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# The Influence of Cell-Matrix Attachment and Matrix Development on the Micromechanical Environment of the Chondrocyte in Tissue-Engineered Cartilage

Mehdi Khoshgoftar, PhD, Keita Ito, MD, ScD, and Corrinus C. van Donkelaar, PhD

Insufficiency of mechanical properties of tissue-engineered (TE) cartilage grafts is still a limiting factor for their clinical application. It has been shown that mechanostimulation of chondrocytes enhances synthesis of extracellular matrix (ECM) and thereby improves the mechanical properties of the grafts. However, the optimal mechanical loading required to stimulate chondrocytes for sufficient matrix synthesis is still unknown. The properties of the pericellular matrix (PCM) and the ability of the chondrocytes to attach to its adjacent matrix may importantly determine the stimulation of the cell in loaded tissue. The aim of the present study is to numerically investigate the influence of tissue development and cell-matrix attachment on the mechanical environment of a chondrocyte embedded in agarose. Mechanical environment inside TE constructs is evaluated and compared with that in native cartilage under 10% unconfined compression. A multiscale finite element modeling approach in conjunction with a validated nonlinear fiber-reinforced poroviscoelastic swelling cartilage model is used. Results indicate that without cell attachment, excessive local strains may be induced in the cell. With PCM development and the establishment of focal adhesions at the cell surface, the cell is strained more homogeneously upon external loading. However, compared with chondrocytes in native cartilage, the transmission of the external compression to the cells in TE constructs is less. This suggests that, over time, the loading magnitude may be increased to continue stimulation of chondrocytes at the physiological or even higher levels to possibly enhance matrix synthesis. These findings improve our insights into the micromechanical environment of cells in tissue engineering cultures.

## Introduction

ARTICULAR CARTILAGE COVERS the surfaces of bones in diarthrodial joints. The primary function of articular cartilage is to bear load and to provide a smooth lubricated surface for joint motion. Treatment of cartilage injuries is still a challenge. Replacing damaged cartilage with tissue-engineered (TE) cartilage has promising potential.<sup>1</sup> For successful long-term cartilage replacement, an important requirement for TE cartilage is to withstand *in vivo* loading conditions postimplantation. To be mechanically functional, TE cartilage should contain sufficient extracellular matrix (ECM), with an appropriate spatial and structural organization.<sup>2</sup> It is known that ECM synthesis can be increased by mechanically stimulating the chondrocytes.<sup>3–8</sup> Therefore, dynamic mechanical stimulation is common in tissue engineering protocols. Even though matrix synthesis can be stimulated, it is not yet possible to produce TE cartilage with native amounts of ECM. To optimize the mechanical stimulation, regime requires time-consuming comparative ex-

perimental work with multiple stimulation groups. However, interpretation of such data remains difficult because the micromechanical environment of the chondrocytes changes with ongoing matrix development. Consequently, to optimize the stimulation regime throughout the culture period likely requires updating the protocol over time to the local changes in ECM.<sup>9</sup>

In native cartilage, the pericellular matrix (PCM) has been suggested to serve as a mechanotransducer. Specific mechanical properties of PCM compared to that of ECM, and also interaction of type VI collagen present in PCM with cell integrins have important roles in the transmission of the loads to the cells.<sup>4,10–13</sup> In cartilage TE, the isolation of chondrocytes from their surrounding matrix may disrupt the cell-PCM interface altering the cell's physical microenvironment.<sup>14</sup> It is unknown how the change or disruption of PCM and/or the attachment of the cells to the surrounding matrix may influence the micromechanical environment of the chondrocytes upon cyclic compression before the PCM is formed. It is known, however, that integrin attachment

during the first days of culture has an effect on the transduction of mechanical stimulation.<sup>15</sup>

Further insights into the correlation between externally applied loading, cell attachment, PCM development, and the micromechanical environment of chondrocytes may help to characterize the optimal loading regimes in cartilage tissue engineering strategies. However, to experimentally quantify the mechanical parameters in the microenvironment of the cells during culture under loading conditions is challenging. Quantification by means of computational modeling can provide valuable insight, which may help to refine or enhance the interpretation of experimental results.

The aim of the present study is therefore to explore the influence of tissue development and cell-matrix attachment on the modulation of mechanical stimulation applied to the construct to that experienced by a chondrocyte embedded in agarose hydrogel. We hypothesize that the transfer of applied load at the level of a tissue engineering construct to the chondrocytes substantially depends on cell attachment and on the presence and development of PCM.

## Materials and Methods

### General approach

Using a multiscale modeling approach, in which the boundary conditions of a microscale model are obtained from the solution of a macroscale (millimeter-scale) model,<sup>16</sup> we simulated chondrocyte-seeded cartilage TE constructs under unconfined compression loading. The macroscopic mechanical conditions within TE constructs were compared to those in mature cartilage explants under the same loading condition. At the microscale cellular level, first, mechanical conditions in chondrocyte-seeded 2% agarose constructs were simulated to represent the initial stage of tissue engineering when there is a lack of PCM around chondrocytes. To study the influence of the presence or lack of cell attachment to agarose, for instance, as a result of RGD-peptide functionalization,<sup>17</sup> unbound (frictionless) versus focal adhesion and continuously bound contact conditions between the chondrocytes and their surrounding gel were examined. Then, the influence of the development of immature and mature PCM around chondrocytes embedded in 2% agarose gel was studied. The micromechanical environment of a native chondron (chondrocyte-PCM inclu-

sion) embedded in various depths (superficial layer, middle zone, and deep zone) of native cartilage ECM was explored and compared with the tissue engineering case. For all cases the calculated mechanical strain fields were compared, considering the potential role of strain as a sensitive mechanical variable that may be involved in cellular signal transduction.<sup>16</sup>

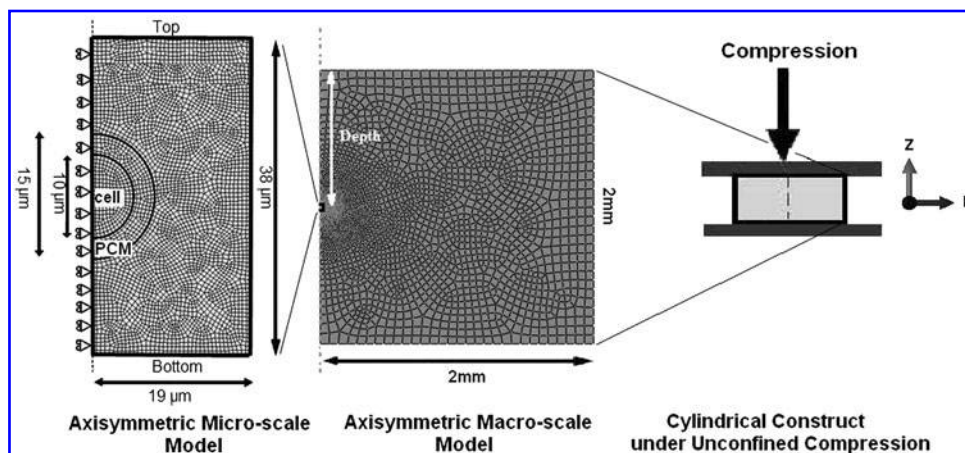
### Macroscale finite element mesh

A macroscale axisymmetric finite element model was created in Abaqus v6.11 (Dassault Systemes, Providence, RI) to either represent a cylindrical 2% agarose TE construct (diameter = 4 mm; height = 2 mm) similar to the dimensions often used in tissue engineering studies,<sup>18,19</sup> or a cartilage explant with native ECM content and distribution (Fig. 1). In the modeled TE construct, a single chondrocyte with its surrounding was modeled at the center of the macroscale model. When considering the native cartilage, the location of the cell was considered to be at the superficial layer (top 5% tissue depth), middle zone (50% tissue depth), or deep zone (90% tissue depth) of the explant.

Simulations consisted of a free-swelling equilibrium step (2000 s) followed by 10% unconfined compression, applied in 0.5 s to simulate maximum loading during a 1 Hz dynamic compression regime. Compression was applied by an impermeable plate, connected to the top surface by frictionless contact. The displacements of the nodes at the symmetry axis were confined in radial direction. Bottom nodes were confined in vertical direction. The nodes on the sample lateral edge were prescribed to zero pore pressure to simulate free fluid flow.

### Microscale finite element mesh

A microscale axisymmetric finite element model of a single chondrocyte with its surrounding was modeled (Fig. 1) (radius = 19  $\mu\text{m}$ ; height = 38  $\mu\text{m}$ ) similar to the dimensions used previously.<sup>16</sup> Chondrocyte diameter and PCM outer diameter were 10 and 15  $\mu\text{m}$ , respectively. The influence of the cell size was studied by varying the cell diameter to 5 and 15  $\mu\text{m}$ , while the thickness of PCM was remained constant. Focal adhesions were modeled as local tie constraints between particular nodes at the cell surface and the surrounding matrix. Adhesion points were assumed



**FIG. 1.** Axisymmetric microscale (left) and macroscale (middle) finite element mesh of a cylindrical construct (right) under 10% unconfined compression.

to be evenly distributed across the surface of the cell. The number of the focal adhesion was arbitrary chosen to be 6 and 11 across one line on the membrane along half the cell surface in an axisymmetric configuration.

To prescribe the boundary conditions of the microscale model, Abaqus v6.11 submodeling was used to prescribe the displacement and pore fluid pressure fields at the boundary of the microscale model based on the solution of the macroscale model.

### Material model

Simulations were performed with the distinct material properties for each component prescribed in an Abaqus User Material subroutine. Agarose gel was modeled as a biphasic, compressible Neo-Hookean material with initial Young's modulus of 15 kPa and Poisson's ratio of 0.12.<sup>18,19</sup> Native cartilage was modeled using a composition-based fibril-reinforced poro-elastic swelling model, which consisted of a fluid phase and a porous solid matrix with swelling properties. The porous matrix of the biphasic tissue consisted of a swelling nonfibrillar ground substance that contains proteoglycans (PGs), and a fibrillar part representing the collagen network.<sup>20-22</sup>

The governing stress equation was<sup>21</sup>

$$\boldsymbol{\sigma}_{tot} = -\mu^f \mathbf{I} + n_{s,0} \left( \left( 1 - \left( \sum_{i=1}^{totf} n_{col}^i \right) \right) \boldsymbol{\sigma}_{nonf} + \sum_{i=1}^{totf} n_{col}^i \sigma_{col}^i \right) - \Delta\pi \mathbf{I} \quad (1)$$

Where  $\mu^f$  was the water chemical potential,  $\mathbf{I}$  the unit tensor,  $\Delta\pi$  the osmotic pressure gradient,  $n_{s,0}$  the initial solid volume (in the unloaded and nonswollen state),  $\boldsymbol{\sigma}_{nonf}$  the stress in the nonfibrillar ground substance,  $\sigma_{col}^i$  the fibril stress in the  $i$ th fibril direction,  $\rho_c^i$  the volume fraction of the non-linear, viscoelastic collagen fibrils with respect to the total solid volume,  $i$  denoted the number of the fibril com-

partment, and  $totf$  the total number of the fibrils. Depth-dependent composition and mechanical description of the collagen fibers and the nonfibrillar matrix can be found in Wilson *et al.*<sup>22</sup>

The osmotic pressure gradient  $\Delta\pi$  as a result of the fixed negative charge concentration in GAGs was given by<sup>23</sup>

$$\Delta\pi = \phi_{int} RT \sqrt{c_{F,exf}^2 + 4 \frac{\gamma_{ext}^{\pm 2}}{\gamma_{int}^{\pm 2}} c_{ext}^2} - 2\phi_{ext} RT c_{ext} \quad (2)$$

The external salt concentration ( $c_{ext}$ ) was 0.15 M, the temperature was ( $T$ ) 310 K, and the gas constant ( $R$ ) was 8.3145 N·m/mmol. Effective fixed charge density  $c_{F,exf}$  based on the extrafibrillar fluid fraction was given by

$$c_{F,exf} = \frac{n_f c_F}{n_{exf}} \quad (3)$$

with  $n_f$  the total fluid fraction,  $n_{exf}$  the extrafibrillar fluid fraction, and  $c_F$  the normal fixed charge density in mEq per mL total fluid. Fixed charge density  $c_F$  was expressed as a function of the tissue deformation, as

$$c_F = c_{F,0} \frac{n_{f,0}}{n_{f,0} - 1 + J} \quad (4)$$

Where  $n_{f,0}$  was the initial fluid fraction and  $c_{F,0}$  was the initial fixed charge density. Further details about determining the extrafibrillar fluid fraction, osmotic ( $\phi_z$ ), and activity coefficients ( $\gamma_z$ ) can be found in Wilson *et al.*<sup>22</sup>

Input parameters of the macroscale model were summarized in Table 1.

At the microscale, PCM was considered to have fluid fraction of 0.85, fixed charge density of 0.2 (mEq/mL), a total compressive Young's modulus of 0.039 MPa, Poisson's ratio of 0.15, and permeability of  $1.9 \times 10^{-15}$  m<sup>4</sup>/N·s.<sup>24</sup> The collagen fibril stiffness of the PCM was  $\sim 10\%$

TABLE 1. INPUT PARAMETERS OF THE MACROSCALE MODEL FOR A 2% AGAROSE CONSTRUCT AND A NATIVE CARTILAGE EXPLANT

Parameter	Description	Value
<i>Agarose construct</i>		
$n_{s,0}$	Initial solid volume fraction	2%
$E_{agr}^*$	Young's modulus of 2% agarose gel	0.015 (MPa)
$K_{agr,0}$	Initial permeability	$2.65 \times 10^{-12}$ m <sup>4</sup> /N·s
<i>Native cartilage</i>		
$n_{f,0}$	Initial fluid volume fraction	0.9–0.2 ( $Z^\#$ )
$n_{s,0}$	Initial solid volume fraction	$1 - n_{f,0}$
$C_{F0}$	Initial fixed charge density	$0.1 (-Z^\#)^2 - 0.24 (Z^\# + 0.035)$
$n_{col}$	Depth-dependent collagen fraction	$1.4(Z^\#)^2 - 1.1 (Z^\# + 0.59)$
$G_m^*$	Shear modulus of PG matrix	0.903 (MPa)
$K_{ECM,0}$	Initial permeability	$1.767 \times 10^{-17}$ m <sup>4</sup> /N·s
$E_J^*$	Collagen material constant	4.316 (MPa)
$K_J^*$	Collagen material constant	16.85
$E_2^*$	Collagen material constant	19.97 (MPa)
$K_2^*$	Collagen material constant	41.49
$\eta^*$	Collagen viscoelasticity dashpot constant	$1.424 \times 10^5$ (MPa·s)

\*Mathematical equations for these parameters can be found in Khoshgoftar *et al.*<sup>20-22</sup> and Wilson *et al.*<sup>20-22</sup>

<sup>#</sup>Z was the normalized depth of the sample (0 at the articular surface and 1 at the bottom of the sample).

ECM, extracellular matrix; PGs, proteoglycans.

TABLE 2. INPUT PARAMETERS OF THE MICROSCALE MODEL FOR PERICELLULAR MATRIX AND CHONDROCYTE<sup>24-26</sup>

Parameter	Description	Value
<b>PCM</b>		
$n_{f,0}$	Initial fluid volume fraction	0.85
$C_{F0}$	Initial fixed charge density	0.2 (mEq/mL)
$E$	Young's modulus	0.039 (MPa)
$\nu$	Poisson's ratio	0.15
$K$	Permeability	$1.9 \times 10^{-15} \text{ m}^4/\text{N}\cdot\text{s}$
$E1^*$	Collagen material constant	0.4316 (MPa)
$K_1^*$	Collagen material constant	16.85
$E_2^*$	Collagen material constant	1.997 (MPa)
$K_2^*$	Collagen material constant	41.49
$\eta^*$	Collagen viscoelasticity dashpot constant	$1.424 \times 10^5 \text{ (MPa}\cdot\text{s)}$
<b>Chondrocyte</b>		
$n_{f,0}$	Initial fluid volume fraction	0.83
$E$	Young's modulus	0.001 (MPa)
$\nu$	Poisson's ratio	0.4
$K$	Permeability	$1 \times 10^{-12} \text{ m}^4/\text{N}\cdot\text{s}$

\*Mathematical equations for these parameters can be found in Khoshgoftar *et al.*<sup>2,9</sup> and Wilson *et al.*<sup>20-22</sup>  
PCM, pericellular matrix.

(mainly type VI collagen) of that of the ECM (mainly type II collagen).<sup>24</sup> The chondrocyte was modeled as a homogeneous biphasic material with Young's modulus of 0.001 MPa, Poisson's ratio of 0.4, solid fraction of 0.17, and permeability of  $1 \times 10^{-12} \text{ m}^4/\text{N}\cdot\text{s}$ .<sup>25,26</sup>

To study the influence of the gradual development of PCM during culture in TE constructs, the matrix content within PCM was varied from 0.25 to 0.5, 0.75, and 1.0 of that in the PCM in native cartilage. The increase in the PCM matrix content was implemented by replacing PCM fluid content with the desired solid matrix content. Fixed-charge density of the PCM was also varied subsequently for each studied case.

Input parameters of the microscale model were summarized in Table 2.

**Results**

At the macroscopic level, in the cartilage explants under 10% compression, internal osmotic swelling pressure was 0.007 MPa in the superficial layer increasing to 0.31 MPa in the deep zone (Fig. 2a). This corresponded to the higher content of fixed charged density (0.175 mEq/mL) in the deep zone compared with that in the superficial layer (0.035 mEq/mL) (Fig. 2b). Similarly, an inhomogeneous strain field emerged (Fig. 3a) where the compressive strain reduced from 17% in the superficial zone to 4% in the deep zone. The tensile and shear strain profiles were also depth dependent, with largest strains in the middle zone (6%) or in the periphery (4.5%), respectively. In the TE construct, without matrix, the osmotic pressure was zero and the strain fields were homogeneous, reaching values for the compressive, tensile, and shear strains of 11%, 5.4%, and 0.53%, respectively (Fig. 3b).

At the microscale, in the initial stage without PCM, the strain profile depended on chondrocyte attachment (Fig. 4). Without attachment, a gradient of compressive and tensile strains from the membrane toward the center of the cell was observed with the highest strains at the center (33% and 15%, respectively). When the cell attached to the matrix, the intracellular strain field became more homogenous with an increasing number of focal adhesion points. Continuous attachment resulted in homogeneous compressive (23%), tensile (12%), and shear (1%) strains inside the cell.

Maturation of the PCM matrix content from 0.25 to 0.5, 0.75, and 1.0 that in native PCM led to the transmission of more compressive strain and less perpendicular tensile strain (Fig. 5a). Cell diameter did not influence the intracellular strain magnitudes (Fig. 5b).

In native cartilage, strain magnitudes in superficial cells were distinct from those in middle- and deep-zone cells (Fig. 6). Superficial cells experienced more lateral tensile strain (16% vs. 8%) and less compressive strain (20% vs. 35%).

Comparison between TE constructs (Fig. 5a) and native cartilage (Fig. 6) revealed that the compressive strain that was induced in cells in TE constructs resembled those in the superficial cells of the mature cartilage (26%), but was distinct from those in cells located in the deep and middle cartilage zones where it reached 35%. Peak tensile strain at the cell poles was in the same range (13%) in TE construct and in native cartilage. However, the center of the chondrocyte experienced tensile strain of 16% in the TE

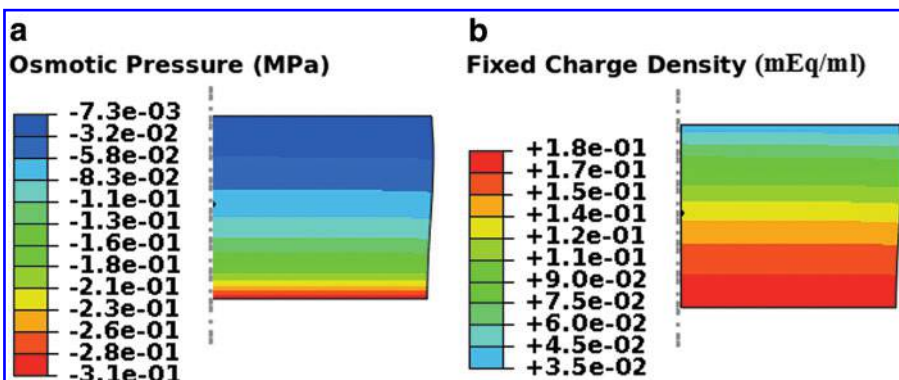
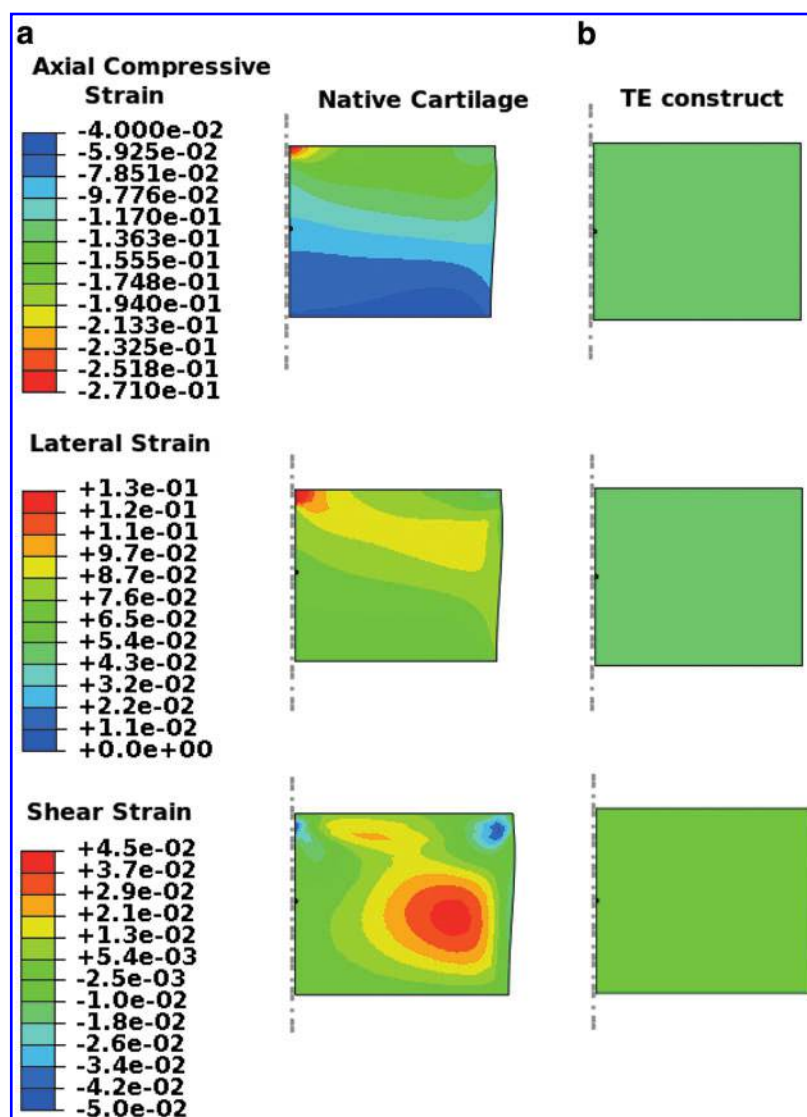


FIG. 2. Osmotic swelling pressure (a) and depth-dependent fixed charge density (b) in native cartilage explants under 10% unconfined compression. Note that the deformed geometry of the explant is shown. Color images available online at [www.liebertpub.com/tea](http://www.liebertpub.com/tea)

**FIG. 3.** Inhomogeneous compressive, tensile, and shear strains in a native cartilage explant (a) and a tissue-engineered (TE) construct (b) under 10% unconfined compression. Color images available online at [www.liebertpub.com/tea](http://www.liebertpub.com/tea)



construct, which was three times higher than that experienced by a chondrocyte embedded in native ECM in mature cartilage. The peak strains and their location calculated for the studied cases were summarized in Table 3.

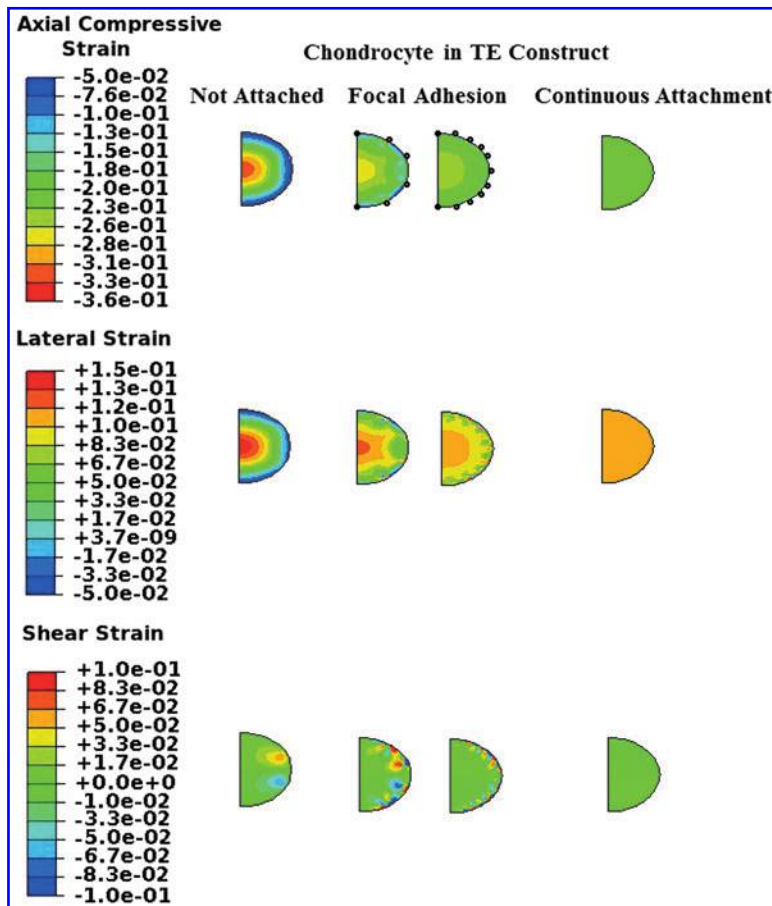
### Discussion

Optimizing the mechanical stimulation regime to enhance chondrocyte metabolism during cartilage tissue engineering requires insight into the transfer of externally applied load to the chondrocytes at both the macroscopic and microscopic scales. This study shows that at the macroscopic scale the absence of an osmotic pressure gradient in TE constructs causes significant differences between cartilage and TE constructs. Consequently, chondrocytes are exposed to different mechanical conditions depending on their position in cartilage, but they will be exposed to the same mechanical environment after isolation and seeding in TE constructs. This may have implications for their response. At the microscopic scale, our model suggests that cell attachment plays an important role in the distribution of strains in the

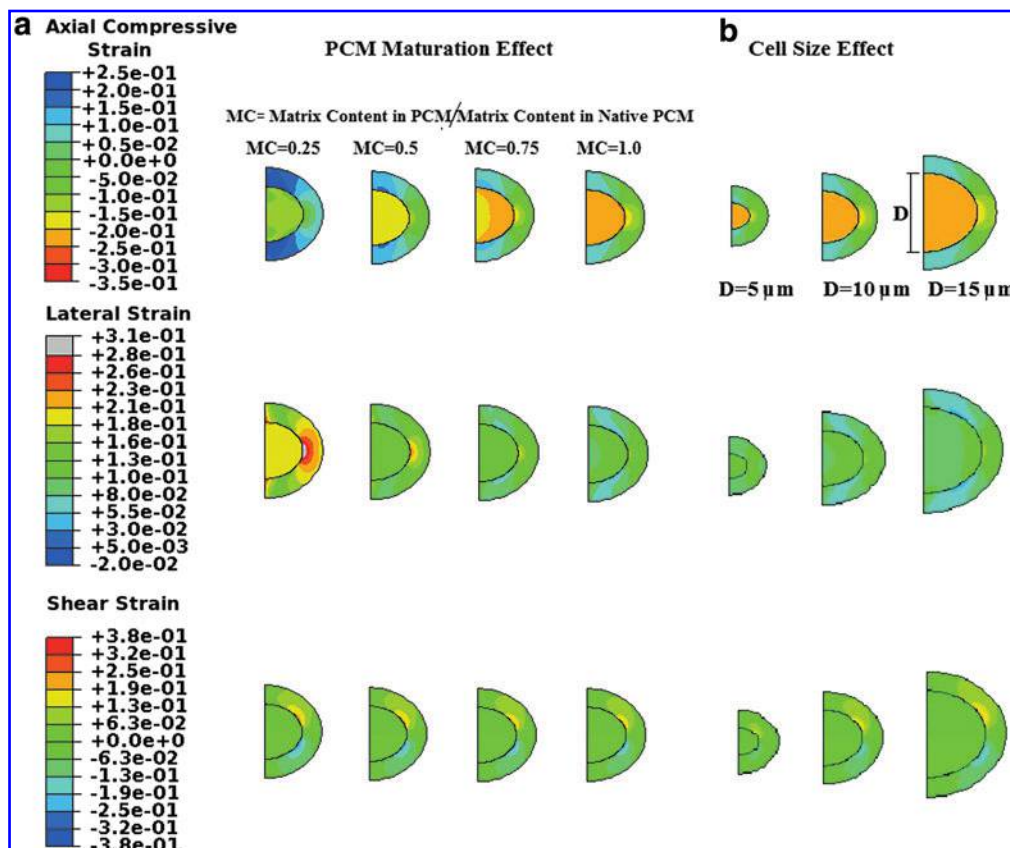
cell. Initially, when no PCM is formed, the transmission of the construct-level applied strain to the chondrocytes is profoundly dependent on whether or not the chondrocyte is attached to the surrounding gel. Lack of cell-matrix attachment may induce excessive distortional shear strains in the cell. Finally, this study suggests that the presence of PCM in TE constructs is insufficient to generate strain pattern similar to those in normal cartilage, although cell attachment and the formation of PCM change the strain field into the right direction.

Shear has been shown to elevate expression of interleukin-6, a marker typical of osteoarthritis cartilage.<sup>27</sup> The present study predicts that shear strains are larger in cells that do not attach to the matrix. It would be interesting to explore experimentally whether indeed interleukin-6 levels rise if cultures are loaded immediately after cell seeding. This may then partly explain why loading during TE should be started few days after cell seeding, in order to have an anabolic effect.<sup>6,28</sup>

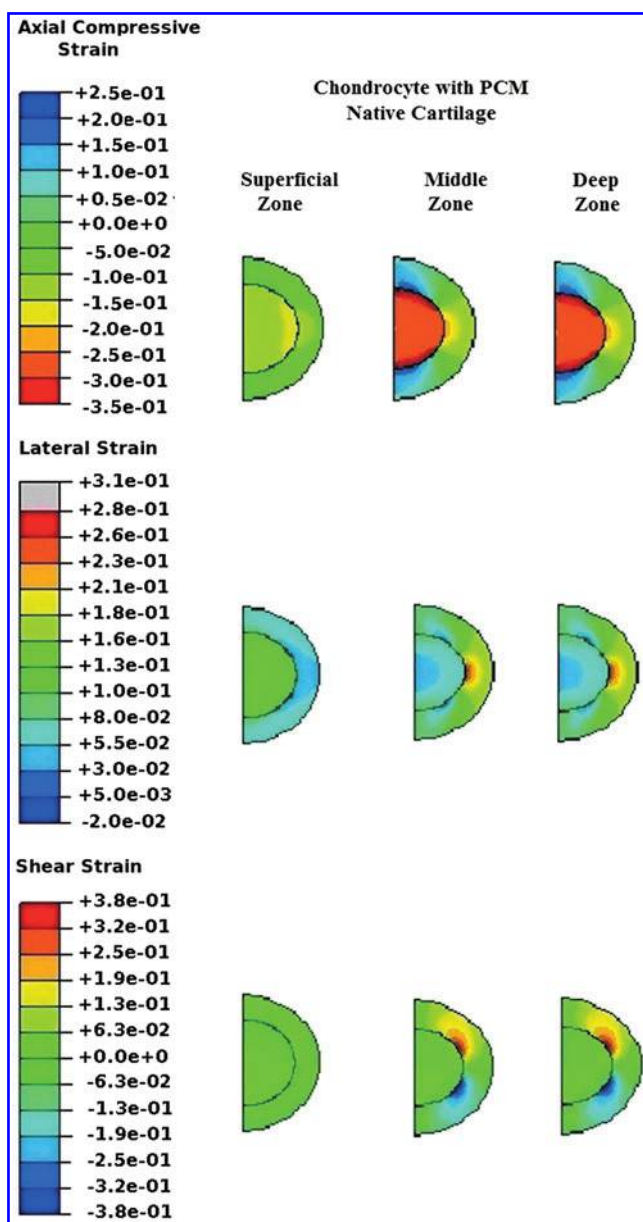
Numerical predictions further indicate that with PCM maturation, the externally applied compression is more



**FIG. 4.** Compressive, tensile, and shear strains induced on chondrocyte embedded in a TE construct without cell-matrix attachment, with focal adhesions or continuously bound contact condition. Color images available online at [www.liebertpub.com/tea](http://www.liebertpub.com/tea)



**FIG. 5.** The influence of pericellular matrix (PCM) maturation (a) and cell size (b) on compressive, tensile, and shear strains induced on chondrocyte embedded in a TE construct. Color images available online at [www.liebertpub.com/tea](http://www.liebertpub.com/tea)



**FIG. 6.** Comparison between compressive, tensile, and shear strains induced on chondrocytes embedded in the superficial, middle, and deep zones of native cartilage. Color images available online at [www.liebertpub.com/tea](http://www.liebertpub.com/tea)

effectively transmitted to the cell (i.e., strain magnification), while at the same time, PCM protects the cell from excessive tensile strains in the direction perpendicular to the loading direction. These effects are independent of cell size.

In native cartilage, magnification of the compressive strain occurs in cells located at the middle and deep zones. Interestingly, strains induced in cells in TE constructs are more comparable to those in superficial-zone cells of the native cartilage where less strain magnification occurs. This may explain why after the first 2–3 weeks of culture, the mechanical loading becomes less effective. This suggests that over time, larger loading magnitude may be required to continue stimulation of chondrocytes.

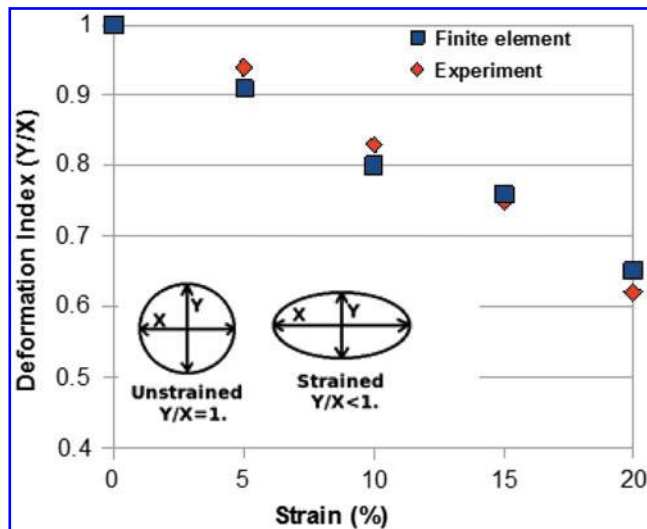
The herein predictions on cell deformations in agarose-chondrocyte constructs concur with those in experimental measurements.<sup>28,29</sup> Cell deformation indexes, defined as the size of the cell in the direction of compression divided by the perpendicular length, compare well when constructs are subjected to 5–20% strain (Fig. 7). Further, a recent study by De Vries *et al.*<sup>28</sup> showed that during culture, PCM becomes protective for cell deformation, so the cell deformation in the direction perpendicular to the loading direction becomes less if the matrix around the cell becomes more mature. This is consistent with our finding (Fig. 5a) where we showed that PCM maturation results in a reduction in the lateral deformation of the cell under axial compressive loading. The deformation index at the presence of PCM was predicted to be in the range of 0.94–0.97 (depending on the PCM maturation) which is in agreement with the experimental findings.<sup>28</sup> Besides these agreements, it is also worth mentioning that the constitutive material model for native ECM has previously been validated and used for studies at both the macroscopic tissue<sup>21,22,30</sup> and the microscopic cell level.<sup>24,31–36</sup>

The different macroscopic and microscopic depth-dependent strain fields between native cartilage and TE constructs may have implications for the mechano-response of the chondrocytes to mechanical stimulation. Cells may have adapted over time to particular *in vivo* environments, which may or may not resemble those that are exposed to in TE constructs, depending on the location where they end up after seeding. This agrees with the findings that superficial and deeper chondrocytes respond differently to the same tissue engineering conditions.<sup>37,38</sup> Two ways to induce a depth-dependent environment to the cells in culture are by varying the stiffness of the scaffold in a layer-wise,<sup>39,40</sup> or by changing the loading regime to, for instance, sliding indentation.<sup>1,41</sup> Future modeling or experimental studies

**TABLE 3.** PEAK COMPRESSIVE, TENSILE, AND SHEAR STRAINS CALCULATED FOR THE STUDIED MICROSCALE CASES

Surrounding matrix	Cell-matrix attachment	Compressive strain	Tensile strain	Shear strain
Agarose	No	33% at cell center 5% at cell periphery	15% at cell center	6% at cell periphery
Agarose	Yes	23% homogeneous	12% homogeneous	1% homogeneous
PCM + agarose	Yes	26% homogeneous	16% at cell center 13% at cell poles	1% homogeneous
PCM + ECM	Yes	26% at cell center 35% at cell poles	5.5% at cell center 13% at cell poles	1% homogeneous





**FIG. 7.** Comparison between model predictions for cell deformation index in 5–20% externally applied strain against experimental measurements of Lee and Bader.<sup>29</sup> Color images available online at [www.liebertpub.com/tea](http://www.liebertpub.com/tea)

may further elucidate effects of cell-matrix attachments or PCM development under such conditions.

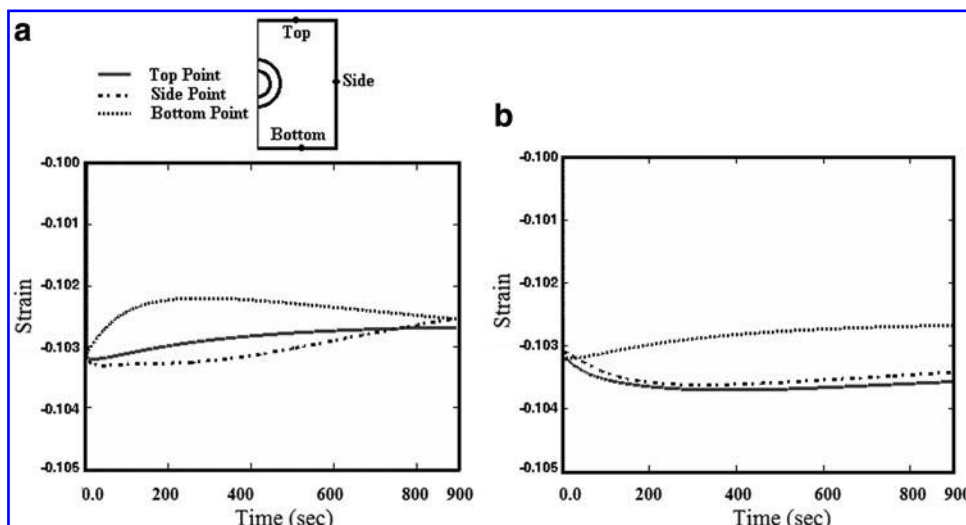
The importance of chondrocyte attachment for mechanotransduction initially during cartilage tissue engineering cultures agrees with a study in which 1 Hz dynamic loading in the first days of culture significantly enhanced the synthesis of ECM in the long term, compared with samples in which integrin binding sites were blocked.<sup>15</sup> The difference in strain magnitude and distribution in and around the chondrocytes as shown by the present study may provide an explanation for these experimental results. Interestingly, the same study showed that a loading frequency of 0.33 Hz did not increase PG synthesis, regardless of integrin blocking. The present simulations show that the peak strain transmitted from the macroscale model to the boundaries of the microscale model is insensitive to the applied frequency (Fig. 8). Thus, the observed difference in response to

loading frequency and cell-matrix attachment may not be due to the global strain magnitude the cells are exposed to, but may have another cause.

One of the limitations in this and other<sup>26,36</sup> modeling studies is that the chondrocyte is idealized as a homogeneous continuum. Subcellular components, such as the nucleus and cytoskeletal fibers, modulate the intracellular strain field. Therefore, the presented intracellular strain fields should not be interpreted as the actual strains, but should rather be considered to indicate that the intracellular strains may be significantly different, depending on variation in ECM and cell attachment. Models incorporating subcellular components, such as the nucleus and cytoskeletal fibers, may provide further insights in intracellular mechanotransduction mechanisms.<sup>42</sup> For such models, the assumption of continuous attachment between chondrocytes and their environment should also be updated to discrete focal cell-matrix attachment sites,<sup>43</sup> to not only include focal adhesion points with the ECM, but also to use these sites for attachment of the actin cytoskeleton.<sup>44</sup> This study presents a first step toward a model that addresses the simulation of focal adhesions at the cell surface and their influence in the transmission of external mechanical strains to the cells in tissue engineering applications.

Compared to the current state of the art,<sup>45,46</sup> a distinct contribution of the present study is the implementation of an advanced and validated material model in a multiscale large deformation framework. In this material model, influences of the fiber reinforcement by the nonlinear viscoelastic collagen network and swelling effects induced by the PG matrix in ECM and PCM have been accounted for. Adaptation of such a detailed material description for cartilage matrix allows more realistic predictions of the micromechanical environment of the cells in native and TE cartilage.

In conclusion, this study shows that the transfer of applied load at the level of a tissue engineering construct to the chondrocytes strongly depends on cell attachment and on the presence of PCM. Thus, these microscopic phenomena should be taken into consideration when developing mechanical loading protocols for cartilage tissue engineering.



**FIG. 8.** Variations in peak compressive strain transmitted from the macroscale model to the boundaries of the microscale model [on arbitrary selected nodes at top, lateral side, and bottom of the microscale model, as illustrated (a)] under 15-min dynamic unconfined compression of 10%, with frequency of 1 Hz (a) and 0.33 Hz (b).

## Disclosure Statement

No competing financial interests exist.

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