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The Local Matrix Distribution and the Functional Development of Tissue Engineered Cartilage, a Finite Element Study

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Abstract—Assessment of the functionality of tissue engineered cartilage constructs is hampered by the lack of correlation between global measurements of extra cellular matrix constituents and the global mechanical properties. Based on patterns of matrix deposition around individual cells, it has been hypothesized previously, that mechanical functionality arises when contact occurs between zones of matrix associated with individual cells. The objective of this study is to determine whether the local distribution of newly synthesized extracellular matrix components contributes to the evolution of the mechanical properties of tissue engineered cartilage constructs. A computational homogenization approach was adopted, based on the concept of a periodic representative volume element. Local transport and immobilization of newly synthesized matrix components were described. Mechanical properties were taken dependent on the local matrix concentration and subsequently the global aggregate modulus and hydraulic permeability were derived. The transport parameters were varied to assess the effect of the evolving matrix distribution during culture. The results indicate that the overall stiffness and permeability are to a large extent insensitive to differences in local matrix distribution. This emphasizes the need for caution in the visual interpretation of tissue functionality from histology and underlines the importance of complementary measurements of the matrix's intrinsic molecular organization.

Keywords—Cartilage, Tissue Engineering, Extracellular Matrix, Homogenization, Mechanical Properties.

INTRODUCTION

Articular cartilage has only a limited capacity for repair after damage. Current therapies aimed at aiding the natural repair process yield inconsistent, often unsatisfactory long-term results.³⁰ Tissue engineering of cartilage can be used to study the process of cartilage formation in a controlled in vitro environment and can potentially provide transplant material for cartilage reconstruction.¹⁸ A prerequisite for clinical use is that a cultured cartilage construct meets, or has the capacity to adapt to, *in-vivo* functional

requirements.⁸ The construct's mechanical properties can be modulated by adjusting the parameters that define the culture environment, such as scaffold material,²⁵ hydrodynamic conditions,^{36,59} mechanical loading,^{5,37,39} addition of growthfactors,^{37,44} cell seeding density and nutrition.⁴⁰ Although it has been shown that mechanical properties which approach those of natural cartilage can be obtained after long term culture,¹⁷ there is still a lack of control over the functional development of tissue engineered constructs.

The general concept is that natural cartilage derives its resistance to mechanical loading from a fibrous network consisting mainly of collagen type II, which is prestressed due to the osmotic or electrostatic swelling pressure exerted by a high concentration of negatively charged glycosaminoglycans (GAG's), immobilized in the form of large proteoglycan (PG) aggregates.^{1,6,24,41} Based on this relation between the molecular composition and function of natural cartilage, the most frequently measured quantities in tissue engineering experiments are the collagen and GAG content. However, even though the presence of these molecular components is prerequisite, their amount does not fully determine the construct's global mechanical properties. Besides collagen and PG's, cartilage contains also noncollagenous proteins such as for example link protein which play a role in the functional assembly of the extracellular matrix.^{24,41} Significant differences in the Poisson's ratio have been found between calf and adult bovine cartilage, which had equal collagen content. This discrepancy could be attributed to the difference in crosslink density, which determines to a large extent the functionality of the collagen network.⁶³ Moreover natural cartilage displays a highly organized zonal structure with different molecular composition, fibril diameter and organization. The anisotropic collagen fiber orientation ranges from perpendicular at the bone interface to parallel to the cartilage surface near the joint cavity. Within these zones a structural distinction can be made between a thin layer of pericellular matrix surrounding the cells, the territorial matrix farther away and the interterritorial matrix, which connects the regions of cell associated matrix.²⁴

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In cartilage tissue engineering the extracellular matrix (ECM) formed by chondrocytes within an artificial scaffold is initially less organized. Newly synthesized matrix molecules like procollagen and PG's are secreted by the cells and transported away while undergoing an assembly process to form fibers and large immobilized molecular aggregates.^{15,41,47,51,56} In addition the thus formed extracellular matrix is also subject to physical, chemical and chondrocyte mediated degradation, leading to a constant matrix remodeling.^{10,50,53} In the course of time the construct's collagen and proteoglycan content can approach a steady state,¹⁶ resulting from a balance between synthesis, incorporation and degradation and/or product inhibited synthesis.^{23,43,62}

The relation between the content of extracellular matrix constituents and biomechanical function has been investigated in various studies. For mature natural cartilage the aggregate modulus has been correlated to GAG content primarily^{12,41,58} and to both GAG and collagen density.⁴⁹ For the hydraulic permeability an inverse relationship with fixed charge density, i.e. PG content, has been demonstrated,³⁴ on the other hand also no significant dependence has been found.⁴⁹ For developing articular cartilage, relationships for the aggregate modulus and permeability as a function of GAG and collagen concentration have been established.⁶¹ It was concluded that changes in biomechanical properties during development were primarily associated with the increase in collagen content. In tissue engineering studies the aggregate -and Young's moduli and permeability of chondrocyte seeded constructs have been correlated with the amount of GAG, collagen and water present.^{9,38,59} A strong dependence of the stiffness on either collagen³⁸ or GAG was observed.^{9,59}

Although the relations between tissue averaged ECM content and mechanical properties are highly instructive, the introduction of additional parameters is necessary to account for the variability in regression slope and large amount of scatter in the data. Besides difference in the molecular organization such as crosslinking, part of this variability may arise from the fact that only tissue averaged quantities were considered, while clear gradients in matrix deposition can occur between the construct's periphery and center.³⁵ Another factor, which has received relatively little attention, is that also the local, cell scale, matrix distribution potentially plays an important role. Patterns of matrix deposition around individual cells in agarose have been quantified and observed to spread outwards in time.^{5,37,47} It has been hypothesized that mechanical functionality arises when contact occurs between zones of matrix associated with individual cells.^{5,37,40,47} In addition it has been speculated that one of the mechanisms by which dynamic loading could contribute to improved mechanical functionality is the dispersion of matrix constituents by compression induced fluid flow, leading to a local matrix redistribution.^{37,46}

The main question is whether assessment of tissue functionality from the main ECM constituents requires in the first place additional information about the local matrix distribution from histology, or complementary global measurements of molecular organization. Therefore the objective of this study is to determine whether the local distribution of newly synthesized extracellular matrix contributes to the evolution of the global mechanical properties of tissue engineered cartilage constructs, in particular the aggregate modulus H_a and hydraulic permeability K . More specifically, if mechanical functionality arises as a result of contact between zones of cell associated matrix, a relatively sudden increase in stiffness at a certain point in time during culture is to be expected. Also, if mechanically induced matrix redistribution can improve tissue functionality, the same amount of matrix with the same properties, only distributed more homogeneously, should give rise to a significant improvement of the mechanical properties. To answer these questions a computational approach is particularly useful, since it enables to distinguish matrix distribution from other important factors that affect functionality, such as for example in the case of dynamic compression, an increase in the amount of matrix synthesized and the production of more link molecules.^{7,14,32,37}

METHODS

A finite element homogenization approach based on the concept of a periodic representative volume element (RVE) was adopted.^{2,29,55} The global material behavior can be captured by modeling only a limited, but sufficiently large part of an inhomogeneous microstructure, in which the microstructural components can be considered as a continuum. Local cellular ECM synthesis and passive transport were modeled. The local stiffness and permeability were adjusted according to the amount of ECM present. Subsequently the averaged, global, material properties H_a and K were derived. The transport parameters were varied to assess the effect of the evolving matrix distribution during culture.

The RVE geometry is based on the case of 2% agarose seeded with 10 million cells cm^{-3} .³⁷ To allow for representative cellular structures, a two dimensional cross sectional area of 200 μm squared is considered. Assuming the cells are stacked in the in-plane dimension, the rounded number of cells in this area is 19 and the interlayer spacing is 46.4 μm . Cells are randomly placed and the cell size is set to 10 μm .^{22,33} An example of an RVE is shown in Fig. 1(a). The mesh was generated using the MATLAB PDE toolbox v1.0.3.

To describe the process of tissue formation at the cellular scale, a model was adopted which was initially proposed by Dimicco to interpret the spatially varying matrix distribution in articular cartilage at the tissue scale.¹⁵ It is assumed that transport of newly synthesized matrix

FIGURE 1. (a) Finite element mesh of a representative volume element (RVE), consisting of randomly distributed cells embedded in a hydrogel, modeled as a porous medium. (b) The homogenized Ha and K were derived for 5 different RVE's in two directions.

components can be described by a process of diffusion, binding and degradation.¹⁵ No distinction is made between collagen and proteoglycans. Only a mobile matrix component c_m and an immobilized, bound, matrix component c_b are distinguished. Transport of the mobile ECM concentration c_m [g cm⁻³] is described by Eq. (1):

$$\frac{\partial c_m}{\partial t} - \vec{\nabla} \cdot (D \vec{\nabla} c_m) = q - k_b c_m, \quad (1)$$

where D [cm² s⁻¹] is the diffusion coefficient, q [g cm⁻³ s⁻¹] the matrix synthesis rate and k_b [s⁻¹] the first order binding constant. k_b Controls the speed at which the newly synthesized matrix components become immobilized and assembled into a functional matrix. The immobilized matrix concentration c_b [g cm⁻³] is described by Eq. (2):

$$\frac{\partial c_b}{\partial t} = k_b c_m - k_d c_b, \quad (2)$$

in which the first term in the right hand side is the matrix assembly term from Eq. (1). The second term accounts for matrix catabolism, where k_d [s⁻¹] is the degradation constant which determines the matrix turnover speed. It was assumed that degraded matrix molecules are no longer able to bind again. In case of proteoglycan aggregates, cleavage by matrix metalloproteinases and aggrecanases results in aggrecan fragments that cannot reattach due to the missing link protein.^{10,15,50}

For the reference binding k_b^{ref} and degradation rate k_d^{ref} the same parameters as in the work of Dimicco¹⁵ were adopted, which were derived based on proteoglycans in cartilage: $k_b^{\text{ref}} = 1.2 \times 10^{-5}$ s⁻¹,^{15,52} $k_d^{\text{ref}} = 3.7 \times 10^{-7}$ s⁻¹.^{15,53} To evaluate the sensitivity, also variations of these parameters were carried out. The diffusion coefficient D of aggregating proteoglycans in solution lies in the order of 10^{-8} – 10^{-9} cm² s⁻¹.^{13,19,20} The reference diffusion coefficient D_{ref} was set to 10^{-10} cm² s⁻¹ since molecular movement will be severely hindered by the gel, given the hydrodynamic radius of a proteoglycan subunit of

54–80 nm¹³ and the estimated pore size of 2% agarose 124–206 nm.⁴⁵ To investigate the influence of matrix distribution D has been varied to $D_{\text{ref}} \times 10$ and $D_{\text{ref}}/10$. The matrix production rate per cell q^{cell} was set to 3×10^{-12} g cell⁻¹ h⁻¹. This value for q^{cell} was derived based on the case of an initial cell seeding density of 10 million cells cm⁻³, free swelling culture in 2% agarose with medium containing 10% serum, where matrix accumulation showed a linear increase in time over 42 days,⁴⁰ the percentage wet weight GAG and collagen were pooled and a construct density of 1 g cm⁻³ was assumed (natural cartilage: 1.15 g cm⁻³).¹⁶ Note that the value for q^{cell} is an underestimate, since it was based on matrix accumulation data in the construct and did not account for matrix molecules lost to the medium.

To evaluate the functional properties resulting from matrix accumulation, the chondrocyte seeded construct was modeled as a porous medium, assuming linear elasticity. Hooke's law in confined compression is given by Eq. (3):

$$\sigma_e = Ha \varepsilon_z, \quad (3)$$

where σ_e [Pa] is the effective stress, Ha [Pa] the aggregate modulus and ε_z [–] the engineering strain. Darcy's law is given by Eq. (4):

$$Q = -K \vec{\nabla} p, \quad (4)$$

where Q [m s⁻¹] is the specific discharge, or apparent fluid flux, K [m⁴ N⁻¹ s⁻¹] the hydraulic permeability and p [Pa] the fluid pressure. It was assumed that only the bound matrix component c_b contributes to the construct's mechanical properties and that the matrix is immediately functional upon immobilization. Based on global, i.e. tissue scale, correlations, expressions of the following form were selected for the local mechanical properties as a function of c_b .^{38,59,61}

$$Ha = Ha_0 + A_{Ha} c_b, \quad (5)$$

where H_{a0} is the scaffold aggregate modulus and A_{Ha} the proportionality constant with c_b .

$$\log_{10}(K) = \log_{10}(K_0) - A_K c_b, \quad (6)$$

where K_0 is the scaffold permeability and A_K the proportionality constant with c_b . Values for A_{Ha} ranging from $2.2 \text{ MPa cm}^3 \text{ g}^{-1}$ to $10 \text{ MPa cm}^3 \text{ g}^{-1}$ can be derived based on global correlation relations^{38,49,59,61} and the GAG to collagen synthesis ratio from the case considered for q , see above.⁴⁰ The lower value of $A_{Ha} = 2.2 \text{ MPa cm}^3 \text{ g}^{-1}$ was used, which was derived for agarose culture,³⁸ reflecting a lower degree of molecular ECM organization than mature tissue. Since the global effective stiffness can underestimate the local stiffness, depending on the local distribution, also $A_{Ha} = 5 \text{ MPa cm}^3 \text{ g}^{-1}$ was considered. In a similar manner $A_K = 19 \text{ cm}^3 \text{ g}^{-1}$ was derived,⁶¹ which was also varied to $A_K = 50 \text{ cm}^3 \text{ g}^{-1}$.

The aggregate modulus Ha of the cell free 2% agarose gel was set to 6 kPa ^{37,39} and the permeability K to $6.61 \times 10^{-13} \text{ m}^4 \text{ N}^{-1} \text{ s}^{-1}$.²¹ For the Poisson's ratio ν of the matrix and scaffold a value of 0.1 was used, based on fetal and calf cartilage.⁶³ The experimental variability in cell properties has only a limited effect on the effective global modulus and permeability.⁶⁴ Therefore the cell properties were kept constant at the following values: $Ha = 1 \text{ kPa}$, $K = 10^{-15} \text{ m}^4 \text{ N}^{-1} \text{ s}^{-1}$ and $\nu = 0.4$.^{22,26}

The finite element package SEPRAN was employed for the numerical implementation.⁵⁴ The transport problem Eq. (1) and (2) was solved for a period of 56 days. Matrix synthesis was prescribed as a line-source on the cell edge, assuming an RVE thickness equal to the in-plane cell stacking distance. No transport within the cells was considered. Periodical boundary conditions were applied for the concentration on the RVE edges. 6-Node triangular elements and Crank-Nicholson time integration with a time step of 8 h were used. For the elasticity problem plane strain, linear elastic, 3-node triangular elements were used. The Darcy problem was solved employing lowest-order Raviart-

Thomas mixed finite elements,^{27,57} for the resulting system of equations an iterative solver (BiCGstab) was used. Coefficients were taken constant per element and adjusted according to the element-averaged bound concentration c_b .

To determine the RVE-averaged aggregate modulus a homogenization technique was adopted, which was developed by Smit in the context of a micro-macro multilevel finite element approach.^{2,29,55} Briefly, periodical boundary conditions for the RVE edge displacements are applied. Because of these periodical boundary conditions the averaged RVE stress and deformation can be described by the displacements and forces in three RVE corner points. The averaged stiffness can then be determined via a variation expression of the averaged RVE stress, which involves the static condensation of the full RVE stiffness matrix in order to determine the relation between corner point force and displacement. The RVE averaged hydraulic permeability in the undeformed state was evaluated using Darcy's law by applying an arbitrary pressure gradient over the RVE and monitoring the resulting total edge flux. Periodical boundary conditions were prescribed for the fluid flux through the RVE edges. To account for different patterns of cell distribution, the averaged aggregate modulus and permeability were determined every two days for 5 different cell distributions in two directions (Fig. 1(b)). Unless explicitly stated otherwise, the reference parameters have been used.

RESULTS

A representative distribution of the bound matrix c_b at $t = 56$ days is depicted in Fig. 2(a)–(c), for the different PG diffusion coefficients considered (D_{ref} , $D_{\text{ref}} \times 10$, $D_{\text{ref}}/10$). Figure 3 shows the resulting global aggregate modulus and permeability as a function of culture time. The standard deviations in Fig. 3, 5 and 6 are due to the averaging over the different microstructures (Fig. 1(b)). The homogeneous matrix distribution (Fig. 2(a)) results in approximately the same global functional properties (Fig. 3)

FIGURE 2. Concentration of immobilized extracellular matrix components, c_b [g cm^{-3}], at $t = 56$ days for different diffusion coefficients, (a); $D_{\text{ref}} \times 10$, (b); D_{ref} , (c); $D_{\text{ref}}/10$. Periodical boundary conditions were applied at the RVE edges. Note that the colorbar limits do not represent the full concentration range.

FIGURE 3. Evolution of the overall aggregate modulus Ha (a) and hydraulic permeability K (b) as a function of time, for different diffusion coefficients. Results were averaged over 5 RVE's in two directions.

as the same amount of matrix distributed more inhomogeneously (Fig. 2(b)). Only for the case with very localized pericellular matrix deposition (Fig. 2(c)), the global aggregate modulus is significantly lower (Fig. 3(a)). In addition the standard deviation increases, which is a measure of the influence of the microstructure. The global permeability however is not affected (Fig. 3(b)). This is an unexpected result since the local flow pattern is clearly altered by zones of more intense matrix accumulation (Fig. 4).

Increasing the influence of the bound matrix concentration on the aggregate modulus and permeability (higher A_{Ha} and A_K) leads to generally the same observations. The global permeability however, shows more variation as a function of matrix distribution (Fig. 5). The effect of varying the binding coefficient k_b and degradation coefficient k_d can be seen in Fig. 6(a)–(b) and (c) respectively. Since the effective transport distance is determined by a combination of diffusion and binding, k_b has been varied to a faster

binding rate of $3.4 \times 10^{-5} \text{ s}^{-1}$, a value corresponding to a lowest half-time of 5.7 h reported for the conversion of low-affinity proteoglycan monomers to a form with a high binding affinity,⁵¹ which has been assumed to be rate limiting.¹⁵ Comparing Fig. 3(a) with Fig. 6(a), the faster binding results in a larger standard deviation for the reference diffusion coefficient and produces a small difference between D_{ref} and $D_{ref} \times 10$. Thus the faster binding rate extends the influence of the microstructure to higher diffusion coefficients, due to more localized matrix deposition. As can be observed in Fig. 6(b), a slower binding rate $k_b^{ref}/10$ results in an initial delay and eliminates any diffusion dependence. Even slower binding increases the delay and reduces the absolute value of Ha reached within the computed time-scale (data not shown). For a slower degradation rate $k_d^{ref}/10$, Ha does not level off as a function of time (Fig. 6(c)). The dependence on the local matrix distribution remains the same however (Figs. 3(a) and 6(c)). In general, differences in averaged

FIGURE 4. Visualization of the fluid flux patterns resulting from microscopic inhomogeneity at $t = 56$ days, (a); $D_{ref} \times 10$, (b); D_{ref} , (c); $D_{ref}/10$. A negative pressure gradient was applied from left to right, the fluid flux was subjected to periodical boundary conditions and deformation was suppressed. Gray values indicate the absolute value of the local Darcy flux. Given values provide only a relative comparison. Depicted streamlines contain equal mass flux. Note that streamlines on the left and right edge do not necessarily coincide.

FIGURE 5. Parameter variation assuming a greater influence of the bound matrix concentration on the local mechanical properties, Eqs. (5) and (6); $A_{Ha} = 5 \text{ MPa cm}^3 \text{ g}^{-1}$, $A_K = 50 \text{ cm}^3 \text{ g}^{-1}$. (a); Global aggregate modulus H_a , (b); Hydraulic permeability K .

FIGURE 6. Parameter variation of matrix assembly and turnover time, Eq. (1) and (2). Global aggregate modulus, (a); $k_b = 3.4 \times 10^{-5} \text{ s}^{-1}$, (b); $k_b = k_b^{ref}/10$, (c); $k_d = k_d^{ref}/10$. Differences in permeability are much less pronounced, data not shown.

permeability between the cases with different diffusion coefficients tend to be less pronounced than differences in the aggregate modulus (data not shown).

DISCUSSION

A computational homogenization approach was adopted to determine to which extent the evolution of the functional properties of tissue engineered cartilage constructs can be attributed to the microscopic distribution of newly synthesized matrix components. To assess the influence of the extracellular matrix distribution, different local distributions were considered from which global mechanical properties were derived.

The computed ECM patterns for the lower diffusion coefficients (D_{ref} , $D_{ref}/10$) were qualitatively similar to experimental distributions of proteoglycans found in agarose culture, with newly synthesized proteoglycans located primarily in the pericellular region (Fig. 2(b) and (c)).^{5,37,47}

The collagen deposition on the other hand tends to be more homogeneous, which correspond with the $D_{ref} \times 10$ case (Fig. 2(a)).^{37,47} Pericellular GAG profiles in agarose, determined by quantitative autoradiography, were of similar shape as those computed in the model.⁴⁷ Moving outwards from the cell, the sharp drop in matrix concentration found experimentally, is in accordance with a diffusion-binding model as employed in this study. Also the experimental finding that the main PG deposition occurred within $10 \mu\text{m}$ from the cell membrane corresponds very well with the reference case in Fig. 2(b), where this distance is determined by diffusion and binding. The time course of matrix formation has been studied in alginate culture, where it has been observed that by 6 weeks multiple islands of matrix have joined together as can be seen in Fig. 2(b) for the model at 56 days.¹¹ At 12 weeks homogeneous GAG staining was observed which was attributed to alginate degradation. Related to this, the importance of matrix diffusivity has been demonstrated by modulating the degradation behavior of

PEG hydrogels.³ It was found that collagen II was either localized in the pericellular region or became distributed throughout the construct as a function of the hydrogel degradation properties.

In general the calculated increase in aggregate modulus H_a and decrease in permeability K are well within the range found for cartilage tissue engineering, considering the major differences in culture conditions.^{5,36–40,59} Based on the distinct matrix distributions in Fig. 2(a) and (b) and the similar resulting mechanical properties in Fig. 3, it can be concluded that the overall stiffness and permeability are to a large extent insensitive to differences in local matrix distribution. From the parameter variations in binding and degradation rate, it appears that the amount of matrix and the intrinsic assembly and turnover time of the matrix components are more important in determining the evolution of the functional properties. Therefore the redistribution of matrix components by dynamic mechanical compression may play only a limited role. Alternatively, for the same ECM content compression may also passively increase tissue properties by altering the functional organization and entanglement of the ECM network. A continuous increase in stiffness with time has been observed in several studies,^{5,11,36} while others have found an initial increase followed by a plateau.^{37,38,40,48} As shown in Fig. 6(c) a continuous increase in stiffness can simply be the result of a lack of degradation. A sudden rise in stiffness due to contact between cell associated matrix zones cannot be observed for the diffusion-binding model, indicating an important role of progressing ECM molecular organization. The results in Fig. 6(b) show that an initial lag time, followed by an increase in stiffness which gradually levels off, can be explained from a binding and degradation process without the need for a distribution related effect. However this does not mean that the diffusion of ECM does not need to be considered for cartilage tissue engineering. As shown in this study, the importance of matrix distribution will depend to a large extent on the diffusive properties of the construct. The $D_{\text{ref}} \times 10$ corresponds in fact to the situation in which the gel is absent, while for $D_{\text{ref}}/10$ the mechanical properties are affected considerably by distribution as the matrix is very localized (Figs. 2(c) and 3(a)).

In general, the model assumption to apply global relations between tissue constituents and stiffness at the local level will underpredict global stiffness, in case the local matrix distribution plays a role. To have an indication of the predictive value of the model, the increase in Young's modulus over 56 days ($\Delta E \sim 40$ kPa) in the case from which the matrix synthesis rate was derived,⁴⁰ was compared with the model predictions, which use a relation based on the correlation between global matrix content and aggregate modulus established in a separate study by the same group.³⁸ Although the predicted values ($\Delta E = 39$ kPa, $\Delta E = 37$ kPa) for $D_{\text{ref}} \times 10$ and D_{ref} corresponded very well for this specific case, no general agreement could be ob-

served for different culture conditions and in the absence of a distribution related effect the model reduces to the global relations.

The primary objective of this study however, was not to give an accurate prediction of the evolution of the mechanical properties of a tissue engineered construct, but to isolate and assess the effect of matrix distribution. Therefore, as a clear reference, simple assumptions were made for the dependence of the functional properties on the matrix concentration and a constant matrix synthesis was assumed, neglecting any cellular regulation mechanisms. A model aiming at predicting the temporal development of the mechanical properties more precisely should include the functional interactions between the separate PG and collagen components and should take into account the time course of the synthesis of specific matrix components, which is affected by factors like mechanical stimulation, growth factors, the amount of matrix present, nutrient availability and cell proliferation.^{5,37,40,43,60} For example, in contrast to the constant matrix synthesis rate assumed here, it has been demonstrated in several studies that GAG synthesis decreased as a function of time,^{5,37} while collagen production can also increase in later stages of culture.³⁷

To provide a clear comparison between the different matrix distribution cases only the initial, linear elastic, stiffness and undeformed permeability were compared. The relation between the local aggregate modulus H_a and the amount of ECM present was assumed to be linear based on global correlations, while for example the proteoglycan swelling pressure as a function of PG concentration and the collagen network tensile behavior are known to be highly nonlinear.^{1,6} A nonlinear increase in local stiffness as a function of ECM concentration would increase the sensitivity to the microscopic matrix distribution. The matrix distribution may also have more effect on the averaged stiffness at lower cell concentrations, since transport distances are longer. At higher cell concentrations the matrix distribution will be more homogeneous. Although the basic mechanisms remain the same, the results may be affected by the fact that only a two-dimensional RVE has been considered. In general, contact between homogeneous cylindrical zones will be stiffer than in case of spheres. In addition the probability of aligned contact is higher. On the other hand, since the same amount of matrix is distributed by diffusion in a cylindrical, respectively spherical manner, the gradients in local stiffness will be sharper for the spherical case.

In the present study it has been assumed that the diffusion coefficients remain constant during the culture period, while in reality the accumulation of ECM components can also progressively decrease the diffusivity,^{4,31,42} leading to a more localized matrix distribution. In addition it has been shown that diffusion of proteoglycans is highly dependent on concentration.¹³ The concentration consists of both mobile newly synthesized matrix molecules as well

as degraded matrix molecules. However, in the model we have, as a first estimate, considered the diffusion of matrix molecules to be independent of concentration (constant D). Nonbinding degraded matrix molecules can therefore be considered as a separate species which does not influence the transport of mobile newly synthesized matrix molecules. For this reason, transport of degraded matrix molecules was not further considered in this study. When such concentration dependency is introduced in the model also the transport of degraded matrix molecules must be included. In agarose culture it has been observed that at high concentrations matrix components not only diffuse through the gel, but that newly formed matrix which is immobilized pericellularly, can also push away the gel material.⁵ To account for this the modeling approach should be extended to include volumetric growth, possibly stress free, or the results of PG swelling, in which case separate PG and collagen components need to be distinguished.^{1,28}

It can be concluded that the evolution of the global permeability and aggregate modulus of tissue engineered cartilage constructs appears to a large extent insensitive to the microscopic matrix distribution. Based on this low sensitivity it is also unlikely that dispersion of matrix components by mechanical compression alone, can significantly enhance tissue properties. It has been shown however that the global stiffness can be significantly reduced in case the microscopic matrix distribution is very localized, which can occur when matrix accumulation poses increasingly severe restrictions on transport. In addition a larger sensitivity to matrix distribution cannot be excluded in case of a non-linear dependence of the local stiffness on matrix constituents. For cartilage tissue engineering the results imply that the diffusive properties of the construct will determine to a large extent whether matrix distribution becomes important. Modeling matrix deposition as a simple diffusion, binding and degradation process does not result in a sudden increase in global stiffness as a result of contact between cell associated matrix, instead the time evolution of the global aggregate modulus is dominated by the matrix's binding and degradation behavior. The present results emphasize the need for caution in the visual interpretation of tissue functionality from histology. Also, since the matrix distribution itself cannot fully explain the lack of correlation between global measurements of collagen and PG content and the mechanical properties of tissue engineered constructs, complementary global measurements of intrinsic matrix properties like crosslink density remain essential.²⁸ Apart from molecular organization and matrix distribution on the microscopic scale, measurements of mechanical properties may be complicated by tissue scale inhomogeneities in matrix distribution, which may be dominating measured mechanical properties.

Microscopic computational models can be used for the optimization of bioreactor culture conditions and protocols, with respect to functionality and culture time, as a func-

tion of the initial cell seeding density. Since, in principle, the time evolution of matrix deposition in relation to tissue functionality can be predicted, also the effectiveness of stimulating matrix synthesis, by growth factors and/or mechanical compression, at different times in culture can be assessed. It is also known that the presence of a pericellular matrix plays an important role in mediating the cell response to mechanical stimulation. On the other hand, the development of a pericellular matrix during culture, will gradually shield cells from externally applied deformation,⁵ possibly reducing the effect of mechanical stimulation protocols. Model predictions of the local cell deformation can be used to adjust the level of global deformation necessary to achieve optimal stimulation. Apart from tissue engineering protocols, model applications lie also in the optimization of, possibly larger pore, scaffolds in relation to matrix deposition. In cartilage explants compression has been shown to introduce direction dependent matrix deposition at the cellular level.⁴⁶ Since effects of compression induced direction dependent diffusion, convective transport and cell alignment may be readily incorporated, the computational approach adopted in the present study may prove especially useful to study and modulate the development of anisotropy in tissue engineered constructs.

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REFERENCES

- ¹Basser, P. J., R. Schneiderman, R. A. Bank, E. Wachtel, and A. Maroudas. Mechanical properties of the collagen network in human articular cartilage as measured by osmotic stress technique. *Arch. Biochem. Biophys.* 351(2):207–219, 1998.
- ²Breuls, R. G. M., B. G. Sengers, C. W. J. Oomens, C. V. C. Bouten, and F. P. T. Baaijens. Predicting local cell deformations in engineered tissue constructs: A multilevel finite element approach. *J. Biomech. Eng.* 124:198–207, 2002.
- ³Bryant, S. J. and K. S. Anseth. Controlling the spatial distribution of ECM components in degradable PEG hydrogels for tissue engineering cartilage. *J. Biomed. Mater. Res.* 64A:70–79, 2003.
- ⁴Bursac, P. M., L. E. Freed, R. J. Biron, and G. Vunjak-Novakovic. Mass transfer studies of tissue engineered cartilage. *Tissue Eng.* 2(2):141–150, 1996.
- ⁵Buschmann, M. D., Y. A. Gluzband, A. J. Grodzinsky, and E. B. Hunziker. Mechanical compression modulates matrix biosynthesis in chondrocyte/agarose culture. *J. Cell Sci.* 108:1497–1508, 1995.
- ⁶Buschmann, M. D. and A. J. Grodzinsky. A molecular model of proteoglycan-associated electrostatic forces in cartilage mechanics. *J. Biomech. Eng.* 117:179–192, 1995.
- ⁷Buschmann, M. D., Y. J. Kim, M. Wong, E. Frank, E. B. Hunziker, and A. J. Grodzinsky. Stimulation of aggrecan synthesis in cartilage explants by cyclic loading is localized to regions of high interstitial fluid flow. *Arch. Biochem. Biophys.* 366(1):1–7, 1999.

- ⁸Butler, D. L., S. A. Goldstein, and F. Guilak. Functional tissue engineering: The role of biomechanics. *J. Biomech. Eng.* 122:570–575, 2000.
- ⁹Carver, S. E. and C. A. Heath. Influence of intermittent pressure, fluid flow, and mixing on the regenerative properties of articular chondrocytes. *Biotechnol. Bioeng.* 65:274–281, 1999.
- ¹⁰Caterson, B., C. R. Flannery, C. E. Hughes, and C. B. Little. Mechanism involved in cartilage proteoglycan catabolism. *Matrix Biol.* 19:333–344, 2000.
- ¹¹Chang, S. C. N., J. A. Rowley, G. Tobias, N. G. Genes, A. K. Roy, D. J. Mooney, C. A. Vacanti, and L. J. Bonassar. Injection molding of chondrocyte/alginate constructs in the shape of facial implants. *J. Biomed. Mater. Res.* 55:503–511, 2001.
- ¹²Chen, S. S., Y. H. Falcovitz, R. Schneiderman, A. Maroudas, and R. L. Sah. Depth-dependent compressive properties of normal aged human femoral head articular cartilage: Relationship to fixed charge density. *Osteoarthritis Cartilage* 9:561–569, 2001.
- ¹³Comper, W. D. and R. P. W. Williams. Hydrodynamics of concentrated proteoglycan solutions. *J. Biol. Chem.* 262(28):13464–13471, 1987.
- ¹⁴Davisson, T., S. Kunig, A. Chen, R. Sah, and A. Ratcliffe. Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. *J. Orthop. Res.* 20:842–848, 2002.
- ¹⁵Dimicco, M. A. and R. L. Sah. Dependence of cartilage matrix composition on biosynthesis, diffusion, and reaction. *Transport Porous Media* 50:57–73, 2003.
- ¹⁶Freed, L. E., A. P. Hollander, I. Martin, J. R. Barry, R. Langer, and G. Vunjak-Novakovic. Chondrogenesis in a cell-polymer-bioreactor system. *Exp. Cell Res.* 240:58–65, 1998.
- ¹⁷Freed, L. E., R. Langer, I. Martin, N. R. Pellis, and G. Vunjak-Novakovic. Tissue engineering of cartilage in space. *Proc. Nat. Acad. Sci. USA* 94:13885–13890, 1997.
- ¹⁸Freed, L. E., I. Martin, and G. Vunjak-Novakovic. Frontiers in tissue engineering: In vitro modulation of chondrogenesis. *Clin. Orthop.* 367S:S46–S58, 1999.
- ¹⁹Gribbon, P. and T. E. Hardingham. Macromolecular diffusion of biological polymers measured by confocal fluorescence recovery after photobleaching. *Biophys. J.* 75:1032–1039, 1998.
- ²⁰Gribbon, P., B. C. Heng, and T. E. Hardingham. The molecular basis of the solution properties of hyaluronan investigated by confocal fluorescence recovery after photobleaching. *Biophys. J.* 77:2210–2216, 1999.
- ²¹Gu, W. Y., H. Yao, C. Y. Huang, and H. S. Cheung. New insight into deformation-dependent hydraulic permeability of gels and cartilage, and dynamic behavior of agarose gels in confined compression. *J. Biomech.* 36:593–598, 2003.
- ²²Guilak, F. and V. C. Mow. The mechanical environment of the chondrocyte: A biphasic finite element model of cell-matrix interactions in articular cartilage. *J. Biomech.* 33:1663–1673, 2000.
- ²³Hascall, V. C., J. D. Sandy, and C. J. Handley. “Regulation of proteoglycan metabolism in articular cartilage.” In: *Biology of the Synovial Joint*, edited by C.W. Archer, B. Caterson, and M. Benjamin. Amsterdam: Harwood Academic Pub., 1999, pp. 101–120.
- ²⁴Hasler, E. M., W. Herzog, J. Z. Wu, W. Müller, and U. Wyss. Articular cartilage biomechanics: Theoretical models, material properties, and biosynthetic response. *Crit. Rev. Biomed. Eng.* 27(6):415–488, 1999.
- ²⁵Hutmacher, D. W. Scaffolds in tissue engineering bone and cartilage. *Biomaterials* 21:2529–2543, 2000.
- ²⁶Jones, W. R., H. P. Ting-Beall, G. M. Lee, S. S. Kelley, R. M. Hochmuth, and F. Guilak. Alterations in the young’s modulus and volumetric properties of chondrocytes isolated from normal and osteoarthritic human cartilage. *J. Biomech.* 32:119–127, 1999.
- ²⁷Kaasschieter, E. F. and A. J. M. Huijben. Mixed-hybrid finite elements and streamline computation for the potential flow problem. *Num. Meth. Part. Diff. Eq.* 8:221–266, 1992.
- ²⁸Klisch, S. M., S. S. Chen, R. L. Sah, and A. Hoger. A growth mixture theory for cartilage with application to growth-related experiments on cartilage explants. *J. Biomech. Eng.* 125:169–179, 2003.
- ²⁹Kouznetsova, V., W. A. M. Brekelmans, and F. P. T. Baaijens. An approach to micro-macro modeling of heterogeneous materials. *Comp. Mech.* 27(1):37–48, 2001.
- ³⁰LeBaron, R. G. and K. A. Athanasiou. Ex vivo synthesis of articular cartilage. *Biomaterials* 21:2575–2587, 2000.
- ³¹Leddy, H. A. and F. Guilak. Site-specific molecular diffusion in articular cartilage measured using fluorescence recovery after photobleaching. *Ann. Biomed. Eng.* 31:753–760, 2003.
- ³²Lee, C. R., A. J. Grodzinsky, and M. Spector. Biosynthetic response of passaged chondrocytes in a type ii collagen scaffold to mechanical compression. *J. Biomed. Mater. Res.* 64A:560–569, 2003.
- ³³Lee, D. A., M. M. Knight, J. F. Bolton, B. D. Idowu, M. V. Kayser, and D. L. Bader. Chondrocyte deformation within compressed agarose constructs at the cellular and sub-cellular levels. *J. Biomech.* 33:81–95, 2000.
- ³⁴Maroudas, A. Physicochemical properties of cartilage in the light of ion exchange theory. *Biophys. J.* 8:575–595, 1968.
- ³⁵Martin, I., B. Obradovic, L. E. Freed, and G. Vunjak-Novakovic. Method for quantitative analysis of glycosaminoglycan distribution in cultured natural and engineered cartilage. *Ann. Biomed. Eng.* 27:656–662, 1999.
- ³⁶Martin, I., B. Obradovic, S. Treppo, A. J. Grodzinsky, R. Langer, L. E. Freed, and G. Vunjak-Novakovic. Modulation of the mechanical properties of tissue engineered cartilage. *Biorheology* 37:141–147, 2000.
- ³⁷Mauck, R. L., S. B. Nicoll, S. L. Seyhan, G. A. Athesian, and C. T. Hung. Synergetic action of growth factors and dynamic loading for articular cartilage tissue engineering. *Tissue Eng.* 9(4):597–611, 2003.
- ³⁸Mauck, R. L., S. L. Seyhan, G. A. Athesian, and C. T. Hung. Influence of seeding density and dynamic deformational loading on the developing structure/function relationships of chondrocyte-seeded agarose hydrogels. *Ann. Biomed. Eng.* 30:1046–1056, 2002.
- ³⁹Mauck, R. L., M. A. Soltz, C. C. B. Wang, D. D. Wong, P. H. G. Chao, W. B. Valhmu, C. T. Hung, and G. A. Athesian. Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. *J. Biomech. Eng.* 122:252–260, 2000.
- ⁴⁰Mauck, R. L., C. C. B. Wang, E. S. Oswald, G. A. Athesian, and C. T. Hung. The role of cell seeding density and nutrient supply for articular cartilage tissue engineering with deformational loading. *Osteoarthritis Cartilage* 11:879–890, 2003.
- ⁴¹Mow, V. C. and A. Ratcliffe. “Structure and function of articular cartilage and meniscus.” In: *Basic Orthopaedic Biomechanics*, edited by V. C. Mow and W.C. Hayes. New York: Raven Press, 1997, pp. 113–177.
- ⁴²Nimer, E., R. Schneiderman, and A. Maroudas. Diffusion and partition of solutes in cartilage under static load. *Biophys. Chem.* 106:125–146, 2003.
- ⁴³Obradovic, B., J. H. Meldon, L. E. Freed, and G. Vunjak-Novakovic. Glycosaminoglycan deposition in engineered cartilage: Experiments and mathematical model. *AIChE J.* 46(9):1860–1871, 2000.

- ⁴⁴Pei, M., G. Seidel, G. Vunjak-Novakovic, and L. E. Freed. Growth factors for sequential cellular de- and re-differentiation in tissue engineering. *Biochem. Biophys. Res. Commun.* 294:149–154, 2002.
- ⁴⁵Pluen, A., P. A. Netti, R. K. Jain, and D. A. Berk. Diffusion of macromolecules in agarose gels: Comparison of linear and globular configurations. *Biophys. J.* 77:542–552, 1999.
- ⁴⁶Quinn, T. M., A. J. Grodzinsky, M. D. Buschmann, Y. J. Kim, and E. B. Hunziker. Mechanical compression alters proteoglycan deposition and matrix deformation around individual cells in cartilage explants. *J. Cell Sci.* 111:573–583, 1998.
- ⁴⁷Quinn, T. M., P. Schmid, E. B. Hunziker, and A. J. Grodzinsky. Proteoglycan deposition around chondrocytes in agarose culture: Construction of a physical and biological interface for mechanotransduction in cartilage. *Biorheology* 39:27–37, 2002.
- ⁴⁸Rotter, N., L. J. Bonassar, G. Tobias, M. Lebl, A. K. Roy, and C. A. Vacanti. Age dependence of biochemical and biomechanical properties of tissue-engineered human septal cartilage. *Biomaterials* 23:3087–3094, 2002.
- ⁴⁹Sah, R. L., S. B. Trippel, and A. J. Grodzinsky. Differential effects of serum, insulin-like growth factor-i, and fibroblast growth factor-2 on the maintenance of cartilage physical properties during long-term culture. *J. Orthop. Res.* 14:44–52, 1996.
- ⁵⁰Sah, R. L. Y., J. Y. H. Doong, A. J. Grodzinsky, A. H. K. Plaas, and J. D. Sandy. Effects of compression on the loss of newly synthesized proteoglycans and proteins from cartilage explants. *Arch. Biochem. Biophys.* 286(1):20–29, 1991.
- ⁵¹Sah, R. L. Y., A. J. Grodzinsky, A. H. K. Plaas, and J. D. Sandy. Effects of tissue compression on the hyaluronate-binding properties of newly synthesized proteoglycans in cartilage explants. *Biochem. J.* 167:803–808, 1990.
- ⁵²Sandy, J. D., J. R. O'Neill, and L. C. Ratzlaff. Acquisition of hyaluronate-binding affinity in vivo by newly synthesized cartilage proteoglycans. *Biochem. J.* 258:875–880, 1989.
- ⁵³Sandy, J. D. and A. H. K. Plaas. Age-related changes in the kinetics of release of proteoglycans from normal rabbit cartilage explants. *J. Orthop. Res.* 4:263–272, 1986.
- ⁵⁴Segal, G. *SEPRAN User's Manual*. Leidschendam, The Netherlands: Ingenieursbureau SEPRA, 2003, pp. 648.
- ⁵⁵Smit, R. J. M., W. A. M. Brekelmans, and H. E. H. Meijer. Prediction of the mechanical behavior of non-linear heterogeneous systems by multi-level finite element modeling. *Comp. Meth. Appl. Mech. Eng.* 155:181–192, 1998.
- ⁵⁶Stockwell, R. A. "Metabolism of cartilage." In: *Cartilage, Volume 1: Structure, Function, and Biochemistry*, edited by B.K. Hall. Orlando: Academic Press, 1983, pp. 253–280.
- ⁵⁷Thomas, J. M. Sur l'analyse numérique des méthodes d'éléments finis hybrides et mixtes. *Ph.D. thesis, Université Pierre et Marie Curie, Paris* 1977.
- ⁵⁸Treppo, S., H. Koepp, E. C. Quan, A. A. Cole, K. E. Kuettner, and A. J. Grodzinsky. Comparison of biomechanical and biochemical properties of cartilage from human knee and ankle pairs. *J. Orthop. Res.* 18:739–748, 2000.
- ⁵⁹Vunjak-Novakovic, G., I. Martin, B. Obradovic, S. Treppo, A. J. Grodzinsky, R. Langer, and L.E. Freed. Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. *J. Orthop. Res.* 17:130–138, 1999.
- ⁶⁰Vunjak-Novakovic, G., B. Obradovic, I. Martin, P. M. Bursac, L. E. Langer, and R. Freed. Dynamic cell seeding of polymer scaffolds for cartilage tissue engineering. *Biotechnol. Prog.* 14:193–202, 1998.
- ⁶¹Williamson, A. K., A. C. Chen, and R. L. Sah. Compressive properties and function-composition relationships of developing bovine articular cartilage. *J. Orthop. Res.* 19:1113–1121, 2001.
- ⁶²Wilson, C. G., L. J. Bonassar, and S. S. Kohles. Modeling the dynamic composition of engineered cartilage. *Arch. Biochem. Biophys.* 408:246–254, 2002.
- ⁶³Wong, M., M. Ponticciello, V. Kovanen, and J. S. Jurvelin. Volumetric changes of articular cartilage during stress relaxation in unconfined compression. *J. Biomech.* 33:1049–1054, 2000.
- ⁶⁴Wu, J. Z., W. Herzog, and M. Epstein. Modelling of location- and time-dependent deformation of chondrocytes during cartilage loading. *J. Biomech.* 32:563–572, 1999.