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Programmable hydrogels for cell encapsulation and neo-tissue growth to enable personalized tissue engineering

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Abstract

Biomimetic and biodegradable synthetic hydrogels are emerging as a promising platform for cell encapsulation and tissue engineering. Notably, synthetic-based hydrogels offer highly programmable macroscopic properties (e.g., mechanical, swelling and transport properties) and degradation profiles through control over several tunable parameters (e.g., the initial network structure, degradation kinetics and behavior, and polymer properties). One component to success is the ability to maintain structural integrity as the hydrogel transitions to neo-tissue. This seamless transition is complicated by the fact that cellular activity is highly variable among donors. Thus, computational models provide an important tool in tissue engineering due to their unique ability to explore the coupled processes of hydrogel degradation and neo-tissue growth across multiple length scales. In addition, such models provide new opportunities to develop predictive computational tools to overcome the challenges with designing hydrogels for different donors. In this report, programmable properties of synthetic-based hydrogels and their relation to the hydrogel's structural properties and their evolution with degradation is reviewed. This is followed by recent progress on the development of computational models that describe hydrogel degradation with neo-tissue growth when cells are encapsulated in a hydrogel. Finally, the potential for predictive models to enable patient-specific hydrogel designs for personalized tissue engineering is discussed.

Keywords

Hydrogel; Degradation; ECM Synthesis; Computational Models; Personalized Medicine

1. Introduction

Synthetic-based hydrogels that are biomimetic and biodegradable are promising platforms for cell encapsulation and tissue engineering. Hydrogels are highly water swollen polymer networks whose water content can easily exceed eighty percent of their total weight. This high water content mimics most tissues in the human body. Hydrogels that are used to encapsulate cells are typically fabricated from hydrophilic and cytocompatible multi-

functional macromolecular monomers (referred herein as macromers).^[1] Many different natural and synthetic polymers have been functionalized with reactive groups to create a wide range of macromers that are suitable for cell encapsulation.^[2] In addition, biological molecules such as proteins, glycosaminoglycans, and peptides have been functionalized to enable their tethering into a hydrogel.^[3] To create biodegradable hydrogels, chemical bonds that are susceptible to cleavage by water or enzymes can be readily introduced into the crosslinks of the polymer network.^[1] Another promising feature is that hydrogels are often amenable to *in situ* polymerization in direct contact with living tissue and thus can be used as an injectable platform to deliver cells *in vivo*.^[2,4] Collectively, the synthetic-based crosslinked network serves as the structural base with which to control many of the macroscopic properties and degradation profiles, while the incorporated biological molecules create an environment that mimics the native extracellular matrix (ECM) of tissue. Thus, synthetic-based biomimetic and biodegradable hydrogels enable encapsulation of cells in hydrogels with programmable macroscopic properties (e.g., mechanics, swelling, and transport), degradation profiles, and chemistries (e.g., polymer properties) that can be tailored to a particular cell type to promote neo-tissue growth (Figure 1A).

While promising, challenges exist with designing degradable hydrogels for cell encapsulation and tissue engineering. A successful tissue engineering strategy that employs a scaffold containing cells must transition from a state that is initially comprised solely of the scaffolding material to a state that is comprised purely of neo-tissue. Ideally, this transition is seamless such that the structural integrity of the construct is maintained throughout the process. When cells are encapsulated in a hydrogel, the crosslinked network entraps the cells within the three-dimensional polymer matrix. This polymer is composed of a mesh-like network that contains pores on the nanometer length scale (Figure 1A). This size scale is smaller than many of the extracellular matrix (ECM) macromolecules that make up tissues. Thus, this mesh network restricts transport of the large ECM molecules through the hydrogel.^[5] To achieve a seamless transition, degradation of the polymer network must closely couple to the synthesis, transport and assembly of ECM molecules. This requirement presents a major challenge when considering the activity of cells. For example, age, genetics, sex, ethnicity, health status, and level of activity of the donor are all factors that can influence activity of cells and subsequently their rates of ECM synthesis.^[6-8] Given the high variability among donors, a universal hydrogel design will be insufficient to achieve successful tissue engineering across all donors.

An approach that enables programmable, patient-specific hydrogels has the potential to overcome the shortcomings associated with donor variability and improve tissue engineering outcomes for all. Such an approach would, however, be difficult and labor intensive using experimental determinants only. This is further complicated by the fact that no donor is alike. On the contrary, computational models have the capability to personalize tissue engineering. Computational models provide the unique opportunity to explore the coupled processes of hydrogel degradation and neo-tissue growth across multiple length-scales (Figure 1B). This knowledge can then be used to develop predictive computational tools for tissue engineering. However to create a successful computational model that predicts patient-specific hydrogel designs, a fundamental understanding of the hydrogel structure and its corresponding properties, their evolution in space and time, and their impact on growth of

neo-tissue is necessary. The focus of this progress report is to a) review programmable properties of synthetic-based hydrogels and their relation to the hydrogel's structural properties and their evolution with degradation and b) describe recent progress that has been made to develop computational models that describe neo-tissue growth when cells are encapsulated in a hydrogel and recent lessons learned. Significant efforts have been made by our group and others to study the role of the hydrogel structure and degradation on neo-tissue growth for cartilage tissue engineering. This progress report uses cartilage cells (i.e., chondrocytes) encapsulated in synthetic-based degradable hydrogels and cartilage neo-tissue growth as an example. The fundamental principles presented herein, however, are applicable across many cell types that are embedded within an ECM including but not limited to cartilage, bone, skin, tendon, heart valve tissue, and the blood vessel wall.

2. Programming Hydrogel Properties

Hydrogels consist of crosslinked polymer chains that swell in aqueous solvents. The network structure and the interaction of the polymer chains with water ultimately dictate the macroscopic properties of hydrogels. These properties include the degree of swelling, which describes the amount of water the hydrogel imbibes, the mechanical behavior of the hydrogel such as stiffness, and diffusive transport of molecules through the hydrogel. Thus, the chemistry and crosslink density define how the hydrogel functions in a given environment. As crosslinks are cleaved during degradation, the hydrogel's crosslink density evolves over time leading to structural changes in the hydrogel and subsequent changes to the macroscopic properties. By controlling the initial crosslink density and the choice of degradable bond, the desired macroscopic properties and their evolution with time can be programmed for a given hydrogel chemistry. The following sub-sections review the constitutive equations that relate hydrogel structure via crosslink density to hydrogel properties and their evolution over time during degradation.

2.1 Hydrogel Structure

The combined theories of equilibrium swelling and rubber elasticity have been extensively used to elucidate the structure of hydrogels, specifically crosslink density (ρ_x) from the macroscopic properties and to describe transport of biologically relevant molecules through the hydrogel.^[10,11] From Flory-Rehner theory,^[12] crosslink density or ρ_x described in moles of crosslinks per volume of polymer is determined from the equilibrium volumetric swelling ratio (Q) and the polymer-solvent interaction parameter (χ_{12}) given by

$$\rho_x = \frac{[\ln(1 - v_{2,s}) + v_{2,s} + \chi_{12} v_{2,s}^2]}{V_1 (v_{2,s}^{1/3} - v_{2,s}/2)} \quad (1)$$

where $v_{2,s}$ is the polymer volume fraction or $1/Q$ and V_1 is the molar volume of solvent (i.e., water). It is important to note that the equation as written assumes dangling chain ends can be neglected. The polymer-solvent interaction parameter depends on chemistry and has been shown to be sensitive to polymer volume fraction and polymer molecular weight.^[13–16] The mechanical properties of a hydrogel and specifically the shear modulus (G) of a swollen

hydrogel have been related to its crosslink density (ρ_x) and equilibrium volumetric swelling ratio (Q) by^[17]

$$G = \rho_x RT / Q^{1/3} \quad (2)$$

where R is the ideal gas constant and T is the absolute temperature. Finally, diffusivity of molecules through a hydrogel (D_g) has been estimated using a free-volume approach described by Lustig and Peppas by^[18]

$$D_g = D_\infty \left(1 - \frac{r_s}{\xi}\right) \exp\left(-Y \left(\frac{1}{Q-1}\right)\right) \quad \text{for } r_s \leq \xi \quad (3)$$

and

$$D_g = 0 \quad \text{for } r_s > \xi \quad (4)$$

where D_∞ is the diffusivity of the diffusing species in the swelling solvent, r_s is the hydrodynamic radius of the diffusing species, Y is the ratio of the critical volume required for a successful translational movement of the diffusing species to the average free volume per molecule of the liquid for which a reasonable approximation is one, and ξ is the mesh size of a swollen hydrogel, which describes the average distance between two adjacent crosslinks. The solute of interest diffuses through the hydrogel only when its radius is less than or equal to the mesh size. The mesh size is related to the equilibrium volumetric swelling ratio and crosslink density by^[19]

$$\xi = \left(\frac{n\ell C_n \rho^P}{M_r}\right) \frac{Q^{1/3}}{\sqrt{\rho_x}} \quad (5)$$

where n , ℓ and M_r are the number of bonds, average bond length, and molecular weight per repeat unit, C_n is the characteristic ratio of the polymer, and ρ^P is the density of the polymer. Taken together, these constitutive equations describe that with an increase in the degree of crosslinking, ρ_x , for a given polymer chemistry, the shear modulus increases, the equilibrium swelling and hence water content decreases, and diffusivity is reduced.

The initial degree of crosslinking can be controlled through the selection of macromers and formulation conditions. Decreasing the molecular weight and/or increasing the functionality of a macromer leads to higher crosslinking. For example, increasing the molecular weight of a poly(vinyl alcohol) macromer from 16,000 to 27,000 g/mol while maintaining the same functionalization (i.e., methacrylation in this example) per molecule led to a ~60% lower compressive modulus.^[20] Increasing the functionalization of a poly(vinyl alcohol) macromer from four to seven methacrylate groups per molecule increased the compressive modulus 3-fold.^[20] The concentration of macromer in solution prior to hydrogel formation offers

another mechanism to control the initial crosslink density. Specifically, the presence of solvent molecules affects crosslinking efficiency. Most notably, this leads to cyclization, a phenomenon that occurs during polymerization when intramolecular reactions are favored over intermolecular reactions resulting in cycles instead of effective crosslinks.^[21] For example, increasing the concentration of a poly(ethylene glycol) dimethacrylate macromer in solution prior to polymerization from 10 to 40% by weight increased the compressive modulus 40-fold.^[22] Similarly, increasing the concentration of the macromer, 4-arm PEG functionalized with vinylsulfones, from 5 to 40% along with its corresponding PEG dithiol crosslinker, led to a 10-fold increase in shear modulus.^[23] In step-growth polymerizations, such as thiol-ene polymerizations, the ratio of thiol to 'ene' is another mechanisms to control the degree of crosslinking.^[24] Thus, through simple changes in the choice macromer and formulation conditions, the initial crosslink density can be tuned which in turn enables programming of the initial macroscopic properties including swelling, mechanical, and transport properties.

2.2. Hydrogel Degradation

An important programmable component to the design of hydrogels used for cell encapsulation and tissue engineering is degradation (Figure 2A). Degradation can be programmed into a hydrogel by engineering in labile bonds that are sensitive to, for example, water^[25] or enzymes (e.g., secreted by cells^[26,27] or exogenously delivered^[28]). Regardless of the type of labile bond, crosslink density decreases over time corresponding to changes in macroscopic properties of swelling, mechanical, and transport properties. Hydrogels that undergo bulk degradation lead to relatively uniform changes in crosslink density throughout the hydrogel. On the contrary, hydrogels that degrade locally due to release of enzymes by encapsulated cells lead to complex degradation patterns that vary in space and time. Mathematical models have been developed to describe structural changes (e.g., ρ_x) in the hydrogel during degradation as a function of time.^[29–33] When combined with the constitutive equations described above, evolution of the macroscopic properties can be predicted as a function of time.^[30–33]

2.2.1. Bulk Hydrogel Degradation—The simplest form of degradation is bulk degradation. Hydrolytically degradable hydrogels are the most common type of hydrogel that leads to bulk degradation where chemical bonds such as esters are incorporated into the crosslinks and undergo chain scission by water. Esters have been introduced during the synthesis of various macromers using chemistries such as poly(l-lactide),^[25] ϵ -caprolactone,^[34] and succinic anhydride.^[35] The overall degradation behavior of the hydrogel is dependent on several factors that include the hydrolysis kinetics of the labile bond, concentration of water in the case of high crosslinked hydrogels ($Q < 10$),^[29,30] and the structure of the hydrogel. The latter influences degradation through the number and location of crosslinks in the polymer backbone and the presence of imperfections in the network (e.g., cyclization).^[29,30,36] Statistical-kinetic models have been developed that combine hydrolysis kinetics, network structure, and network imperfections to predict the evolution of crosslink density as a function of time.^[29,30,36,37] When combined with the constitutive equations described previously, macroscopic properties are predicted. For example, modeling results predicted an increase in Q with time for hydrogels formed from a step-

growth polymerization of multi-arm PEG macromers functionalized with vinylsulfones and PEG dithiol macromers. The increase in Q was slower when the number of vinylsulfone groups per macromer was increased.^[30] The model predicted the experimental behavior by using the same hydrolysis kinetic constant for the labile bond but accounting for differences in the initial network structure and how it evolves during degradation.^[30] This example of experimental and modeling results is shown in Figure 2B. Bulk degradation can also be achieved in enzyme-sensitive hydrogels, but only under conditions when diffusion of the enzyme is rapid such that the concentration of enzyme within the hydrogel is constant and uniform throughout the hydrogel.^[38]

A characteristic feature of bulk degrading hydrogels is the transition from a crosslinked hydrogel to a highly branched soluble polymer (Figure 2C). Initially during degradation, crosslinks are cleaved leading to a reduction in crosslink density, but minimal changes in polymer mass. As more crosslinks are cleaved smaller polymer chains (e.g., crosslinks) are eroded from the hydrogel and polymer mass is lost. Eventually, a point is reached where there is no longer a sufficient number of crosslinks to maintain a solid structure and the remaining highly branched chains dissolve resulting in a sharp increase in mass loss. This phenomenon is referred to as reverse gelation. The point at which reverse gelation occurs can be controlled through several factors. For example, in chain-growth polymerization of multi-functional macromers, reverse gelation can be shifted to a higher mass loss if the length of the kinetic chains is increased (Figure 2C).^[36] The length of the kinetic chain can be controlled through the rate of initiation by decreasing initiator concentration, for example. This leads to a lower number of, but longer propagating chains (referred to as the kinetic chains). In step-growth polymerization, the point of reverse gelation is directly related to network connectivity, which has been described by a statistical network formation model.^[39] By changing the functionality of each macromer (e.g., A-terminated macromer and B-terminated macromer) and/or the stoichiometric ratio (e.g., A to B), the point at which reverse gelation occurs can be tuned. Moreover, mix-mode polymerizations that combine both chain and step growth mechanisms provide further tunability over the time at which reverse gelation occurs and the mass loss at reverse gelation.^[40] In both chain-growth and step-growth polymerizations, increasing solvent concentration induces cyclization, which leads to fewer effective crosslinks. Thus causes a decrease in the number of crosslinks per chain, which in turn affects reverse gelation.^[29,36] Thus by controlling how the crosslinked network structure forms it is possible to control the point at which reverse gelation occurs.

2.2.2. Localized Hydrogel Degradation—Enzyme-sensitive hydrogels that degrade by cell-secreted enzymes have emerged as a promising hydrogel platform due to their ability to be tuned to a particular cell type and/or the cell's function. The degradation behavior, however, is spatially and temporally complex. In these hydrogels, enzymes are synthesized by encapsulated cells, released on their boundary and transported into the hydrogel surrounding the cells. Diffusion of the enzyme through the hydrogel depends on the relative size of the enzyme to that of the hydrogel's mesh size and has been modeled by Fickian diffusion.^[33,41] At the same time, enzymes react with their substrate (i.e., enzyme labile bonds), which leads to hydrogel degradation. This reaction has been modeled by Michaelis-Menten kinetics.^[33,42] A competition, thus, arises between the characteristic times of

enzyme diffusion and enzyme reaction. This complex spatiotemporal degradation behavior has been described using a combined experimental and computational approach.^[33] The computational approach is a multiphase mixture model that incorporates the constitutive equations for equilibrium swelling and rubber elasticity theories combined with Michaelis-Menten kinetics.^[31,33,41,43] Three characteristic patterns of spatiotemporal degradation emerge depending on the competition between diffusion and reaction mechanisms (Figure 3). When reaction mechanisms dominate (Figure 3A), enzymes cleave polymer chains in the region immediately near the cell boundary leading to localized reverse gelation of the hydrogel. Beyond this region, the crosslink density sharply increases over a short distance until it reaches the bulk crosslink density of the hydrogel. A degradation front forms that travels over time and in space away from the cell boundary. The speed at which the front travels and its width can be characterized. If both diffusion and reaction mechanisms are important (Figure 3B), a concentration gradient in enzyme forms at the boundary of the cell as enzymes diffuse, while at the same time enzymes cleave polymer chains. This leads to a wide degradation front. At the same time, the crosslink density in the bulk hydrogel decreases. A degradation front also forms and travels over time and in space, but concomitant with an overall decrease in the bulk crosslink density. Finally, when diffusion dominates (Figure 3C), enzymes readily diffuse into and through the hydrogel and crosslink density decreases uniformly as a function of time in the hydrogel mimicking that of an isotropic hydrolytically degradable hydrogel.

The governing equations for enzyme reaction (Michaelis-Menten) and diffusion (Fickian) have been coupled to the constitutive equations for swelling and rubber elasticity theories and non-dimensionalized.^[33] Two dimensionless parameters emerged that were identified as having a significant effect on the spatiotemporal degradation behavior of enzyme-sensitive hydrogels and are as follows^[33]

$$\alpha = \frac{r_e \sqrt{\rho_c}}{(Q^o)^{1/3}} \left(\frac{M_r}{3\ell^2 C_n \rho^p} \right)^{1/2} \quad (6)$$

and

$$\kappa = \frac{k_{cat} C_e^o L^2}{D_\infty \rho_c} (Q^o - 1) e^{1/(Q^o - 1)} \quad (7)$$

where Q^o is the initial volumetric equilibrium swelling ratio prior to degradation, k_{cat} is the kinetic constant associated with substrate turn over in Michaelis-Menten kinetics, and D_∞ is diffusivity of the enzyme in solvent. The dimensionless parameter α is a measure of the relative size of the enzyme to the mesh size of the hydrogel. The dimensionless parameter κ describes the competition between reaction and diffusion, relating k_{cat} , enzyme concentration, diffusivity and critical crosslink density. Notably, these two parameters can be used to describe the degradation front with respect to front width and front speed. For example for very high values of κ , the front width is sharp (i.e., narrow front width) and the front velocity is high regardless of α . For small values of α , the front velocities are fast as

enzymes readily diffuse regardless of the value of κ , but the front becomes sharper (i.e., narrow front width) with increasing κ . For large values of α when the size of the enzyme approaches that of the mesh size of the hydrogel front velocity slows, but the front becomes sharper. These two dimensionless parameters are based on the initial hydrogel design and the targeted enzyme and thus offer a powerful tool to tune the spatiotemporal degradation behavior of enzyme-sensitive hydrogels.

3. Coupling Extracellular Matrix Synthesis to Hydrogel Degradation

A successful tissue engineering strategy must support ECM synthesis and its elaboration into a neo-tissue in concert with hydrogel degradation. Due to the small mesh size of the hydrogel, the structure of the hydrogel has a significant impact on the evolution of ECM and neo-tissue growth. Thus, a close coupling of ECM synthesis to hydrogel degradation is necessary to achieve success. The following sub-sections provide evidence for the role of hydrogel structure and hydrogel degradation in ECM elaboration.

3.1. The Role of Hydrogel Structure in ECM Molecule Transport

When tissue-specific cells are incorporated into a hydrogel, the cells are responsible for the synthesis and secretion of ECM molecules that assemble extracellularly to form neo-tissue. One of the main ECM molecules that makes up the structural ECM in nearly all tissues is collagen. Fibril-forming collagens include type I, II, III, V, and XI and are formed from procollagen molecules that are secreted by cells, which then oligomerize extracellularly to form large collagen macromolecules.^[45,46] For example, collagen type I is formed from heterotrimer procollagen molecules consisting of pro- α 1(I) and pro- α 2(I) chains. While procollagen molecules are relative small (e.g., ~1 nm in diameter and ~300 nm in length),^[47] they assemble to form very large ECM molecules with diameters of ~500 nm, but lengths that can reach one cm.^[48] Similarly, collagen type II is formed from procollagen molecules of pro- α 1(II) chains, which have a diameter ~10 nm^[49] when secreted from chondrocytes and then assemble extracellularly to form large fibers. Proteoglycans are also another important component of the structural ECM. For example, aggrecan monomers in cartilage have molecular weights between 1 and 4 MDa,^[50] which assemble extracellularly along a hyaluronic acid polymer chain to form large aggrecan aggregates that reach molecular weights of 100's of MDa and 1–2 μ m in length.^[51] The organization of the ECM also depends on the interaction between different ECM macromolecules. Thus, for cells to regenerate their ECM, precursor molecules that are secreted by cells must be transported extracellularly where they assemble to form the macromolecules that deposit in a highly organized manner to create the new ECM.

One of the challenges when cells are encapsulated in hydrogels is that the secreted ECM molecules must diffuse through the mesh of the hydrogel. Thus, the relative size of the ECM molecules to the mesh size will largely dictate their transport. Studies have identified procollagen molecules (i.e., the precursors secreted by cells)^[52] and proteoglycans^[5] in the culture medium of stable hydrogels containing encapsulated cells. This observation indicates that the smaller precursor molecules are able to diffuse through the hydrogel.^[52] However, large collagen and proteoglycan (e.g., aggrecan) macromolecules have been shown to be

restricted to the pericellular space between the cell boundary and the hydrogel,^[5] indicating that the macromolecules are unable to diffuse into the hydrogel. These observations are supported by the fact that the size of the macromolecules is much larger than typical mesh size of the hydrogel. Examples of transport of small ECM molecules and restricted transport of large ECM molecules are shown in Figure 4 for chondrocytes encapsulated in a stable PEG hydrogel with a mesh size of ~15 nm. In addition, smaller ECM precursor molecules that are able to diffuse into the hydrogel may not be able to assemble within the hydrogel because their concentration and concentration of supporting molecules that are necessary to facilitate assembly become too low with increasing distance from the cell to facilitate assembly.

3.2. The Role of Hydrogel Degradation in ECM Molecule Transport

With degradation, a reduction in crosslink density leads to a concomitant increase in mesh size of the hydrogel. However, the mesh size still remains too small for most large ECM macromolecules to diffuse through the hydrogel. In addition, increased diffusion leads to an even further decrease in the concentration of the precursor molecules within the hydrogel. As a result, it is only when the hydrogel reaches reverse gelation that transport of large ECM molecules is possible. Thus, coupling ECM synthesis to hydrogel degradation is critical. Ideally, the transfer of mechanical support by the hydrogel to that of the neo-tissue is seamless resulting in a continuous maintenance of the overall integrity of the construct and its corresponding mechanical properties. Three potential scenarios of this transition from hydrogel to neo-tissue are depicted in Figure 5A along with corresponding evidence from experimental data in Figure 5B–D. In stable or very slow degrading hydrogels, neo-tissue growth is restricted to the pericellular space and the overall modulus of the construct is dictated by the hydrogel as minimal neo-tissue growth is possible. This is consistent with experimental observations.^[28,53] For example, when chondrocytes were encapsulated in a stable PEG hydrogel, the overall compressive modulus was maintained with time (Figure 5B).^[53] When degradation is not matched to neo-tissue growth, a large drop in the modulus of the construct occurs as the hydrogel degrades, but ECM remains restricted until the hydrogel reaches reverse gelation. This is consistent with experimental observations.^[53] For example, when chondrocytes were encapsulated in a hydrolytically degradable PEG-co-poly(lactic acid) hydrogel formed from dimethacrylate macromers a 10-fold drop in modulus was reported over the course of four weeks (Figure 5C).^[53] To overcome the challenge of matching ECM synthesis with reverse gelation, other approaches have investigated hydrogels that contain degradable crosslinkers of different degradation kinetics.^[54,55] For example, fast degrading crosslinks were used to enable hydrogel degradation, but a small fraction of slow degrading crosslinks were incorporated as a means to maintain the hydrogel in a state just before reverse gelation.^[54] Even with substantial degradation, the large collagen macromolecules remained restricted to the pericellular space due to the fact that the hydrogel had not undergone reverse gelation. Another approach used low concentrations of exogenously delivered enzymes to control the timing of bulk degradation, where a small bout of enzyme treatment at week four led to a 2-fold increase in the compressive modulus from week 6 to 8.^[28] Nonetheless, studies have reported achieving a seamless transfer in mechanical properties.^[34] For example, when chondrocyte were encapsulated in a hydrolytically degradable PEG-co-caprolactone hydrogel formed from

thiol and norbornene multifunctional macromers a steady increase in modulus was reported (Figure 5D).^[34]

Enzyme sensitive hydrogels due to their spatiotemporal degradation behavior enables greater control over degradation. Several studies have reported promising results leading to macroscopic tissue elaboration when cells are encapsulated in enzyme-sensitive hydrogels.^[56–58] Since the degradation behavior is complex, mathematical models offer a platform from which to explore a much wider parameter space than is possible with experiments. To better understand the spatiotemporal degradation of enzyme-sensitive hydrogels coupled with ECM synthesis and elaboration, a multiphasic model has been developed.^[41,43] In this model, the impact of the competition between enzyme reaction and enzyme diffusion on ECM elaboration in an enzyme sensitive hydrogel with encapsulated cells was investigated.^[43] Select results from this study are shown in Figure 6A. In Figure 6A*i*, a mixed reaction-diffusion mechanism leads to elaboration of ECM surrounding the cell, but which grows as the degradation front of the hydrogel evolves. At the same time, however, a decrease in the overall crosslink density is observed in the bulk hydrogel which leads to a decrease in the mechanical properties. In Figure 6A*ii*, a reaction dominated mechanism leads to elaboration of the ECM surrounding the cell, which also grows as the degradation front of the hydrogel evolves. However, in this condition, the bulk crosslink density is maintained and thus the mechanical properties will remain high.

4. Computational Models for Tissue Growth in Degradable Hydrogels

4.1 Modeling strategies

Theoretical models are extremely useful to understand the key mechanisms of hydrogel-mediated growth and eventually tune hydrogel design for optimal tissue development. Several examples have already been provided in the preceding sections. The mechanics of growth has traditionally been described with continuum approaches that can quantify the competition between the rate of hydrogel degradation and the cell-mediated release, transport and deposition of new tissue. For tissue engineering, it is particularly important to construct models that have the ability to provide a link between microscopic processes and the overall growth dynamics of the construct.^[60] For instance, Treweek *et al.*^[61] proposed a multispecies model of cell-mediated growth in cartilage constructs, pointing out the distinct roles of advection and diffusion fluxes at the microscopic level. A link between transport and mechanics can further be found in refined formulations based on the theory of mixture^[62] and poro-elasticity.^[63] Here, tissues are seen as a mixture of interacting solid and fluid phases. For the past several decades, the literature has shown that such formulations, through their ability to couple mechanics, transport and chemical reactions within a single consistent framework^[59,63,64] are ideal to investigate the homeostasis and growth of biological tissues at a fundamental level.

Despite the apparent maturity of these formulations, it is still not clear today, how to decouple biological processes such as cell activity,^[65] and physical processes driven by transport, assembly and possible degradation of the hydrogel scaffold. This is even more challenging when the scaffold is subjected to finite deformation and possess ever-changing chemical and mechanical properties.^[66] Addressing this issue is of primary importance since

a majority of physical processes can be controlled by the scaffold (e.g., hydrogel) design. This is evident with synthetic hydrogels whose chemistry and mechanics can be accurately controlled. A promising strategy resides in the derivation of multiscale models that can predict the temporal evolution of the construct (macroscale) from the knowledge of the activity of a single cell (micron-level) and the scaffold (e.g., hydrogel) design (molecular level). In a nutshell, such models start by specifying the synthesis rates Γ_e and Γ_m of degrading enzymes (e) and ECM molecules (m), respectively, from the cell membrane. The effect of these molecules on neo-tissue growth is then captured at the molecular scale by constitutive laws that relates the change in construct composition due to hydrogel degradation (e.g., hydrolytic or enzymatic), enzyme and ECM transport (related to the evolving mesh size of the hydrogel) and the deposition of new ECM.

The coupled degradation, transport, and deposition processes yield the general diffusion-reaction equations of the form:

$$\frac{D\rho_x}{Dt} = -k_h\rho_x - k_{cat}c_e \frac{\rho_x}{\rho_x + K_m} \quad (8)$$

$$\frac{\partial c_\alpha}{\partial t} = D_\alpha(\rho_x)\nabla^2 c_\alpha - \gamma_\alpha \quad (9)$$

subjected to the boundary condition, $J_\alpha(t) = \Gamma_\alpha(t)$ at the cell boundary. The flux (J) boundary condition for species α is given in moles per unit time per unit area and is equal to the rate of synthesis (denoted by Γ) of species α by the cell, which is given in moles per unit time per surface area of the cell. The first equation describes the change of the hydrogel's crosslink density ρ_x via hydrolytic degradation at rate k_h and enzyme-mediated degradation, as captured by the Michaelis-Menten kinetics with rate constants, k_{cat} and K_m . The second equation describes the transport of chemical species with diffusivity D_α and used $\alpha = e$ for enzymes and $\alpha = m$ for ECM molecules. Note that the term $\gamma_\alpha(\alpha = m)$ represents the rate of conversion of ECM molecules between their free (or fluid) state and linked (or solid) state. An important feature of this system arises from the fact that molecular diffusivity D_α is coupled with crosslink density (see Equations 3, 4 and 5). This dependency of enzyme and ECM transport on the rate of hydrogel degradation may, in some cases lead to the emergence of highly localized degradation and growth profiles around cells.^[42,43] To further understand how this affects the hydrogel's mechanical integrity, one can numerically solve Equations 8 and 9 to predict the evolution of the hydrogel/ECM mixture surrounding a small group of cells,^[40] in a representative volume element such as that shown in Figure 1B.

The knowledge of the spatial and temporal composition of the construct, as modeled by Equations 8 and 9, may indeed serve at evaluating the local mechanical properties via the concentration-dependent elasticity of the hydrogel (in terms of ρ_x) and the ECM (in terms of concentration of linked ECM molecules).^[42] In the case of small deformation, a linear

elasticity relation can be used to relate the stresses σ_p and σ_m in the polymer (p) and ECM (m), respectively as follows:

$$\sigma_p = 2G \left(\frac{\nu}{(1-2\nu)} \text{tr}(\varepsilon_p) \mathbf{1} + \varepsilon_p \right) \quad (10)$$

$$\sigma_m = c_s (\lambda \text{tr}(\varepsilon_m) \mathbf{1} + 2\mu \varepsilon_m)$$

where ε_p and ε_m are the corresponding strains while G is defined in Equation 2 and ν is the Poisson's ratio for the hydrogel. The coefficient λ and μ are the Lamé constants representing the elasticity of the newly deposited matrix (expressed in Pa per mole of linked ECM). These equations describe the loss in hydrogel stiffness with degradation (as captured by the term ρ_x) and the rise in stiffness with ECM deposition (as captured by the term c_s representing the concentration of solid ECM). Local properties can then be translated to the macroscale (construct scale) using appropriate averaging operations over the representative volume element. Eventually, this strategy enables the prediction of the construct's composition (hydrogel and ECM and their spatial distribution) and the corresponding construct's integrity (for instance, measured by its compressive modulus) in time during the neo-tissue growth process. This process is highlighted in Figure 1B.

4.2. Coupling Experiments and Modeling to Identify New Mechanisms

We have seen that neo-tissue growth in a hydrogel with encapsulated cells is highly coupled to the network structure and degradation behavior of the surrounding hydrogel. Furthermore, modeling can provide spatial and temporal information on the hydrogel and separately on the ECM across multiple length-scales, which is difficult if not impossible to discern through experimental methods alone. Thus, by combining experiments and computational approaches, new mechanisms may emerge that are highly sensitive to the process of interest.

Recently, the presence of heterogeneities within a cell-laden hydrogel was identified as being an important factor in facilitating tissue growth.^[58,67] Heterogeneities emerged via two predominant mechanisms:

- a. Spatial variation in the hydrogel's crosslink density occurs around encapsulated cells. In this mechanism, a decrease in crosslink density surrounding cells was identified, which can arise during cell encapsulation in radical-mediated polymerizations where cells act as chain transfer agents to quench propagating radicals.^[68–70] This decrease in crosslink density was also manifested in an overall decrease in the mechanical properties of the hydrogel post encapsulation, which could not be explained by the incorporation of soft cells. Several studies have reported a lower modulus with the presence of cells.^[21,58,71]
- b. A non-uniform cell distribution occurs when cells were encapsulated in a degradable PEG hydrogel that was formed by a radical-mediated polymerization.^[58,67] In this mechanism, regions of cell clustering at the time of encapsulation

have been observed where cells were in close proximity to each other (*e.g.*, less than one cell diameter or a distance $\sim 10\ \mu\text{m}$).^[33,58,67,72] As an example, a region of a cell cluster of chondrocytes encapsulated in an enzyme-sensitive hydrogel and the local evolution of ECM elaboration over culture time is shown in Figure 6B.^[58] Interestingly, diffusion mechanisms dominated the enzyme degradation behavior due to the low enzyme secretion rates concomitant with a low initial crosslink density; yet an interconnected ECM formed between adjacent cells in cell clusters.

Importantly, the above multi-phasic model was able to capture experimental results when spatial variations in crosslink density and non-uniform cell distribution were considered.^[58,67] The presence of these heterogeneities can also explain the promising results that have been reported for neo-tissue growth by cells encapsulated in hydrolytically degradable hydrogels. Notably, the data shown in Figure 5D, which reports a seamless transition in mechanical properties from hydrogel to neo-tissue, can be achieved with the combination of spatial variations in local crosslink density surrounding the cells and the relative high cell concentration (100 million cells per ml of precursor solution) that was used.^[33]

Heterogeneities have the potential to have profound effects on neo-tissue growth. In clusters, cells are located close together such that regions of reduced crosslinking overlap. As water molecules hydrolyze labile bonds or as cells secrete enzymes that cleave labile bonds, the hydrogel in these regions quickly undergoes reverse gelation providing space for ECM macromolecules to assemble and form a connected ECM between cells. Therefore in the regions of high cell density, it is possible for degradation to match neo-tissue growth (*i.e.*, Scenario 3 in Figure 5A). When cells are spaced far apart, degradation behavior differs for hydrolytically and enzymatically degradable hydrogels. In hydrolytically degradable hydrogels, the initial spatial variation in hydrogel crosslinking surrounding cells spans only a short distance while the remaining hydrogel is at a uniform crosslink density. In this scenario, a dramatic loss in mechanical properties will occur over time as ECM interconnectivity is not achievable until the hydrogel undergoes reverse gelation (*i.e.*, Scenario 2 in Figure 5A). In enzymatically degradable hydrogels where enzymes are secreted by encapsulated cells, the initial spatial variation in hydrogel crosslinking surrounding cells can instead propagate due to the concentration gradient in enzyme. In this scenario, degradation can be matched to neo-tissue growth (*i.e.*, Scenario 3 in Figure 5A). As cell-laden hydrogels can have regions of cell clusters and regions of non-clusters of cells, the degradation patterns that evolve in space and over time rapidly become complex. Thus, computational models play an important role in understanding how ECM evolves as hydrogel network structure evolves with degradation. Once validated, computational models can be used to more effectively design hydrogels such that the ideal scenario is achievable where hydrogel degradation matches to neo-tissue growth.

5. The Future: Computational Models to Personalize Hydrogel Designs for Improved Tissue engineering

While computational models are critical for identifying potential growth mechanisms and guiding experimental effort, tomorrow's models are expected to play a major role in

enabling personalized tissue engineering. A change in cell activity may strongly affect growth and degradation dynamics of a construct; this means that each individual, according to genetics, age, gender, health, etc. is likely to need a different hydrogel to regenerate damaged tissue. Due to the sensitivity of the hydrogel design on growth, computational models, such as those discussed herein, are therefore invaluable to not only explore how changes in hydrogel designs affect growth for a particular cell, but to further optimize the scaffold design. A vision of the process is illustrated in Figure 7 in which cells would be sampled from specific patients and their activities monitored and analyzed in a few preselected hydrogels. Once quantified, these activities can be used as an input in the computational model such that different growth outcomes can be predicted by exploring the hydrogel design space. Using optimization techniques, a best design can be identified in order to maximize an objective function that favors a high growth rate and high construct's mechanical properties over time. The predicted design can finally be fabricated and injected into the patient.

To enable this technology, new generation of computer models need to be developed, with the capacity to be trained on large experimental datasets in order to become predictive. Indeed, the predictive power of computational models relies on two key pillars: 1) the accuracy of the mechanisms that it can capture (for instance, hydrogel degradation, ECM transport and assembly) and 2) the accuracy of the physical parameters (such as crosslink density) that cannot be measured through direct experiments. An accurate evaluation of these parameters and their statistical distribution has been and is still a recurrent issue. Although, a solution may be found in models that “actively learn” from experimental data with the goal of objectively evaluating the value of physical parameters and improve their predictive power. The development of such algorithms could rely on classifying model parameters in two ways.^[15] First, the design parameters refer to physical properties of a material (hydrogel) that can be experimentally tuned. Second, the latent parameters refer to properties that drive mechanical and transport phenomena but cannot be easily controlled or measured. The targeted macroscopic outputs refer to quantities that can be measured experimentally. The objective of a self-learning algorithm^[73] will therefore be to determine the set of latent parameters that minimizes the distributional distance between experimental and modeling outcomes for all values of design parameters. Such a problem can now be tackled with existing machine learning techniques,^[74,75] raising the hope that computationally-based personalized medicine could soon become a reality.

6. Conclusions

Overall, synthetic-based degradable hydrogels are promising scaffolds to deliver cells *in situ* through minimally invasive methods. However, as cells are encapsulated in the hydrogels, unique challenges arise due to the requirement that hydrogel degradation must be closely coupled to ECM synthesis, transport and deposition. This challenge can be mitigated in part by relying on computational models that can identify key parameters, such as reverse gelation, that are critical to optimizing hydrogel designs for neo-tissue growth. Furthermore, by combining experimental approaches with computational methods, new insights emerge. For example, recent studies have identified that heterogeneities in network structure provide new knobs that can be used to tune hydrogel degradation with neo-tissue growth. Thus,

computational models provide an important component for advancing hydrogel designs for cell encapsulation and tissue engineering. Their ability to decipher the complex and interrelated processes between hydrogel degradation and neo-tissue growth, where experiments are unable, is critical. Current models of hydrogels relate structure and chemistry to macroscopic properties that include mechanics, swelling, and transport. Moreover, there are a number of hydrogel parameters that can be tuned (e.g., polymer properties, initial hydrogel structure, degradation kinetics, and reverse gelation) to achieve optimal neo-tissue growth. Clinical translation, however, will rely on *in vitro* and *in vivo* experimental development and identification of chemistries (including precursors and the degradation products) that are biocompatible. Moreover, computational models that include different polymer chemistries as well as the fundamental structure-function relationships as described herein will be critical to expanding hydrogel design. An important role of computational models in the near future is their ability to enable patient-specific hydrogel designs. Ultimately, predictive models provide the opportunity to create personalized hydrogel designs that will enable tissue engineering for patients of all ages and health status.

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Figure 1. Cell encapsulation in degradable hydrogels and neo-tissue growth

A) A schematic of the process of cell encapsulation in biomimetic and biodegradable hydrogels formed from multi-functional macromers. A variety of multi-functional macromers with different functional groups, labeled as F, has been designed and investigated for cell encapsulation. Common examples of hydrogels formed by chain-growth polymerizations include acrylates and methacrylates functionalized polymers, which react to form long chains of polyacrylates or polymethacrylate, respectively, that are referred to as kinetic chains. Hydrogels formed by step-growth polymerization require the reaction between two unique functional groups. Common examples include vinyl (e.g., acrylate, vinyl sulfone, norbornene) functionalized polymers that react with thiol (i.e., SH) functionalized polymers. During polymerization, a polymer network forms around cells and encapsulates them in space. Closely coupled processes of hydrogel degradation and ECM synthesis, transport and deposition lead to the formation of neo-tissue. B) Computational models offer the ability to study the coupled degradation and growth mechanisms governing hydrogel mediated tissue growth. They include estimations of local and global hydrogel degradation and matrix deposition and the evolution of the construct's mechanical properties (compressive modulus is shown here). Reproduced with permission from The Royal Society of Chemistry.^[9]

Figure 2. Characteristic degradation behavior of bulk degrading hydrogels

A) A schematic of a crosslink (i.e., insoluble) network, where crosslinks are initially cleaved leading to increases in swelling, but minimal mass loss. Next, mass is lost where soluble polymer chains are released, but an insoluble network remains that continues to swell. Eventually, there are no longer a sufficient number of crosslinks to maintain a 3D network and the hydrogel transitions to highly branched and soluble polymer chains. B) Experimental (points) and modeling (line) results for equilibrium mass swelling ratio (q) as a function of time for a PEG hydrogel formed from a step-growth polymerization of multi-arm PEG vinyl sulfone and PEG dithiol macromers with increasing vinyl sulfone functional groups per molecule of three (diamonds), four (squares), and eight (circles). Reproduced with permission.^[30] C) Example of characteristic mass loss profiles as a function of degradation time. Modeling results are shown for mass loss as a function of time for a poly(vinyl alcohol) (PVA) hydrogel formed from chain polymerization of multi-vinyl PVA macromers with increasing kinetic chain length from lines (a) to (b) to (c). Reproduced with permission.^[33] A statistical-kinetic model was used in (B) and (C).

Figure 3. Spatiotemporal degradation patterns of enzyme-sensitive hydrogels

A pictorial representation and modeling results for three different degradation regimes of enzyme-mediated degradation of A) reaction mechanisms dominate diffusion, B) reaction and diffusion mechanisms are important, and C) diffusion mechanisms dominate reaction. A multi-phasic modeling approach was used and the data are adapted with permission from Skaalure et al.^[33]

Figure 4. Hydrogel structure via the mesh size (ξ) dictates transport of ECM macromolecules through the hydrogel

In this example of cartilage ECM molecules, small aggrecan monomers and/or fragments of aggrecan monomers staining positive for chondroitin sulfate (red) are seen in the pericellular region and diffuse through the hydrogel. On the contrary, large aggrecan macromolecules (red) and collagen II (green) are restricted to the pericellular region. Here, chondrocytes were encapsulated in a PEG hydrogel with a mesh size of 15 nm and the corresponding compressive modulus (E) and volumetric swelling ratio (Q) are also given. The cell-laden hydrogels were cultured for four weeks and stained by immunohistochemical techniques where nuclei were counterstained with DAPI (blue). Scale bar is 50 μm . Reproduced with permission.^[5]

Figure 5. The evolution of modulus as a function of culture time for cell-laden hydrogels

A) Three different scenarios are depicted. Scenario 1 illustrates restricted tissue growth that is common in stable (i.e., non-degrading) hydrogels. Scenario 2 illustrates the evolution of modulus when degradation is not matched to tissue growth. Scenario 3 illustrates the evolution of modulus when degradation is closely matched to tissue growth. In each scenario, the modulus is depicted for the entire construct (blue line) and its individual components consisting of the hydrogel (gray) and the neotissue (red). B) Experimental evidence for Scenario 1 where chondrocytes were encapsulated in a stable PEG hydrogel. Reproduced with permission.^[53] C) Experimental evidence for Scenario 2 where chondrocytes were encapsulated in a hydrolytically degradable hydrogel formed by chain-growth polymerization of poly(lactic acid)-*b*-PEG-*b*-poly(lactic acid) dimethacrylate macromers. Reproduced with permission.^[53] D) Experimental evidence for Scenario 3 where chondrocytes were encapsulated in a hydrolytically degradable hydrogel formed by step-growth polymerization of 8-arm PEG-*co*-oligo(caprolactone) endcapped with

norbornene and PEG dithiol macromers. Reproduced with permission.^[34] In B–D, representative confocal microscopy images are shown for collagen II (green) and nuclei (blue) by immunohistochemical techniques. Scale bar is 50 μm . In B–C, the compressive modulus represents the slope of the linear region of the stress-strain curve between 10 and 15% strain. In D, the compressive modulus represents the secant modulus at 15% strain.

Figure 6. Coupled ECM Synthesis and Hydrogel Degradation for Enzyme-Sensitive Hydrogels

A) Modeling results demonstrate the spatiotemporal response in the concentration of free ECM (c_m), which describes ECM precursor molecules that are able to diffuse, concentration of linked ECM (c_m^*), which describes the ECM molecules that have assembled into macromolecules and deposited, and the hydrogel's crosslink density (ρ_x) as a function of time and space. Reproduced with permission from The Royal Society of Chemistry.^[43] B) A combined experimental and computational campaign of chondrocytes encapsulated in an enzyme-sensitive hydrogel. This work identified two types of heterogeneities in the cell-laden hydrogel, which were necessary for the model to explain the experimentally observed data. The heterogeneities included the presence of cell clustering, which is shown in the 1 day immunohistochemical image, and spatial variation in hydrogel crosslink density in the

immediate vicinity of the cell (as shown in the representative volume element (RVE) at day 1 of crosslinking). Representative immunohistochemical images from confocal microscopy that are stained for aggrecan (red) and nuclei (blue) are shown for day 1 and week 12. Scale bar is 50 μm . Simulations results are of RVEs for crosslink density and ECM at day 1 and week 12. Reproduced with permission.^[59] A multiphasic model combined with finite elemental analysis was used in A and B.

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Figure 7. A Proposed Approach for Personalized Hydrogel Design in Tissue Engineering

In this approach, a biopsy of tissue would be taken from the patient and their cells isolated. These autologous cells would be analyzed for their cellular activities. Once quantified, these activities can be used as an input in the computational model such that different growth outcomes can be predicted by exploring the hydrogel design space. Using optimization techniques, a best design can be identified in order to maximize an objective function that favors a high growth rate and the target outcome (e.g., high construct's mechanical properties over time). The predicted design can finally be fabricated and used to deliver autologous cells into the patient where the 3D hydrogel serves as a temporary support to facilitate neo-tissue growth.