartilage interface was measured by displacing the scaffold from the cartilage explant and measuring the stress until failure. Data are shown as mean $\pm$ standard error. * denotes difference from day 42–0 cycles per day ($p < 0.001$).

Fig. 4 Biochemical quantification of s-GAG, collagen, and cellular content within the scaffolds. Biochemical quantification was performed for (a) s-GAG content, (b) collagen content (OHP assay), and (c) cell number ($n = 8$ per group). Bars are shown as mean $\pm$ standard error. * denotes difference from unloaded group ($p < 0.05$).

any of the mechanically loaded groups. There were also no differences found between any of the mechanically loaded groups and cultured scaffold–cartilage explants at days 0 and 28.

3.2 Biochemistry. Scaffold proteoglycan (s-GAG) content decreased in groups that were subjected to mechanical loading (Fig. 4(a)). s-GAG content in the unloaded group ($28.8 \pm 5.2 \mu\text{g}$) was higher than in all the loaded groups regardless of number of loading cycles, but these differences were only significant at 900 cycles/day ($11.1 \pm 2.3 \mu\text{g}$) and 7200 cycles/day ($6.0 \pm 0.5 \mu\text{g}$). Collagen content (Fig. 4(b)) as measured by OHP was not significantly affected by mechanical loading (unloaded, $11.0 \pm 3.2 \mu\text{g}$; 900 cycles/day, $14.5 \pm 5.7 \mu\text{g}$; 1800 cycles/day, $4.9 \pm 0.4 \mu\text{g}$; 3600 cycles/day, $17.6 \pm 6.3 \mu\text{g}$; 7200 cycles/day, $4.8 \pm 0.6 \mu\text{g}$). The number of cells did not change significantly with mechanical loading (Fig. 4(c)).

3.3 Histological Analysis at Day 42. Histological staining was difficult because of the tendency of the scaffold to become dislodged from the articular cartilage explant during sample preparation, particularly in the loaded samples. Nonetheless, representative images of the unloaded group revealed the presence of chondrocytes within the pores of the scaffolds, which were sporadically surrounded by a collagen-rich matrix (Fig. S1, which is available under the “Supplemental Materials” tab for this paper in the ASME Digital Collection).

3.4 In Silico Model. After 150 loading cycles, the displacement of the loading platen varied less than 0.5% between consecutive cycles, thus indicating that the scaffold–cartilage construct had reached steady-state (Fig. 5). As such, even if higher numbers of loading cycles were modeled, the micromechanical conditions within the scaffold or at the scaffold–cartilage interface will not change.

At steady-state, the top zone of the articular cartilage deformed in the direction of the interface with the scaffold, whereas the middle and bottom zones of the scaffold expanded laterally toward the articular cartilage (Fig. 6(a)). The magnitude of vertical displacement of the articular cartilage at the scaffold–cartilage interface was lower than the vertical displacement of the scaffold at the same depth. Thus, micromotion (i.e., relative movement) occurred at the scaffold–cartilage interface (Fig. 6(b)). During the cyclic loading process, peak micromotion always occurred under the top zone and decreased toward the top and bottom surfaces (Fig. 6(c)). Peak micromotion of $175 \mu\text{m}$ occurred beneath the top zone. Over 90% of the applied load was distributed through the native cartilage, and articular cartilage had much greater stresses than the PVA scaffold (Figs. 6(d) and 6(e)). An 80 kPa discontinuity in solid normal stress occurred at the scaffold–cartilage interface (Fig. 6(e)). Of note, fluid exchange was observed during the cyclic loading: fluid flowed from PVA to the native cartilage

when load was increasing, and fluid flowed from the native cartilage to the PVA when load was decreasing.

4 Discussion

The optimal method to integrate scaffolds with articular cartilage has not yet been identified [13]. This situation is caused in part by the lack of understanding about the mechanical and biological conditions that affect integration. The objective of this study was to quantify the effect of mechanical loading on integration between a scaffold and articular cartilage. We hypothesized that increased number of loading cycles would have a detrimental effect on interface integrity. To test this hypothesis, we applied a combined in vitro and in silico approach to a scaffold–cartilage construct subjected to varying numbers of mechanical loading cycles. We established that mechanical loading creates a complex interplay of micromotion and abrupt changes in stresses and fluid pressure at the scaffold–cartilage interface, which reach steady-state conditions at 150 cycles. Because of these conditions, mechanical loading, regardless of the number of load cycles applied, disrupted matrix formation and led to a reduction in interfacial strength.

In the quest to create a strong interface between scaffolds and articular cartilage, a number of elegant techniques have been suggested. Yu et al. [37] and Sharma et al. [38] developed concepts based on aldehyde-amine Schiff-base reactions to chemically bond polymers to extracellular matrix components. Allon et al. [19] augmented polymers with adhesion proteins while Maher et al. [18] used growth factor-augmented nanofibers. Fortier et al. [16] and Rackwitz et al. [39] augmented scaffolds with growth

Fig. 5 Vertical displacement of the loading platen varied with the number of loading cycles. After 100 loading cycles, the displacement of the loading platen varied less than 0.5% between cycles, indicating that the construct reached steady-state.

Fig. 6 (a) Distribution of vertical displacement through the scaffold, cartilage construct under peak load, (b) Distribution of vertical displacement at the horizontal midline (dotted line) of the model, (c) micromotion as a function of normalized tissue depth. Peak relative micromotion of 175 μm occurred beneath the top zone, (d) distribution of fluid pressure (kPa) in PVA and articular cartilage, and (e) distribution of compressive stress (kPa) in PVA and articular cartilage.

factors and stem cells, respectively, while Erickson et al. [40] modulated the extent of mesenchymal stem cell maturation in the hopes of optimizing integration. Hunziker and Kapfinger [21] chose to partially, enzymatically digest the host cartilage in an effort to free chondrocytes that would be capable of migrating to the scaffold interface. Ng et al. [20] augmented enzymatic digestion with a scaffold designed to create a tight press-fit with surrounding tissue. More recently, Makris et al. [41] demonstrated that a treatment regime which included the use of a digestive enzyme (chondroitinase-ABC), TGF- β 1, and the collagen cross-linking agent lysyl oxidase could result in improved scaffold–cartilage integration. However, the efficacy of these techniques was assessed in unloaded tissue culture models, or minimally and unquantified in vivo conditions. Therefore, it is unclear if any of these options will result in a robust scaffold–cartilage interface capable of functioning in the heavily loaded environment of the joint into which they will ultimately be used.

Articular joints can experience forces of up to five times body weight during activities of daily living [24]. These forces are distributed across the articular cartilage and in the case of the knee joint, for example, can result in peak contact stresses of up to 8 MPa [42] or more widespread contact stresses of up to 1 MPa [43]. Any scaffold intended for implantation into a defect in articular cartilage should be capable of withstanding joint loads, and also should be able to integrate with the host tissue and maintain that integration under the applied loading conditions. Despite this requirement, there is a paucity of information about how mechanical loading can affect the cartilage–scaffold interface. To start to address this deficit in our knowledge, we developed an approach of combining in silico models (which allow us to quantify the micromechanical conditions within the scaffold–cartilage construct) with in vitro tissue culture models (in which we can directly analyze the response of the interface to prescribed loading conditions).

To simplify our modeling approach and interpretation of the data, rather than choosing to use a degradable scaffold (the properties and morphology of which would change throughout the duration of the study), we choose to use a nondegradable porous scaffold. Nondegradable hydrogels, such as PVA have been tested

for many orthopedic applications including meniscal replacement [44–48] and nucleus pulposus replacement for an intervertebral disk [49,50]. More recently, the porous PVA scaffold tested in this study was demonstrated to integrate with articular cartilage in a static culture cartilage–scaffold, unloaded tissue culture model [20]. Based on this earlier work, we chose to incubate the scaffold–cartilage explants for 28 days to ensure that sufficient time had passed to allow chondrocytes to migrate into the scaffold and begin to lay down matrix [20].

The in silico bFEM was designed to mimic the inhomogeneous and biphasic nature of native articular cartilage. The following characteristics of the tissue were included: fluid flow through cartilage and through the porous scaffold, surface-to-depth variation in compressive modulus, and strain-dependent permeability. While our original goal was to model the number of cycles as applied in the physical in vitro experiment (up to 7200 cycles), we realized that steady-state of the construct in the bFEM was reached at 150 cycles. Therefore, modeling any more loading cycles would not change the results of the model. In a practical sense, this result corroborates the finding from the physical model, which found that number of cycles (within the range of 900–7200 cycles) were all equally detrimental to the interface. Our results further suggest that this conclusion would hold, even if the number of loading cycles were decreased to 150. But to understand why as few as 150 loading cycles could be detrimental to the interface, we must consider: (I) the distribution in applied load between the scaffold and the cartilage and (II) the effect of pre-culture interface conditions on the micromechanical conditions within the scaffold and articular cartilage.

(I) *The distribution in applied load between the scaffold and the cartilage:* The material properties of the scaffold and the surrounding articular cartilage are different. Specifically, the modulus of the scaffold is an order of magnitude lower than that of the articular cartilage, and scaffold permeability is substantially higher. Thus, upon loading, higher compressive stresses and fluid pressures occur in the articular cartilage compared to that in the scaffold, resulting in abrupt changes in compressive stress and fluid pressure at the interface (Figs. 6(d) and 6(e)). Moreover, the scaffold has a homogeneous structure, while native cartilage is inhomogeneous with depth-dependent properties which lead to

depth-dependent inhomogeneities in stress and fluid flow patterns that are absent in the scaffold. Such differences in mechanical properties between scaffolds intended for cartilage repair and the host articular cartilage are common [16,18,19,37–39], and while it is difficult to say exactly which of these property differences led to the dramatic change in mechanics along the interface, it is clear that an abrupt change in stresses along the interface occurred. Interestingly, decreased matrix production from cells within the scaffold also occurred, which may be the result of high regional deformations within the softer scaffold.

(II) *The effect of preculture interface conditions on the micro-mechanical conditions within the scaffold and articular cartilage:* A key input parameter to our in silico model is the boundary conditions at the scaffold–cartilage interface. Interfacial strength at the interface will vary as a function of time. For the purposes of this study, we used interface strength input values to match that quantified from day 28 cultured samples (1.817 kPa), which resulted in peak interface micromotion of 115 μm at the first loading cycle. Had loading commenced on day 0, interfacial strength would have been 0.885 kPa, and resulted in peak interface micromotion of 180 μm at the first loading cycle (see supplemental data, Fig. S2, which is available under the “Supplemental Materials” tab for this paper in the ASME Digital Collection). The effect of micromotion on osseointegration (i.e., integration between bone and artificial implant) has been extensively studied since the 1970s, and it was found that there is a critical threshold micromotion of approximately 100 μm , above which osseointegration is inhibited [51]. Though the effect of micromotion on the scaffold–cartilage integration is unknown, it is possible that even if scaffold mechanical properties cannot be designed to match that of native cartilage, by varying the time in culture before the application of load may be a powerful way to modulate interface micromotion and prevent mechanical disruption.

The study has several limitations. The load profile input to the bioreactor and applied to the bFEM was a simplified sinusoidal wave and as such does not necessarily represent the in vivo loading. More recently, physiological load profiles have been quantified from simulated gait [43,52] and in future studies, these load profiles will be input. Due to the difficulty of processing the PVA scaffold–cartilage explant, we could not generate good quality immunohistochemical images—thus a full image of the distribution of collagen was not possible. Additionally, due to the small size and elasticity of the scaffolds, the biochemical assays included the entire scaffold and therefore it was not possible to localize the results to the actual scaffold–cartilage interface. Moreover, the biochemical assays were not performed on the cartilage explants to understand the changes in articular cartilage with loading; this analysis will be performed in future studies. It should be noted that the superficial and deep zones were removed from the cartilage explants to ensure flat, parallel surfaces suitable for mechanical loading. Recognizing that the percent of tissue physically removed from each explant varies and that there exists a depth-dependent variation in juvenile bovine articular cartilage properties [32,53], even without consideration of the superficial and deep zones (Fig. 2), the bFEM included both the superficial and deep zones. But the properties as input were average values from literature and not specimen-specific properties. Whether or not specimen-specific input values are needed is yet unclear. Finally, our study was developed using a nondegradable scaffold, to avoid the complexities of modeling time-dependent changes in scaffold properties. While nondegradable porous scaffolds are being developed for cartilage repair [20,54], applicability of this model to the more commonly used degradable scaffolds would strengthen its implications.

In summary, using a combined in vitro and in silico approach, we have established that mechanical loading creates a complex interplay of micromotion and abrupt changes in shear stress, fluid pressure, and principal stress at the scaffold–cartilage interface that reaches steady-state at 150 cycles. Because of these conditions, mechanical loading, regardless of the number of load cycles

applied, disrupted matrix formation and led to a reduction in interfacial strength. As we try to engineer functional scaffolds for cartilage repair, the modeling approach herein described could act as a platform for optimization of the mechanobiological environment of this clinically relevant interface.

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Nomenclature

bFEM = biphasic finite element model
 DMEM = Dulbecco’s modified Eagle’s medium
 E = Young’s modulus
 J = volume ratio
 k = strain-dependent permeability
 k_0 = initial permeability
 m = permeability parameter
 OHP = orthohydroxyproline
 PVA = poly(vinyl alcohol)
 s-GAG = sulfated-glycosaminoglycan
 ν = Poisson’s ratio

References

- [1] Simon, T. M., and Jackson, D. W., 2006, “Articular Cartilage: Injury Pathways and Treatment Options,” *Sports Med. Arthroscopy Rev.*, **14**(3), pp. 146–154.
- [2] Flanagan, D. C., Harris, J. D., Trinh, T. Q., Siston, R. A., and Brophy, R. H., 2010, “Prevalence of Chondral Defects in Athletes’ Knees: A Systematic Review,” *Med. Sci. Sports Exercise*, **42**(10), pp. 1795–1801.
- [3] Kotlarz, H., Gunnarsson, C. L., Fang, H., and Rizzo, J. A., 2009, “Insurer and Out-of-Pocket Costs of Osteoarthritis in the U.S.: Evidence From National Survey Data,” *Arthritis Rheum.*, **60**(12), pp. 3546–3553.
- [4] Freedman, K. B., Nho, S. J., and Cole, B. J., 2003, “Marrow Stimulating Technique to Augment Meniscus Repair,” *Arthroscopy*, **19**(7), pp. 794–798.
- [5] Szerb, I., Hangody, L., Duska, Z., and Kaposi, N. P., 2005, “Mosaicplasty: Long-Term Follow-Up,” *Bull. Hosp. Jt. Dis.*, **63**(1–2), pp. 54–62.
- [6] Bedi, A., Feeley, B. T., and Williams, R. J., 2010, “Management of Articular Cartilage Defects of the Knee,” *J. Bone Jt. Surg. Am.*, **92**(4), pp. 994–1009.
- [7] Brittberg, M., Peterson, L., Sjogren-Jansson, E., Tallheden, T., and Lindahl, A., 2003, “Articular Cartilage Engineering With Autologous Chondrocyte Transplantation: A Review of Recent Developments,” *J. Bone Jt. Surg. Am.*, **85A**(Suppl. 3), pp. 109–115.
- [8] Widuchowski, W., Lukasik, P., Kwiatkowski, G., Faltus, R., Szyluk, K., Widuchowski, J., and Koczy, B., 2008, “Isolated Full Thickness Chondral Injuries. Prevalence and Outcome of Treatment. A Retrospective Study of 5233 Knee Arthroscopies,” *Acta Chir. Orthop. Traumatol. Cech.*, **75**(5), pp. 382–386.
- [9] Mithoefer, K., Hamby, K., Della Villa, S., Silvers, H., and Mandelbaum, B. R., 2009, “Return to Sports Participation After Articular Cartilage Repair in the Knee: Scientific Evidence,” *Am. J. Sports Med.*, **37**(Suppl. 1), pp. 167–176.
- [10] Moseley, J. B., Jr., Anderson, A. F., Browne, J. E., Mandelbaum, B. R., Micheli, L. J., Fu, F., and Erggelet, C., 2010, “Long-Term Durability of Autologous Chondrocyte Implantation: A Multicenter, Observational Study in U.S. Patients,” *Am. J. Sports Med.*, **38**(2), pp. 238–246.
- [11] Solheim, E., Hegna, J., Oyen, J., Austgulen, O. K., Harlem, T., and Strand, T., 2010, “Osteochondral Autografting (Mosaicplasty) in Articular Cartilage Defects in the Knee: Results at 5 to 9 Years,” *Knee*, **17**(1), pp. 84–87.
- [12] Frenkel, S. R., and Di Cesare, P. E., 2004, “Scaffolds for Articular Cartilage Repair,” *Ann. Biomed. Eng.*, **32**(1), pp. 26–34.
- [13] Athanasiou, K. A., Responde, D. J., Brown, W. E., and Hu, J. C., 2015, “Harnessing Biomechanics to Develop Cartilage Regeneration Strategies,” *ASME J. Biomech. Eng.*, **137**(2), p. 020901.
- [14] Boushell, M. K., Hung, C. T., Hunziker, E. B., Strauss, E. J., and Lu, H. H., 2017, “Current Strategies for Integrative Cartilage Repair,” *Connect. Tissue Res.*, **58**(5), pp. 393–406.
- [15] Pabbruwe, M. B., Esfandiari, E., Kafienah, W., Tarlton, J. F., and Hollander, A. P., 2009, “Induction of Cartilage Integration by a Chondrocyte/Collagen-Scaffold Implant,” *Biomaterials*, **30**(26), pp. 4277–4286.
- [16] Fortier, L. A., Nixon, A. J., and Lust, G., 2002, “Phenotypic Expression of Equine Articular Chondrocytes Grown in Three-Dimensional Cultures

- Supplemented With Supraphysiologic Concentrations of Insulin-Like Growth Factor-1," *Am. J. Vet. Res.*, **63**(2), pp. 301–305.
- [17] Luo, Z., Jiang, L., Xu, Y., Li, H., Xu, W., Wu, S., Wang, Y., Tang, Z., Lv, Y., and Yang, L., 2015, "Mechano Growth Factor (MGF) and Transforming Growth Factor (TGF)- β 3 Functionalized Silk Scaffolds Enhance Articular Hyaline Cartilage Regeneration in Rabbit Model," *Biomaterials*, **52**, pp. 463–475.
- [18] Maher, S. A., Mauck, R. L., Rackwitz, L., and Tuan, R. S., 2010, "A Nanofibrous Cell-Seeded Hydrogel Promotes Integration in a Cartilage Gap Model," *J. Tissue Eng. Regen. Med.*, **4**(1), pp. 25–29.
- [19] Allon, A. A., Ng, K. W., Hammoud, S., Russell, B. H., Jones, C. M., Rivera, J. J., Schwartz, J., Hook, M., and Maher, S. A., 2012, "Augmenting the Articular Cartilage-Implant Interface: Functionalizing With a Collagen Adhesion Protein," *J. Biomed. Mater. Res. A*, **100**(8), pp. 2168–2175.
- [20] Ng, K. W., Wanivenhaus, F., Chen, T., Hsu, H. C., Allon, A. A., Abrams, V. D., Torzilli, P. A., Warren, R. F., and Maher, S. A., 2012, "A Novel Macroporous Polyvinyl Alcohol Scaffold Promotes Chondrocyte Migration and Interface Formation in an In Vitro Cartilage Defect Model," *Tissue Eng. Part A*, **18**(11–12), pp. 1273–1281.
- [21] Hunziker, E. B., and Kapfinger, E., 1998, "Removal of Proteoglycans From the Surface of Defects in Articular Cartilage Transiently Enhances Coverage by Repair Cells," *J. Bone Jt. Surg., Br. Vol.*, **80**(1), pp. 144–150.
- [22] Wernike, E., Li, Z., Alini, M., and Grad, S., 2008, "Effect of Reduced Oxygen Tension and Long-Term Mechanical Stimulation on Chondrocyte-Polymer Constructs," *Cell Tissue Res.*, **331**(2), pp. 473–483.
- [23] Ng, K. W., Mauck, R. L., Wang, C. C., Kelly, T. A., Ho, M. M., Chen, F. H., Ateshian, G. A., and Hung, C. T., 2009, "Duty Cycle of Deformational Loading Influences the Growth of Engineered Articular Cartilage," *Cell Mol. Bioeng.*, **2**(3), pp. 386–394.
- [24] Kutzner, I., Heinlein, B., Graichen, F., Bender, A., Rohlmann, A., Halder, A., Beier, A., and Bergmann, G., 2010, "Loading of the Knee Joint During Activities of Daily Living Measured In Vivo in Five Subjects," *J. Biomech.*, **43**(11), pp. 2164–2173.
- [25] Torzilli, P. A., 1990, "Measurement of the Compressive Properties of Thin Cartilage Slices: Evaluating Tissue Inhomogeneity," *Methods in Cartilage Research*, A. Maroudas and K. Kuettner, eds., Academic Press, London, pp. 304–308.
- [26] Torzilli, P. A., Bhargava, M., and Chen, C. T., 2011, "Mechanical Loading of Articular Cartilage Reduces IL-1-Induced Enzyme Expression," *Cartilage*, **2**(4), pp. 364–373.
- [27] van de Breevaart Bravenboer, J., In der Maur, C. D., Bos, P. K., Feenstra, L., Verhaar, J. A., Weinans, H., and van Osch, G. J., 2004, "Improved Cartilage Integration and Interfacial Strength After Enzymatic Treatment in a Cartilage Transplantation Model," *Arthritis Res. Ther.*, **6**(5), pp. R469–R476.
- [28] Farndale, R. W., Sayers, C. A., and Barrett, A. J., 1982, "A Direct Spectrophotometric Microassay for Sulfated Glycosaminoglycans in Cartilage Cultures," *Connect. Tissue Res.*, **9**(4), pp. 247–248.
- [29] Reddy, G. K., and Enwemeka, C. S., 1996, "A Simplified Method for the Analysis of Hydroxyproline in Biological Tissues," *Clin. Biochem.*, **29**(3), pp. 225–229.
- [30] Brown, D. A., Chou, Y. F., Beygui, R. E., Dunn, J. C., and Wu, B. M., 2005, "Gelatin-Embedded Cell-Polymer Constructs for Histological Cryosectioning," *J. Biomed. Mater. Res. B*, **72**(1), pp. 79–85.
- [31] Ng, K. W., Torzilli, P. A., Warren, R. F., and Maher, S. A., 2014, "Characterization of a Macroporous Polyvinyl Alcohol Scaffold for the Repair of Focal Articular Cartilage Defects," *J. Tissue Eng. Regen. Med.*, **8**(2), pp. 164–168.
- [32] Chen, A. C., Bae, W. C., Schinagl, R. M., and Sah, R. L., 2001, "Depth- and Strain-Dependent Mechanical and Electromechanical Properties of Full-Thickness Bovine Articular Cartilage in Confined Compression," *J. Biomech.*, **34**(1), pp. 1–12.
- [33] Guo, H., Maher, S. A., and Spilker, R. L., 2013, "Biphasic Finite Element Contact Analysis of the Knee Joint Using an Augmented Lagrangian Method," *Med. Eng. Phys.*, **35**(9), pp. 1313–1320.
- [34] Guo, H., Nickel, J. C., Iwasaki, L. R., and Spilker, R. L., 2012, "An Augmented Lagrangian Method for Sliding Contact of Soft Tissue," *ASME J. Biomech. Eng.*, **134**(8), p. 084503.
- [35] Guo, H., and Spilker, R. L., 2011, "Biphasic Finite Element Modeling of Hydrated Soft Tissue Contact Using an Augmented Lagrangian Method," *ASME J. Biomech. Eng.*, **133**(11), p. 111001.
- [36] Guo, H., and Spilker, R. L., 2014, "An Augmented Lagrangian Finite Element Formulation for 3D Contact of Biphasic Tissues," *Comput. Methods Biomech. Biomed. Engin.*, **17**(11), pp. 1206–1216.
- [37] Yu, F., Cao, X., Du, J., Wang, G., and Chen, X., 2015, "Multifunctional Hydrogel With Good Structure Integrity, Self-Healing, and Tissue-Adhesive Property Formed by Combining Diels-Alder Click Reaction and Acylhydrazone Bond," *ACS Appl. Mater. Interfaces*, **7**(43), pp. 24023–24031.
- [38] Sharma, B., Fermanian, S., Gibson, M., Unterman, S., Herzka, D. A., Cascio, B., Coburn, J., Hui, A. Y., Marcus, N., Gold, G. E., and Elisseeff, J. H., 2013, "Human Cartilage Repair With a Photoreactive Adhesive-Hydrogel Composite," *Sci. Transl. Med.*, **5**(167), p. 167ra6.
- [39] Rackwitz, L., Djouad, F., Janjanin, S., Noth, U., and Tuan, R. S., 2014, "Functional Cartilage Repair Capacity of De-Differentiated, Chondrocyte- and Mesenchymal Stem Cell-Laden Hydrogels In Vitro," *Osteoarthritis Cartilage*, **22**(8), pp. 1148–1157.
- [40] Erickson, I. E., Kestle, S. R., Zellars, K. H., Dodge, G. R., Burdick, J. A., and Mauck, R. L., 2012, "Improved Cartilage Repair Via In Vitro Pre-Maturation of MSC-Seeded Hyaluronic Acid Hydrogels," *Biomed. Mater.*, **7**(2), p. 024110.
- [41] Makris, E. A., MacBarb, R. F., Paschos, N. K., Hu, J. C., and Athanasiou, K. A., 2014, "Combined Use of Chondroitinase-ABC, TGF- β 1, and Collagen Crosslinking Agent Lysyl Oxidase to Engineer Functional Neotissues for Fibrocartilage Repair," *Biomaterials*, **35**(25), pp. 6787–6796.
- [42] Bedi, A., Kelly, N. H., Baad, M., Fox, A. J., Brophy, R. H., Warren, R. F., and Maher, S. A., 2010, "Dynamic Contact Mechanics of the Medial Meniscus as a Function of Radial Tear, Repair, and Partial Meniscectomy," *J. Bone Jt. Surg. Am.*, **92**(6), pp. 1398–1408.
- [43] Wang, H., Chen, T., Torzilli, P., Warren, R., and Maher, S., 2014, "Dynamic Contact Stress Patterns on the Tibial Plateaus During Simulated Gait: A Novel Application of Normalized Cross Correlation," *J. Biomech.*, **47**(2), pp. 568–574.
- [44] Kobayashi, M., 2004, "A Study of Polyvinyl Alcohol-Hydrogel (PVA-H) Artificial Meniscus In Vivo," *Biomed. Mater. Eng.*, **14**(4), pp. 505–515.
- [45] Kobayashi, M., Chang, Y. S., and Oka, M., 2005, "A Two Year In Vivo Study of Polyvinyl Alcohol-Hydrogel (PVA-H) Artificial Meniscus," *Biomaterials*, **26**(16), pp. 3243–3248.
- [46] Kobayashi, M., and Oka, M., 2004, "Characterization of a Polyvinyl Alcohol-Hydrogel Artificial Articular Cartilage Prepared by Injection Molding," *J. Biomater. Sci. Polym. Ed.*, **15**(6), pp. 741–751.
- [47] Kobayashi, M., Toguchida, J., and Oka, M., 2003, "Preliminary Study of Polyvinyl Alcohol-Hydrogel (PVA-H) Artificial Meniscus," *Biomaterials*, **24**(4), pp. 639–647.
- [48] Swieszkowski, W., Ku, D. N., Bersee, H. E., and Kurzydowski, K. J., 2006, "An Elastic Material for Cartilage Replacement in an Arthritic Shoulder Joint," *Biomaterials*, **27**(8), pp. 1534–1541.
- [49] Joshi, A., Fussell, G., Thomas, J., Hsuan, A., Lowman, A., Karduna, A., Vresilovic, E., and Marcolongo, M., 2006, "Functional Compressive Mechanics of a PVA/PVP Nucleus Pulposus Replacement," *Biomaterials*, **27**(2), pp. 176–184.
- [50] Thomas, J., Lowman, A., and Marcolongo, M., 2003, "Novel Associated Hydrogels for Nucleus Pulposus Replacement," *J. Biomed. Mater. Res. A*, **67**(4), pp. 1329–1337.
- [51] Wazen, R. M., Currey, J. A., Guo, H., Brunski, J. B., Helms, J. A., and Nanci, A., 2013, "Micromotion-Induced Strain Fields Influence Early Stages of Repair at Bone-Implant Interfaces," *Acta Biomater.*, **9**(5), pp. 6663–6674.
- [52] Chen, T., Wang, H., Warren, R., and Maher, S., 2017, "Loss of ACL Function Leads to Alterations in Tibial Plateau Common Dynamic Contact Stress Profiles," *J. Biomech.*, **61**, pp. 275–279.
- [53] Bian, L., Lima, E. G., Angione, S. L., Ng, K. W., Williams, D. Y., Xu, D., Stoker, A. M., Cook, J. L., Ateshian, G. A., and Hung, C. T., 2008, "Mechanical and Biochemical Characterization of Cartilage Explants in Serum-Free Culture," *J. Biomech.*, **41**(6), pp. 1153–1159.
- [54] Krych, A. J., Wanivenhaus, F., Ng, K. W., Doty, S., Warren, R. F., and Maher, S. A., 2013, "Matrix Generation Within a Macroporous Non-Degradable Implant for Osteochondral Defects is Not Enhanced With Partial Enzymatic Digestion of the Surrounding Tissue: Evaluation in an In Vivo Rabbit Model," *J. Mater. Sci. Mater. Med.*, **24**(10), pp. 2429–2437.