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Heterogeneity is key to hydrogel-based cartilage tissue regeneration

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Abstract

Degradable hydrogels have been developed to provide initial mechanical support to encapsulated cells while facilitating growth of neo-tissues. When cells are encapsulated within degradable hydrogels, the process of neo-tissue growth is complicated by the coupled phenomena of transport of large extracellular matrix macromolecules and the rate of hydrogel degradation. If hydrogel degradation is too slow, neo-tissue growth is hindered, whereas if it is too fast, complete loss of mechanical integrity can occur. Therefore, there is a need for effective modelling techniques to predict hydrogel designs based on the growth parameters of the neo-tissue. In this article, hydrolytically degradable hydrogels are investigated due to their promise in tissue engineering. A key output of the model focuses on the ability of the construct to maintain overall structural integrity as the construct transitions from pure hydrogel to an engineered neo-tissue. We show that heterogeneity in cross-link density and cell distribution are key elements for this successful transition and ultimately to achieve tissue growth. Specifically, we find that optimally large regions of weak cross-linking around cells in the hydrogel and well-connected and dense cell clusters create the optimum conditions needed for neo-tissue growth while maintaining structural integrity. Experimental observations using cartilage cells encapsulated in a hydrolytically degradable hydrogel are compared with model predictions to show the potential of the proposed model.

Graphical Abstract

A combined computational-experimental approach showing the importance of heterogeneity in hydrogel properties and cell distribution, for interstitial growth of cartilage.

Keywords

Biomechanics; Tissue Engineering; Degradable Hydrogel Scaffold; Multi-scale model; Cartilage; Cell Clustering; Structural Integrity

1. Introduction

Permanent tissue damage related to aging, injury and disease is a serious healthcare burden, which will only increase with the current rises in both population and life expectancy. Because specific tissues such as cartilage are limited in terms of healing and recovery, the pursuit of medical strategies to regrow and restore the original functionality of these tissues has become an important and challenging problem. Tissue engineering has the potential to address this problem through the introduction of populations of cells within a scaffolding material that can be placed in the damaged region in order to facilitate tissue regrowth. In this context, hydrogels offer an ideal material whereby cells are suspended in a liquid precursor solution that is injected to the site of interest and cured *in situ*¹. This minimally invasive process leads to the entrapment of cells within a three-dimensional hydrogel. Synthetic-based hydrogels are particularly promising as they can be engineered with precision while allowing cell encapsulation and supporting extracellular matrix (ECM) synthesis and deposition²⁻⁴.

The main challenge with using hydrogels for cell encapsulation, however, is that the polymer cross-links hinder diffusion of large ECM molecules and confine them to the immediate region around the cells^{6,7}. To address this issue, degradable hydrogels have been designed to provide initial structural support for the entrapped cells, while also increasing the extent of ECM transport. However, degradation can lead to rapid decrease of mechanical properties (e.g., modulus) and eventual loss of structural integrity^{8,9}, i.e. complete collapse of the 3D network. This is especially problematic for hydrolytically degradable hydrogels where the high water contents, typically higher than eighty percent, cause hydrolysis of the polymer network throughout the bulk hydrogel. Indeed, in this situation, since the transport of large ECM molecules is restricted until reverse gelation (i.e., critical point at which the polymer loses its overall connectivity and transitions from a solid to a soluble polymer), the construct's structural integrity can be severely compromised before the neo-tissue forms¹⁰. Interestingly, experimental studies show that hydrolytically degradable gels in some cases

appear to maintain tissue integrity and promote growth^{11–13}, while others have reported a significant drop in mechanical properties during tissue growth⁹. Although, hydrolytically degradable hydrogels are one of the simplest systems in terms of control and design, the coupled mechanisms responsible for growth and construct failure (i.e., when neither the hydrogel nor the neo-tissue can provide structural support) are still poorly understood.

The objective of this study is thus to identify key features within cell-laden hydrogels that prevent construct failure and ultimately improve neo-tissue growth using a theoretical approach. The theory of mixtures has typically been used in previous works to model the variety of components that participate in the scaffold degradation-tissue growth process^{14–16}. For instance, authors such as Wilson et al.¹⁷ and Haider et al.¹⁸ introduced and extended phenomenological models to qualitatively understand the roles of the hydrogel, unlinked and linked ECM in engineering cartilage. The effect of ECM transport, deposition and scaffold degradation on the construct stiffness was also investigated by Sengers et al.¹⁹ with a 2D model. Furthermore, the competition between hydrogel degradation and ECM deposition was studied numerically by Dhote et al.²⁰ and Akalp et al.²¹. While these models gave important information on the mechanisms of construct evolution, our experimental observations based on poly (ethylene glycol) (PEG) hydrogels suggest that construct growth and mechanical integrity is sensitive to the spatial heterogeneity in hydrogel properties and cell distribution. In contrast to previous models, this article therefore explores via computational modelling how the presence of heterogeneity affects the dynamics and properties of the construct over time. We particularly show that non-uniform hydrogel properties can lead to a better local transport and deposition of neo-tissue, while the presence of high-density cell clusters can in some cases, induce growth to progress faster in these regions compared to those with low cell densities. We show that together, these situations are highly favourable to both growth and overall construct stability over time.

The article is organised as follows. The next section introduces experimental observations and describes the modelling methodology to describe tissue growth in degradable hydrogels. The mathematical description of heterogeneity in the cross-link density is discussed in detail in section 3 supported by experimental evidence. The role of heterogeneous cell distribution due to clustering is then investigated in section 4. We further provide qualitative comparisons of the evolution of elastic modulus between experimental data and model predictions.

2. Combined hydrogel scaffold degradation and tissue growth: experiment and model

In this study the construct is originally composed of cartilage cells (i.e., chondrocytes) embedded in a hydrogel made of polymer chains and crosslinks. Cells synthesize and secrete extracellular matrix (ECM) molecules, which are essential to tissue formation and growth. Here, degradation is achieved through hydrolysis of linkages, such as ester bonds, within the hydrogel network. The experimental system is a PEG hydrogel containing caprolactone segments within each crosslink, which in the presence of water undergo ester hydrolysis¹³. Cell-laden hydrogels were cultured and chondrocyte-mediated growth was monitored for a

period up to 12 weeks. For more information on experimental methods, please refer to Appendix A. Experimental results are shown for neo-tissue organization through immunohistochemical staining for collagen II, the main collagen in cartilage (Fig 1(a)) and for construct compressive modulus (Fig 1(b)) as a function of culture time. It can be seen from the immunohistochemical images that growth progresses in time in a localised manner. This observation is particularly evident in the case of low cell density at 4 weeks, where the newly deposited collagen II is localized to the pericellular space. In the lowest cell seeding density, a drop in modulus is observed at four weeks, while the higher cell densities have led to an increase in the overall modulus. By week 12, all three seeding densities result in successful generation of tissue while the construct remains intact the entire time.

Mechanics-based model of tissue growth in degradable hydrogels

Let us now summarize a computational modelling strategy that can capture and elucidate some of the important mechanisms responsible for the combined growth and degradation of a hydrogel-based tissue construct. The model is based on the mechanisms presented in the schematics of Fig. 2. In short, after cells are encapsulated in a hydrogel, hydrolysis of the polymer crosslinks is assumed to occur, following first order kinetics²². As crosslinks are cleaved, the formation of new tissue starts with the release of unlinked (fluid-like) ECM precursors from the cell. These precursors assemble extracellularly to form large ECM molecules of aggrecan and collagens. Herein, we consider the ECM molecules once they have assembled. Depending on the local state of the gel, these molecules may diffuse and deposit to create a solid-like matrix where aggrecan and collagen molecules interact to form the neo-tissue that eventually participates in the mechanical stiffness of the construct. To describe these physics, we have constructed a continuum-based multiphasic model^{16,21,23} that first considers the coupled transport/mechanics problem in the regions near cells for which the main equations are given below:

Degradation Kinetics

Hydrolytic degradation arises from the cleavage of cross-links initiated by hydrolysis. At the micron-scale, this leads to a progressive and spatially uniform decrease in cross-linking density, that is approximated by first order kinetics:

$$\frac{d\rho_x}{dt} = -\kappa\rho_x \quad (1)$$

where ρ_x is the cross-link density, defined as the number of moles of cross-links per unit volume of the hydrogel in its dry state and κ is the degradation constant. After integration, one therefore finds that the cross-link density decreases in time exponentially according to the relation $\rho_x(t) = \rho_x^0 e^{-\kappa t}$, until it reaches reverse gelation. At this critical point, polymer chains lose their network connectedness and the polymer undergoes a transition from a solid-like to a fluid-like medium. This phenomenon is critical for the transport of ECM within the gel since a sharp increase in particle diffusivity occurs at this threshold. In this study, the onset of reverse gelation is captured by the parameter β defined as the ratio of the cross-link density at reverse gelation over the initial cross-link density.

ECM Diffusion and Tissue Growth

As discussed above, tissue growth originates from the synthesis, transport and deposition of ECM in the form of large collagen and proteoglycan molecules. These molecules are initially unlinked and can freely diffuse within the gel depending on the diffusion coefficient D_m . Extracellularly, these molecules assemble to form the large ECM molecules which then link together to form a solid-like neo-tissue. These processes are modelled by the rate of conversion γ_m between unlinked (fluid) ECM macromolecules and linked (solid) ECM¹⁸. The above two processes can therefore be described by a standard reaction-diffusion equation of the form:

$$\frac{\partial c_m}{\partial t} = D_m(\rho_x) \nabla^2 c_m - \gamma_m \quad (2)$$

where c_m is the unlinked ECM concentration and rate of linkage is assumed to linearly depend on this concentration through $\gamma_m = k_m c_m$. We note that the ECM macromolecule diffusion is a function of cross-link density. However, since the hydrodynamic radius of the ECM macromolecules is much larger than the polymer mesh size, their diffusion is highly restricted before reverse gelation (i.e., $D_m = 0$ if $\rho_x > \beta \rho_x^0$). After reverse gelation however, diffusion is fast and can be approximated by the ECM diffusivity D_m^∞ in water (i.e., $D_m = D_m^\infty$ if $\rho_x < \beta \rho_x^0$) assuming Stokes-Einstein relationship. For simplicity, we assume that there is no hindrance in the diffusivity of fluid ECM macromolecules through the linked solid ECM. The rate of ECM synthesis from cell surfaces is finally represented in terms of its mass flux, Q_m , which is defined on the basis of homeostasis as $Q_m = Q_m^0 (1 - c_m/c_m^0)$ in moles per area per time. This definition accounts for a decreasing rate of ECM production with accumulation. This yields a boundary condition for eq. 2 in the form:

$$Q_m = -D_m \nabla c_m \quad (3)$$

The accumulation of solid ECM through the construct is finally captured by integrating the rate γ_m following the equation:

$$\frac{\partial c_s}{\partial t} = \gamma_m \quad (4)$$

where c_s is the concentration of solid ECM (i.e., deposited neo-tissue) at a given point. The solid ECM is the result of a connected network of ECM molecules that form the foundation of the tissue.

Mechanical Properties of the construct's constituents

Understanding the mechanical properties of the construct as it transitions from a gel to a tissue is critical to avoid its failure due to external physiological loads. In this work, we are particularly interested in monitoring how its effective shear modulus changes as the initial

hydrogel vanishes to give way to the new tissue. To explore this, it is first essential to derive mechanical models for the two solid phases responsible for the construct integrity: the hydrogel and the solid ECM. The elastic response of hydrogels is typically captured by Flory's theory²⁴. Due to the small deformations occurring during stress-free growth, the theory can be linearized²¹ to yield a stress-stress relation of the form:

$$\boldsymbol{\sigma}_p = 2G_{gel} \left(\frac{\nu}{(1-2\nu)} \text{tr}(\boldsymbol{\varepsilon}_p) \mathbf{1} + \boldsymbol{\varepsilon}_p \right) \quad (5)$$

where $\boldsymbol{\sigma}_p$ is the stress tensor, $\boldsymbol{\varepsilon}_p$ is the strain tensor, G_{gel} is the shear modulus of the swollen gel and $\mathbf{1}$ is the identity tensor. Note here that since hydrogels are nearly incompressible at short time scales, a Poisson's ratio close to 0.5 is used in our simulations. Moreover, the shear modulus can be related to cross-link density via $G_{gel} = \rho_x RT J_0^{-1/3}$, where J_0 is the equilibrium swelling ratio, R is the gas constant and T is temperature. To further account for the loss of gel stiffness at reverse gelation, the shear modulus is assumed to drop sharply to a zero-value as the cross-link density becomes $\rho_x = \beta \rho_x^0$.

The elastic properties of the neo-tissue are still poorly understood but within the range of small deformation, it can be assumed as an isotropic, linearly elastic material whose stiffness increases continuously with ECM deposition [17]. Following this hypothesis and consistent with previous work^{16,21}, the stress-strain relation of the ECM is taken to be:

$$\boldsymbol{\sigma}_m = c_s (\lambda \text{tr}(\boldsymbol{\varepsilon}_m) \mathbf{1} + 2\mu \boldsymbol{\varepsilon}_m) \quad (6)$$

where c_s is the solid ECM concentration while λ and μ are the Lamé constants expressed in energy per mole. In order to model the porous behaviour of the tissue, a constraint, $\lambda = 0.8\mu$ is used in this study^{25,26}. Now assuming that hydrogel and ECM are interpenetrating and do not strongly interact with one-another, the mechanical response of a material point within the construct follows an additive mixture rule¹⁴, i.e. the total stress arises from additive contribution of each component, as $\boldsymbol{\sigma} = \boldsymbol{\sigma}_p + \boldsymbol{\sigma}_m$. We note here that the presence of chondrocytes throughout the construct (Fig. 2) also participate in its mechanical properties. Experimental images and the literature suggest that these cells can be assumed as incompressible spherical particle whose mechanical response is close to that of a hydrogel. Under small deformation, the constitutive models given by Eq. 5 can therefore be used in which a shear modulus, $G_{cell} = 0.2 \text{ kPa}$, is used in agreement with literature²⁷.

Evolution of construct mechanics

The investigation of the temporal evolution of the construct mechanics can typically be modelled by considering a cubic representative volume element (RVE)²⁸ its evolving structure. At the micron-scale ($\sim 50 \mu\text{m}$), the construct's response depends on the volume fraction $f = V_C/V$ of chondrocytes in the RVE (here V_C is the volume of the cells while V is the total RVE volume). To represent these conditions, a cubic RVE is introduced wherein a number of cells (consistent with the value of f) are randomly placed following a Poisson's

distribution²⁹. The diffusion-reaction equations (1) and (2) are then solved spatially and temporally using the finite difference method. The mechanical properties of the construct at any time during the growth process can then be evaluated using traditional homogenization methods used for composites³⁰. More specifically, to determine the average elastic modulus, the RVE is subjected to an unconfined compressive uniaxial loading by applying displacement boundary conditions on the top and bottom of the RVE. Internal stresses and strains are calculated from the solution of the equilibrium equation given by $\nabla \cdot \boldsymbol{\sigma} = 0$, and the average elastic modulus of the composite is then evaluated by techniques of numerical homogenization^{30,31} as

$$\langle E \rangle = \langle \sigma_{11} \rangle / \langle \varepsilon_{11} \rangle \quad (7)$$

where $\langle \sigma_{11} \rangle$ and $\langle \varepsilon_{11} \rangle$ are the average compressive stress and strain on the RVE in the direction of loading. This operation is performed numerically using the finite element method based on the solution of the cross-link density, ρ_x , and the ECM concentration, c_s , using constitutive models introduced in the previous section. More details regarding the formulation can be found in²¹. We note that since the composition of the construct evolves in time due to gel degradation and ECM linkage, the average elastic modulus is a function of time (t) and is represented by the function $\langle E \rangle(t)$. This function is key to describe the mechanical integrity of the construct in time and is an implicit function of the cell volume fraction f . For convenience, in the remainder of the manuscript, this average modulus is normalised with respect to the initial modulus of the acellular hydrogel, i.e. by $\langle E^* \rangle = \langle E \rangle / E_{gel}$.

Based on the hydrogel properties and growth mechanisms described above, we now explore the prediction of mechanical properties by the current model with the input parameters listed in Table 1 of section 3. The model estimate of the construct's modulus is averaged over multiple cell distributions corresponding to the same cell volume fraction, f (i.e., the cells are homogeneously distributed). In Fig. 3, plots of the average elastic modulus corresponding to cell densities 50 million cells/mL ($f = 0.026$) and 150 million cells/mL ($f = 0.078$) are shown (corresponding to experiments presented in Fig. 1).

The prediction shows failure of the construct at a point where the modulus falls in the failure region shaded in red, when the hydrogel is completely degraded after which growth begins as seen from the distribution of cross-link density and solid ECM concentration. The failure region represents values below the modulus corresponding to the reverse gelation point of the hydrogel. These results predict that, in contrast to experimental observations, hydrolytically degradable scaffolds lose all structural integrity before growth begins. These results can be explained by the fact that ECM diffusion is restricted until the hydrogel reaches reverse gelation. When reverse gelation occurs, however, ECM molecules can diffuse, but the hydrogel has already transitioned from a solid to a liquid. The discrepancies between experimental and modelling results may be attributed to several factors related to model simplifications. We hypothesize that an important difference between the real and the modelled system relates to the presence of inhomogeneity, both at the hydrogel level and at

the cellular level in the experimental system. Indeed, Fig. 1a clearly shows that, in contrast to model assumptions, cell distribution and tissue growth is heterogeneous across the construct. We will show in the next section that when a non-uniform cross-link density is considered in the gel, significant differences arise between the predicted and measured construct modulus, even at very early stages. These considerations provide a motivation to explore and quantify the role of construct inhomogeneity on both tissue growth and the evolution of scaffold properties.

3. The role of inhomogeneous cross-link density

Chondrocytes are able to interfere with radical-mediated polymerization during cell encapsulation in PEG hydrogels^{34–36}. Notably, the cell membrane can react with extracellular free radicals leading to reactions like lipid peroxidation³⁷. This phenomenon has the potential to affect the local cross-link density around each cell and if severe, could affect the overall construct properties. To explore this possibility, we show in Fig. 4(a) an experimental/modelling comparison of the initial compressive modulus of cell-laden hydrogel scaffold for different seeding densities. We note that the model predictions here were made with the above numerical formulation, and was a-priori calibrated with an acellular PEG hydrogel that had the same formulation and was subjected to the same polymerization process. Experimental results were obtained by subjecting the cell-laden hydrogel to unconfined compression at a relatively fast rate, compared to the characteristic time for water diffusion in the gel (poro-mechanical effects are neglected). The figure clearly shows increasing discrepancies as the cell seeding density rises, wherein the scaffold appears to significantly weaken compared to numerical predictions.

Local variations in cross-link density

Based on the argument that cells interfere with the polymerization, we hypothesize that local variations in cross-link density exist in the pericellular regions around chondrocytes. We have, for simplicity, assumed that cross-link density increases as a function of the distance X from the cell surface until it finally reaches its uniform and maximum value ρ_x^0 at long distances. In the present work, we assume this function to take the form of an exponential as:

$$\rho_x(X) = \rho_x^0 (1 - \exp(-kX/R_d)) \quad (8)$$

where R_d is the effective radius of weakly polymerized gel, measured from the cell surface. More precisely, we define it as the distance at which the cross-link density reaches 99% of its maximum value ρ_x^0 ; this implies that $k = \ln(0.01)/R_d$ in Eq. 2. We further note that the choice of this function in Eq. 8 implies that the cross-link density vanishes at the cell surface as shown in Fig. 4b. Implementing this function into the numerical model and simulating an unconfined compression test at initial time, we found that a satisfactory match with experimental measurement was $R_d = 27R_c$, where R_c is the radius of a single cell (Fig 4b).

Role of inhomogeneous cross-links on local degradation and growth

Based on the discussion of the previous sections, we are now able to understand the consequence of an inhomogeneous cross-link density on degradation locally around a cell. To study the effect of a non-zero R_d , we compare the evolution of degradation profiles at different times for $R_d = 0$ and $R_d \neq 0$ locally around a cell. This is obtained by substituting Eq. 8 in the solution of Eq. 1. The cross-link density function defined in Eq. 8 is valid for a distance X away from the cell surface in any direction, implying spherical symmetry in 3D. Using a 1D representation, Fig. 5 illustrates the local cross-link degradation at initial time ($t=0$), and later time points, $t=t_1$ and $t=t_2$, around a cell comparing cases where $R_d = 0$ and $R_d = 2R_c$. The red-shaded region marks the critical cross-link density at which reverse gelation occurs. Given the uniform rate of degradation with degradation constant κ , it is observed that in contrast with the case of uniform cross-link density, the presence of a weak region around cells accelerates the time necessary to reach the onset of reverse gelation. In 3D, this produces the appearance and growth of fluid-like spheres around cells enabling the deposition of matrix in localized regions before the gel dissolves far from cells. The effect of this mechanism on the construct is discussed next.

Role of inhomogeneous cross-links on construct evolution

To evaluate the role of a non-uniform cross-link distribution at the construct scale, let us consider a RVE, composed of a cell population at volume fraction f and a degraded region (characterized by R_d) as seen in Fig. 6. To better understand the evolution in the construct's mechanical properties and particularly, the load transfers between hydrogel and tissue, it is convenient to introduce measures of hydrogel and matrix connectivities and evaluate how they change over time. In this work, the overall connectivity of the neo-tissue (ψ_m) and hydrogel (ψ_g) are respectively defined as:

$$\psi_m = \frac{N_{cells} - N_m}{N_{cells} - 1} \quad \psi_g = \begin{cases} 1/N_{gel} & \text{if } N_{gel} \neq 0 \\ 0 & \text{if } N_{gel} = 0 \end{cases} \quad (9)$$

where N_{cells} is the number of cells in the RVE while N_m and N_{gel} are the number of regions of disconnected solid ECM and cross-linked gel (i.e., $\rho_x > \beta\rho_x^0$), respectively. We note that ψ_g and ψ_m are defined so that their range remains between 0 and 1, with 0 representing no connectivity and 1 representing full connectivity. To explore the role of R_d on construct degradation, growth and connectivity, we present in Figs. 6 and 7, a series of RVE simulations considering different cases of R_d and volume fraction, f . In all these simulations, it is assumed that the intrinsic rate of linkage, k_m , is twice the degradation rate constant, κ . All model parameters used for simulations presented in this section are listed in Table 1, except for the degradation rate constant, $\kappa = 2.5 \text{ day}^{-1}$. This value was chosen such that the time scales of ECM diffusion and gel degradation are comparable (i.e. $D_m/\kappa L^2 \sim 1$ where $L = 25 \mu\text{m}$ is the characteristic length of the RVE), which provides a good candidate to study the competition between degradation and growth. Finally, for the sake of generality, the results are reported in a non-dimensional form following the procedure presented in Appendix B. The overall construct properties (stiffness and connectivity) were determined after averaging

simulation results subject to multiple Poisson cell distributions of the same volume fraction, f .

To help interpret the results, it is useful to realize that depending on the value of R_d , the region of low cross-links density around each cell can come into contact or overlap before the bulk hydrogel reaches reverse gelation. In this case, the overlapping region can lead to connectivity of the growing matrix phase within the degraded regions. This condition implicitly depends on the average inter-cellular distance, d_c and can be roughly expressed by $R_d > d_c/2$, where d_c is related to the volume fraction, f , by $d_c = 2R_c(f^{-1/3}-1)$ (see Appendix C). This overlap condition can be written in terms of the overlap index, η , given by:

$$\eta = \frac{R_d}{d_c/2} = \frac{R_d}{R_c(f^{-1/3}-1)} > 1 \quad (10)$$

In short, $\eta < 1$ implies the absence of an overlapping region while the opposite is true when $\eta > 1$.

To demonstrate the consequence of the overlap condition on the construct, we consider examples in Fig. 6 where Eq. 10 is not satisfied (i.e., $\eta < 1$) and in Fig. 7 where Eq. 10 is satisfied (i.e., $\eta > 1$). Based on the simulation results a few important trends can be identified. First, as illustrated in Fig. 7, when Eq. 10 is satisfied, the accelerated degradation around the cells enable a quick diffusion and deposition locally, while the hydrogel away from the cell is still mechanically supportive. Eventually, these ECM regions connect and participate in the load carrying capacity of the entire construct. In other words, this situation yields a scenario in which both hydrogel and matrix connectivity co-exist in time. This enables a smooth transition of mechanical properties of the construct, from hydrogel-supported to matrix-supported, in contrast to the cases when Eq. 10 is not satisfied. In particular, an $R_d = 0$ will never satisfy Eq. 10 and so can never lead to successful tissue growth. This is also seen from previous estimates in Figs. 3 and 4. Second, while the above observation suggests that a large overlapping region is preferable, this might be detrimental to the construct modulus as a whole. Indeed, excessive overlapping implies that the hydrogel around the cells is weak from the start. The modulus of the construct therefore starts from a significantly low value compared to cases where there is no overlap. It is also seen that in Fig. 7a, even though the overlap index, η , is greater than 1, the predicted construct modulus reduces considerably in time to fall just below the failure region even if not resulting in complete failure. This leads to the conclusion that even in cases when Eq. 10 is satisfied, a small overlap index can result in a behaviour comparable to cases where Eq. 10 is not satisfied, shown in Fig. 6. In Fig. 7, we further show that although optimal overlap conditions can be found to enable smooth transfer of the hydrogel and matrix mechanics while keeping a finite overall construct modulus over time, the construct's modulus is still restricted to small and perhaps insufficient magnitudes over time. The overlap conditions, given in Eq. 10, can be optimized theoretically by tuning the values of R_d and f . While the value of R_d is relatively more complicated to control, we will see next that the value of f can be suitably varied if cells are non-uniformly distributed through the hydrogel.

4. The role of inhomogeneous cell distribution

Microscopy images (Fig. 8a) have shown that when encapsulated in a hydrogel, chondrocytes have the tendency to form clusters. In time, the matrix deposition shows similar trends where pockets of dense matrix are separated by regions in which cartilage cells and tissue are quasi-inexistent. These observations can be appreciated from immunohistochemical images of Fig. 1 showing the evolution of collagen II distribution at weeks 0, 4 and 12 in a hydrogel scaffold. From a mechanical viewpoint, the construct can therefore be classified as an evolving heterogeneous two-phase composite, comprised as a dense (cell cluster) phase and sparse phase, whose overall properties depends on both the stiffness and the morphological features of these phases. To construct a model that incorporates those features, we first note that the characteristic distance between cells remains significantly smaller than the size and spacing of clusters. This enables us to invoke the principle of scale separation and represent the cellular scale (discussed in the previous section) in a homogenized fashion. In other words, at the scale of clusters, cells are represented by their volume fraction, f , the gel by its average cross-link density, $\langle \rho_x \rangle$, and the matrix by its average concentration, $\langle c_s \rangle$, quantities that can be determined, in time from an averaging operation at the cellular scale from RVEs shown in Figs. 6 & 7. For the sake of clarity, details of such averaging operations are given Appendix D. The presence of cell clusters enables a situation in which hydrogel degradation and tissue growth occur at different rates depending on their location. In this section, we study the effect of these spatially non-uniform degradation and growth processes on the construct evolution.

Model

We propose to capture the existence of cell clusters through a simple three-parameter model whose level of detail can be later extended if necessary. In this model, we assume that cells form arbitrarily-shaped clusters of large, but uniform density, f_{cl} that exist on top of a uniform background cell population of density $f_{bg} < f_{cl}$. The clusters are themselves characterized by their volume V_c , shown in Fig. 8a, from which the volume fraction of clusters can be determined by $F = \sum V_c / V$, where V is the volume of the construct and the summation includes all clusters present. Thus, the cell cluster distribution is captured by the following three parameters: the cluster volume fraction F , the cluster contrast c_f and the overall average cell density f_h expressed as

$$c_f = f_{cl} / f_{bg} \quad f_h = f_{cl} F + f_{bg} (1 - F) \quad (11)$$

To represent this cell population, we constructed a macroscopic RVE (mm size) in which clusters are distributed on a grid following a Poisson distribution²⁹ subject to an average cluster spacing $D \approx 0.55 N_c^{-1/3}$ ³⁸, where N_c is the number of clusters per unit volume. The morphology of each cluster was randomly generated about its seed point following a self-avoiding random walk algorithm³⁹ (Fig. 8). Construct evolution could then be modelled by following the local degradation, growth and mechanical properties within clusters and in the background (shown in Fig. 6&7) and using average operations (see Appendix D) to determine its overall response.

Role of cell clusters in construct evolution

To understand the evolution of this evolving composite and its mechanical integrity, it is first important to characterize the connectedness (or stress transfer capacity) of its phases. We will particularly pay attention to the cluster connectivity Ψ defined as

$$\Psi = \frac{N_c - N_{dc}}{N_c - 1} \quad (12)$$

where N_c and N_{dc} are numbers of clusters and disconnected clusters in the RVE, respectively. We see that if the clusters are not connected, $N_{dc} = N_c$ and the connectivity factor vanishes. For a fully connected cluster network however, $N_{dc} = 1$ and the connectivity factor converges to unity. Fig. 8b shows simulation results that illustrate how cluster connectivity changes with total number of clusters and cluster size; it can clearly be seen that the system displays a percolation transition⁴⁰ whose critical cluster fraction N_c varies with cluster size.

We investigated the role of cell clusters on construct evolution by performing simulations (Fig 9) in three cases $F < F_c$ (disconnected clusters), $F \approx F_c$ (weakly connected clusters) and $F > F_c$ (strongly connected clusters). For all cases, we kept a constant contrast parameter, $c_f = 8$, background and cluster concentrations $f_{bg} = 0.028$ and $f_{cl} = 0.22$, respectively and $R_d = 5R_c$. The overlap index, η , for the background and clusters, evaluated using Eq. 10, are therefore 2.2 and 7.6, respectively. Based on the cellular-scale analysis described in the previous section, we could then estimate the evolution of the average modulus, $\langle E^* \rangle$, and the average ECM deposition $\langle c_s^* \rangle$, of the cluster and background phases of the construct. This was then used as input to the macroscale RVE analysis of the construct from which the time evolution of the construct-scale average modulus, $\langle\langle E^* \rangle\rangle$, and average ECM concentration, $\langle\langle c_s^* \rangle\rangle$, could be estimated using the same corresponding definitions.

Taken together, our simulations show that gel degradation and matrix growth occur first in the cluster phase due to its higher overlap index, η , compared to the background. While the above statement is true for all three cases in Fig. 9, as seen from the evolution of $\langle E^* \rangle$, and $\langle c_s^* \rangle$, the overall construct properties evolve differently. Initially, the background phase acts as the primary structural integrity phase due to its higher modulus compared to the clusters. However, as time progresses, we observe that the mechanical integrity of construct with disconnected clusters primarily relies on the background phase throughout time. Since the overall condition (Eq. 10) is not verified in this region, the full construct eventually fails once reverse gelation is reached. Constructs with disconnected clusters display a very different scenario; at early time, their mechanical integrity is ensured by the background phase while degradation and growth occur early in the clusters. Interestingly, at a later time, the new ECM in these clusters is fully linked and able to support the load as the background region fails (Fig. 9). As a result, growth $\langle\langle c_s^* \rangle\rangle$ and evolution of mechanical properties $\langle\langle E^* \rangle\rangle$ of the construct with the highest cluster connectivity, Ψ , in Fig. 9c, show significant improvement over the other two constructs with notably low cluster connectivity, in Figs. 9a

& b. It is important to note that the role of R_d is especially important in determining the overlap index, η , of the clustered regions which enables a fast degradation and growth thereby preserving the integrity of the construct in these regions. Moreover, the construct cannot acquire both benefits of a high initial modulus and successful tissue growth without a strong variation in overlap index, η , of the background and cluster phases. We are thus led to the following conclusions regarding the optimal conditions necessary for effective load transfer from scaffold to tissue in the construct: (1) the original cell clusters need to form a connected network and (2) the cluster contrast, c_f , needs to be large enough for a given R_d , such that the degradation and growth kinetics in the clustered and background regions occur in an out-of-phase fashion.

Model comparisons with experiments

The accuracy of a model is measured by its ability to predict reality as seen through experimental observations. Therefore, we compare experimental measurements of the construct modulus, for three different seeding densities described in section 2, with three different model predictions each under a different assumption. The assumptions of the three models are: (1) homogeneous cross-link density, ρ_x , ($R_d = 0$) and cell volume fraction, f , (2) heterogeneous cross-link density, ρ_x , ($R_d = 27R_c$) and homogeneous cell volume fraction, f , and (3) heterogeneous cross-link density, ρ_x , ($R_d = 27R_c$) and cell volume fraction, f . The construct properties used as input to the models are given in Tables 1 and 2. The cluster volume fraction, F_c and contrast, c_f , were determined by using image processing techniques on histology images. The value of R_d was obtained by calibrating the model prediction of the initial construct modulus with experimental measurements. Details are provided in the supplementary information. Since the intrinsic linkage rate, k_m , could not be directly measured, it was calibrated to match the experimental construct modulus such that $k_m = 5k$, where κ is the hydrogel degradation rate constant. Finally, the model simulations are discontinued shortly after complete degradation of the hydrogel since pure tissue growth is not the object of this study.

When a homogeneous cross-link density and cell volume fraction are considered in the construct, it predicted modulus evolution deviates sufficiently from experimental measurements leading to construct failure, as shown in Fig. 10. Thus, this condition is not able to match the experimental system. With the assumption of heterogeneous cross-link density, regardless of the nature of cell distribution, it is seen that model predictions are reasonably accurate in comparison with experiments. The similarity in these model predictions can be attributed to the low contrast, c_f , of the cell density in clusters compared to the background phase, even though the cluster volume fraction, F , indicates strong cluster connectivity. To further support our hypothesis that a heterogeneous cross-link density exists, we have compared the model prediction from an assumption of heterogeneous cell volume fraction and a uniform global reduction in cross-link density to the experiments. In other words, this condition tests whether the drop in modulus as measured experimentally (Fig 1(c)) is a result of a homogeneous drop in cross-link density across the bulk hydrogel instead of a heterogeneous drop in cross-link density that is restricted to the regions surrounding the cells. The model predicts that construct mechanical failure will occur before sufficient matrix growth and thus provides additional evidence that the interactions between

cartilage cells and the polymerization reaction are best described by local weak cross-links characterized by R_d . Moreover, without overlapping regions ($\eta = 0$), ECM transport is restricted until reverse gelation. Our analysis in Fig. 9 points out that increasing the cluster contrast, c_f could potentially result in an increase of the construct modulus during its transition from hydrogel to neo-tissue, which could eventually improve tissue growth. We finally note that most hydrogel-based biomaterials including this work report an initial hydrogel modulus much lower than native cartilage (between 500 and 1000 kPa⁴¹). This is due to the requirement of low crosslinking (hence low modulus) to allow ECM elaboration. However, we have published several works of chondrocytes encapsulated in high modulus hydrogels without adverse effects on cell viability^{6,10}. Thus, while the hydrogels employed herein have a much lower modulus, the results from the model can be applied to the high modulus hydrogels such that it would be possible to achieve an initial high modulus and which can be maintained through the transition from hydrogel to neo-tissue. This is a focus of our future work.

Model Limitations

A few assumptions in the model create uncertainties in its predictions. First, the diffusivity of fluid-like ECM through the solid ECM matrix reduces with increasing ECM deposition^{42–44}. We neglect this detail in our model as its exact nature is not well understood. However, we understand that it may affect the model prediction of growth rate and modulus evolution. This issue can be addressed in future work by including an ECM concentration-dependent diffusivity derived from appropriate experiments. Second, we note that the intrinsic linkage rate of conversion of fluid ECM to solid ECM is not obtained explicitly from experiments, which affects the predictive ability of the model. Third, the simple cell cluster model while providing critical understanding, can be improved further to account for variability in cluster sizes and spacing in a given construct. Fourth and finally, the model does not explicitly address the existence of cell death or proliferation, which would yield a changing cell distribution in time. Understanding cell proliferation and cell death is complex and will depend on multiple factors that are not yet well understood. Chondrocytes in their native tissue minimally, if at all, proliferate. When encapsulated in a 3D hydrogel, the polymer network that surrounds the cells reduces their ability to proliferate due to physical restraints in the available space. Prior studies with hydrolytically degradable hydrogels have reported constant levels of DNA content over the course of six weeks over which time the hydrogel had completely degraded⁴⁵. Thus, to account for potential variabilities in cell number, we chose to calculate ECM production rate on a per cell basis using the number of live cells as determined by experiments. While the model does not currently account for cell proliferation or cell death, changes in cell number are indirectly accounted. Moreover, the presented model aims at capturing the role of a hydrogel scaffold on the early growth of a neo-tissue and therefore does not intend to represent the mechanisms of tissue growth and remodelling after complete scaffold degradation. The mechanical properties of the neo-tissue increase in time due to tissue remodeling, for example by cross-linking of collagen molecules, as is evident from the experimental system with the highest cell density where the construct modulus continued to increase in time even after complete gel degradation. These aspects could be better described using other models^{14,15,32}.

5. Summary and Final Remarks

In this work, we have used a computational modelling approach to elucidate the dynamics of an evolving cell-laden degradable hydrogel construct. When properly integrated with experimental efforts, the model helped to identify important mechanisms driving construct properties and tissue growth, which can then be used to guide improved experimental developments. Here, we have particularly shown that hydrolytically degradable hydrogels can be effectively used for neo-tissue growth by exploiting the spatial heterogeneities in the polymer cross-link density and cell distribution. Motivated by experimental observations, we included the presence of a weak hydrogel region surrounding the embedded cells at the time of encapsulation, in addition to considering an inhomogeneous clustered distribution of cells. Our model suggests that when the weak pericellular hydrogel regions overlap, local “pockets” of ECM can connect before the bulk hydrogel reaches reverse gelation. This region then enables a smooth transfer of mechanical properties from the hydrogel to the neo-tissue. The scenario however results in a weaker initial construct, increasing the risk of construct mechanical failure before growth. The role of cell clustering was further investigated using a simple three-parameter model. We found that optimal conditions for neo-tissue growth and construct integrity are met when cell clusters are well connected and display a large enough contrast. In this case, hydrogel degradation and growth kinetics occur earlier in clusters compared to background thereby facilitating consistent structural integrity of the construct.

Successful tissue growth was achieved with heterogeneity using hydrolytically degradable hydrogels because of spatiotemporal degradation and growth. Localized, cell-mediated degradation over time is another possible solution, though complicated in terms of design compared to hydrolytic degradation. It is to be noted that while this might provide the necessary conditions for growth at the cellular scale, well connected and distinct cell clusters with a high cluster contrast, c_f can improve growth conditions at the cluster scale similar to the findings in this article. Cell-mediated degradation can be achieved by replacing crosslinks with peptides that are sensitive to enzymes released by the cell^{4,46–48}. The resulting localized degradation region around the cells therefore allows for ECM transport and tissue growth without significantly compromising its overall properties. Several experimental studies have been published on PEG hydrogels with enzyme sensitive peptides for cartilage regeneration^{49–54}. Modelling efforts have been helpful in providing a qualitative understanding of the different processes and characteristics of growth in degradable hydrogel scaffolds with the use of a multi-phasic model^{23,55,56}. Enzymatically degradable hydrogels were studied in detail by Akalp et al.²¹ from a modelling standpoint to provide insights on the hydrogel degradation characteristics and design suggestions for successful tissue growth. Thus, both types of degradation mechanisms (i.e., hydrolysis and enzyme-mediated) when tailored with appropriate degradation kinetics are potential candidates for tissue growth using encapsulated cells. It is finally important to mention that tissue engineering strategies usually aim to use stem cells for encapsulation. The differentiation of these cells to the appropriate type depends on bio-chemical and mechanical cues⁵⁷ that can be provided by the hydrogels. In this context, computational models of cell mechano-sensitivity^{58–61} based on mixture theories⁶², transport and mechanics of

interfaces^{63–65} or structural models^{66,67} can be used to understand the role of gel properties on differentiation. Such approaches can be combined with the gel degradation-tissue growth model proposed in this article to tune hydrogel design that induces appropriate cell differentiation and subsequent tissue growth. This will be the object of future studies. By tuning the hydrogel degradation with neo-tissue growth, which is cell-specific, it may be possible to design hydrogels that are personalized to individual patient.

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Appendix

A. Experimental Data

Juvenile bovine chondrocytes, harvested from the femoral condyles and femoral patellar groove of 1–3 week old calves, were encapsulated at increasing cell densities (50, 100, and 150 million (M) cells per mL) in PEG hydrogels. Synthesis of the PEG_{8-arm,20k}-caprolactone-norbornene (PEG-cap-NB) macromer was carried out as previously described in Neumann et al.¹³. The number of caprolactones per PEG arm was determined to be 1.26 with 64.5% norbornene conjugation through nuclear magnetic resonance spectroscopy. In brief, the polymer precursor solution consisted of 10 wt% (g/g) PEG-cap-NB combined with PEG dithiol (MW 1000) at 1-to-1 stoichiometry of thiol to norbornenes 0.05 wt% (g/g) Iracure 2959 photo-initiator in phosphate-buffered saline (PBS). The precursor was sterilized through a 0.2 μm filter. The solution was polymerized with UV light (352 nm, 5 mW/cm²) for 7 minutes into cylinders (5 mm diameter, 2.5 mm height). The degradation rate constant (κ) was determined from experimental studies. Sterile acellular PEG-caprolactone hydrogels were placed in chondrocyte growth medium and samples were removed at different times. Each sample was measured for the compressive modulus and volumetric swelling ratio, which were then used to determine the crosslinking density at each point. Hydrogels were compressed at 0.5 mm/min until reaching 15% strain. The compressive modulus was taken as the linear region between 10 and 15% strain from the stress/strain curve. The volumetric swelling ratio was determined from the mass swelling ratio (wet weight/dry weight) of each construct using 1.0 g/mL for the solvent density and 1.07 g/mL for the polymer density. The rate constant was the decay constant for the crosslink density over time, which was fit to an exponential function. The ratio of the crosslinking density near reverse gelation and the initial crosslinking density was used to estimate the beta parameter.

Cluster Properties from Microscopy Images

An image analysis of microscopic images taken from different locations of the sample is carried out. A small data collection window is traversed over the image that counts the number of cells enclosed by the window and estimates f in that region. Thus, a spatial distribution of f is obtained from which clusters are located by identifying regions of high f . The average cluster parameters, F and c_f , are then calculated based on the locations and shape of the clusters. We assume that the cluster parameters have the same values when interpolated in 3D. The cluster parameters are averaged over several images of the same sample obtained from different locations.

Determination of R_d

The average cluster parameters found from the histology images of different samples are used to simulate the average modulus of the construct, E_M , at initial time for different values of R_d as shown in Fig. 11. The approximate value of R_d can be estimated by matching the average simulated modulus, E_M , with the average modulus obtained from experiments at initial time.

The estimate of R_d by this process is an approximate value since we are assuming that R_d is the same for all cells. This approximate value is sufficient to estimate other quantities of the system. Because of the standard deviation in the modulus estimate from the experiments, we have a standard deviation and average value for R_d as well as shown in the plot.

The value of R_d was found to be 27 times that of the cell radius.

Additional Notes

The value of homeostatic ECM concentration, c_m^0 , shown in Table 1 of the article was estimated as follows. The concentration of ECM for seeding density of 150 million cells/mL at 12 weeks after which the hydrogel had completely degraded was measured. This value was then scaled by linear interpolation of the average measured construct modulus at 12 weeks ($= 65 \text{ kPa}$) with respect to native cartilage modulus ($\approx 700 \text{ kPa}$ ⁶⁸) to get the homeostatic ECM concentration.

B. Non-dimensionalisation and Modelling Parameters

In order to simplify the computations and data interpretation we non-dimensionalise different quantities that take part in the equations. The length and time parameters after non-dimensionalisation are given by

$$x^* = \frac{x}{L}, \quad t^* = \kappa t \quad (\text{A.1})$$

where L is the characteristic length and depends on the length scale of the particular problem. Here, the average size of the RVE is the length scale that is considered. Given the

non-dimensional time parameter, the resulting non-dimensional degradation rate constant is given by $\kappa^* = 1$. Based on Eqs. 1, 3 and 4, the non-dimensional cross-link density and concentration parameters are given by

$$\rho_x^* = \frac{\rho_x}{\rho_x^0}, \quad c_m^* = \frac{c_m}{c_m^0}, \quad c_s^* = \frac{c_s}{c_m^0} \quad (\text{A.2})$$

where ρ_x^0 is the cross-link density at initial time and c_m^0 is an estimated maximum concentration of ECM that is chosen based on the production. The definition of the above non-dimensional parameters when substituted in Eq. 3 naturally lead to the following

$$D_e^* = \frac{D_e}{\kappa L^2}, \quad k_e^* = \frac{k_e}{\kappa} \quad (\text{A.3})$$

The elastic moduli of the different materials are normalized with that of the pure hydrogel.

$$E_{tissue}^* = \frac{E_{tissue}}{E_{gel}}, \quad E_{cell}^* = \frac{E_{cell}}{E_{gel}}, \quad E_m^* = \frac{E_m}{E_{gel}}, \quad E_M^* = \frac{E_M}{E_{gel}} \quad (\text{A.4})$$

The parameters used for non-dimensionalisation are given in Table 2 along with other model parameters obtained from experiments and literature.

C. Inter-cellular distance

The expression for the average distance between the cell surfaces of spherical cells randomly distributed by a homogeneous Poisson process is derived from the distance between their centers as

$$d_c = D_c - 2R_c \quad (\text{B.1})$$

where d_c is the distance between cell surfaces, D_c is the distance between cell centres and R_c is the cell radius. The distance between cell centers, D_c , can be obtained approximately based on the volume fraction, f , by considering a sphere of diameter, D_c , whose surface passes through the two cell centers at diametrically opposite locations. This implies that approximately half the volume of each spherical cell falls inside the bigger sphere spanning the distance between the cell centers, given $D_c \gg 2R_c$. The volume fraction is then given by

$$f \approx \frac{\frac{4}{3}\pi R_c^3}{\frac{4}{3}\pi (D_c/2)^3} = \frac{(2R_c)^3}{D_c^3} \quad (\text{B.2})$$

Rewriting (B.2) in terms of D_c and substituting in (B.1),

$$d_c \approx 2R_c(f^{-1/3} - 1) \quad (\text{B.3})$$

D. Averaging Operations

Spatial average of construct properties over the RVE is given by

$$\langle \alpha \rangle = \frac{1}{\bar{V}} \int \alpha dV \quad \langle \langle \alpha \rangle \rangle = \frac{1}{\bar{\bar{V}}} \int \langle \alpha \rangle dV \quad (\text{C.1})$$

where $\alpha = \{\rho_x, c_s\}$ and $\bar{V}$ is the volume of the RVE in the cellular scale, $\bar{\bar{V}}$ is now the volume of the macroscale RVE.

Homogenization for Construct Modulus

The average stress derived from homogenization^{30,31} methods is given by

$$\overline{\sigma_{ij}} = \frac{1}{\bar{V}} \int \sigma_{ij} dV = \frac{1}{\bar{V}} \int \frac{1}{2} (\tau_i x_j + \tau_j x_i) dS \quad (\text{C.2})$$

where $\bar{V}$ and $\bar{S}$ are the volume and boundary surface of the appropriate RVE, indices i and j can take values 1,2,3 representing the directions. The vector $\mathbf{x}$ represents the position vector and $\boldsymbol{\tau}$ represents the traction vector at the boundary surface of the RVE.

Fig. 1.

(a). Representative immunohistochemical images taken from confocal microscopy for three different seeding densities at different time points (n=3). Collagen II is stained green and nuclei are counterstained with DAPI (4',6-diamidino-2-phenylindole) and shown in blue. The scale bar represents 200 μm for all images;(b). Compressive modulus (measured at 10–15% strain; see Appendix A) at 0,4 and 12 weeks as a function of culture time. Data are presented as mean with standard deviation as error bars (n=3). The red square, blue circle and green triangle correspond to cell densities 50, 100 and 150 million cell/mL, respectively.

Fig. 2. Diffusion of fluid unlinked ECM and deposition of solid linked ECM (network connectivity). Diffusion is restricted by the mesh size of the hydrogel.

Fig. 3.

Model prediction of the normalized elastic modulus, $\langle E^* \rangle$, for experiments with cell volume fraction of (a). 50 million cells/mL, (b). 150 million cells/mL. The red shaded region represents the failure region. (signifying the region below hydrogel reverse gelation)

Fig. 4.

(a). Experiment Vs model prediction of modulus at initial time assuming uniform cross-link density, (b) Schematic of the initial region of hydrogel degradation of radius R_d around the cell surface. (c) Experiment Vs model prediction of modulus at initial time when $R_d = 27R_c$, where R_c is the cell radius. Experiments are represented by dots with error bars and model predictions are represented by shaded bars.

Fig. 5. Schematic of hydrogel cross-link density degradation around a cell when (a) $R_d = 0$ and (b) $R_d = 2R_c$. The cross-link density is shown in 1D with the assumption of spherical symmetry around the cell. The quantities t_1 and t_2 represent two different points of time after initiation of degradation. The red shaded region represents the critical cross-link density region corresponding to reverse gelation

Fig. 6.

Evolution of gel connectivity, ψ_g , ECM connectivity, ψ_m , and normalized elastic modulus at the cellular scale, E_m , for an overlap index, $\eta < 1$ with (a). $\eta = 0$, $R_d = 0$, $f = 0.15$, and (b). $\eta = 0.93$, $R_d = 2R_c$, $f = 0.032$ where R_c is the cell radius. The grey shaded regions highlight the transition from hydrogel to neo-tissue. The red shaded region represents the failure region. (signifying the region after reverse gelation)

Fig. 7.

Evolution of gel connectivity, ψ_g , ECM connectivity, ψ_m , and normalised elastic modulus at the cellular scale, E_m , for $\eta > 1$ at (a). $\eta = 3.65$, $f = 0.075$, and (b). $\eta = 10.8$, $f = 0.32$, both with, $R_d = 5R_c$ where R_c is the cell radius. The grey shaded region highlights the transition from hydrogel to neo-tissue. The red shaded region represents the failure region. (signifying the region after reverse gelation)

Fig. 8.

(a) Microscopy image of live cells (green) and dead cells (red) showing cell clustering when embedded in the hydrogel (Scale bar represents 100 μm). The clustering is depicted in 3D with a RVE illustrating cells present in clusters with volume fraction, f_{cl} , and background with volume fraction, f_{bg} . A representative cluster depicts the cluster volume, V_c , (b) A plot of the cluster connectivity, Ψ , shows the critical value of $F = 0.05$, that marks percolation transition.

Fig. 9.

Evolution of average growth, $\langle c_m^* \rangle$ and modulus, E_m^* , of cluster and background phases and the overall construct for (a) $F = 0.01 < F_c$, (b) $F = 0.05 \approx F_c$, and (c) $F = 0.30 > F_c$. The contrast parameter, $c_f = 8$ for all cases. The red shaded region denotes the construct failure region.

Fig. 10.

Model Prediction (line plots) Vs Experimental observation (scattered points with error bars representing mean and standard deviation ($n=3$)) of construct modulus for seeding density (a). 50 million cells/mL, (b). 100 million cells/mL, (c). 150 million cells/mL. The red shaded region is the construct failure region.

Fig. 11. Determining R_d from experimental data of modulus at initial time, where R_c is the cell radius.

Table 1**Model Simulation Inputs**

Description	Symbol	Value	Units	Ref.
Hydrogel degradation rate constant	κ	0.066	day ⁻¹	Exp. Data [*]
Network connectivity	β	0.096	-	Exp. data
Radius of chondrocytes	R_{cell}	5	μm	²¹
Hydrodynamic radius of ECM	r_e	20,000	nm	³²
Diffusion constant of ECM in pure solvent	D_e	1.703×10^{-8}	mm ² /s	³³
ECM production rate	Q_m^0	2.0×10^{-17}	mol/cell/day	Exp. data [†]
ECM homeostasis concentration	c_m^0	1.52×10^{-3}	mol/L	Exp. data
Characteristic length at cellular scale	L	25	μm	Exp. data
Elastic modulus of chondrocytes	E_{cell}	0.6	kPa	²⁷
Elastic modulus of hydrogel	E_{Gel}	46.5	kPa	Exp. data

^{*} $\kappa = 2.5 \text{ day}^{-1}$ is used in Figs. 6 and 7 for illustration.

[†] Chosen in the same order as measured values in Table 2

Table 2

Construct Properties used for the simulations presented in Fig. 10.

Property	a	b	c
f_h^* (million cells/mL)	50	100	150
F	0.11	0.12	0.10
c_f	2.28	2.31	2.28
f_{cl}	0.028	0.05	0.077
f_{bg}	0.012	0.022	0.034
Q_m^0 (moles/cell/day)	5.0×10^{-17}	5.0×10^{-17}	5.0×10^{-17}

* Seeding density based on pre-swelling volume