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Modeling the dynamic composition of engineered cartilage

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Abstract

Mathematical models to describe extracellular matrix (ECM) deposition and scaffold degradation in cell–polymer constructs for the design of engineered cartilage were developed and validated. The ECM deposition model characterized a product-inhibition mechanism in the concentration of cartilage molecules, collagen and glycosaminoglycans (GAG). The scaffold degradation model used first-order kinetics to describe hydrolysis (not limited by diffusion) of biodegradable polyesters, polyglycolic acid and polylactic acid. Each model was fit to published accumulation and degradation data. As experimental validation, cell–polymer constructs ($n = 24$) and unseeded scaffolds ($n = 24$) were cultured in vitro. Biochemical assays for ECM content and measurements of scaffold mass were performed at 1, 2, 4, 6, 8, or 10 weeks ($n = 8$ per time point). The models demonstrated a strong fit with published data and experimental results ($R^2 = 0.75$ to 0.99) and predicted the temporal total construct mass with reasonable accuracy (30% RMS error). This approach can elucidate mechanisms governing accumulation/degradation and may be coupled with structure–function relationships to describe time-dependent changes in construct elastic properties.

Keywords: Cartilage; Mathematical model; Tissue engineering; Extracellular matrix; Biosynthesis; Biodegradable polymers

Articular cartilage is a dense, highly hydrated tissue found on the ends of long bones. It provides a low-friction surface for joint motion, aids in the transfer of load between bones, and absorbs some of the compressive stresses associated with joint motion. Damage to the articulating surfaces can occur via traumatic injury or arthritic disease, leading to loss of joint function and pain. The material properties of articular cartilage emerge from an extracellular matrix (ECM)[2] rich in aggrecan, collagen type II, and glycosaminoglycans (GAG), and a relatively simple tissue structure makes articular cartilage suitable for study as an engineered tissue [1]. Understanding the regulatory mechanisms of matrix deposition and remodeling in both native and engineered tissues may contribute to the development of an improved cartilage tissue replacement.

Some published methods of engineering cartilage include seeding chondrocytes onto scaffolds made of hydrogels such as alginate and agarose, or biodegrdable polymers, such as polylactic acid (PLA) or polyglycolic acid (PGA), or a biopolymer such as collagen [2–6]. During in vitro or in vivo culture, the composition of these cell–polymer "constructs" evolves from low to high ECM content and from high to low scaffold content. The scaffold stabilizes construct mechanics and cell phenotype until the chondrocytes synthesize and remodel new tissue. Over time, the scaffold degrades and the newly formed tissue continues to develop. Methods of predicting the temporal changes in construct composition, with regard to scaffold and new tissue mass, would be useful in the design and evaluation of engineered tissues [7]. In addition, predictive parametric models could be used to study the effects of scaffold material properties and degradation on chondrocyte behavior and matrix deposition.

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[2] *Abbreviations used:* ECM, extracellular matrix; GAG, glycosaminoglycans; PLA, polylactic acid; PGA, polyglycolic acid; FCD, fixed charge density; MMPs, matrix metalloproteinases; HA, hyaluronate.

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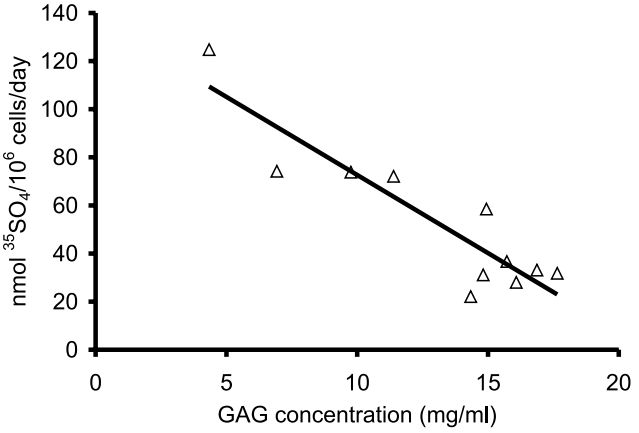

Fig. 1. The product inhibition mechanism as demonstrated in cartilage synthesis. Dose-dependent decrease in GAG synthesis rate (as measured by radiolabeling) concomitant with an increase in GAG concentration [2].

A representative data set from a study of engineered cartilage [2] shows that as the concentration of GAG in the construct increases with time, the GAG synthesis rate drops. A strong negative correlation ($R^2 = 0.82$) between concentration and synthesis rate was found (Fig. 1). This response has also been observed in chondrocytes cultured in monolayer with exogenous proteoglycans and was shown to be similarly dose dependent [8]. The negative correlation between synthesis rate and concentration of GAG in the matrix suggests that the deposition of GAG and perhaps other matrix molecules is governed by a negative feedback loop. We hypothesize that there is a "target" or steady state concentration of each matrix molecule that chondrocytes seek to maintain for a given set of biomechanical, physicochemical, and culture conditions. As the concentration of each matrix molecule approaches the target concentration, net production of the molecule is downregulated by reducing synthesis and/or increasing turnover. The expected kinetics of the hypothesized mechanism are consistent with product inhibition and a growing body of data demonstrating an exponential, asymptotic increase in the content of structural matrix molecules with time in culture.

The biochemical and physical parameters regulating matrix deposition are under investigation. GAG and collagen molecules, when densely packed around a cell, provide a mechanically competent native tissue-like housing, capable of changing pericellular osmolarity, permeability, and pH [9]. These local physicochemical changes, which gradually occur with matrix molecule accumulation, may control cell metabolism by interfering with nutrient uptake and waste withdrawal, adjusting cell volume, or changing ion channel activity [9,10]. Chondrocytes have also been shown to alter their biosynthetic activity in response to static and dynamic compression and hydrostatic pressure, in both engineered constructs and explants [11–14]. The mechanisms of this apparent mechanotransductive response are unclear and may involve deformation of the cell membrane or nucleus, generation of streaming potentials, or compaction of the deposited matrix.

Chondrocytes respond to the mechanics and chemistry of their environment by changing the rates and ways in which matrix molecules are synthesized and deposited. The understanding of this process and the manipulation of it through mechanical bioreactors is often the focus for new construct designs. It has been shown that cyclic compression of cartilage explants and engineered cartilage constructs can stimulate or suppress GAG or protein synthesis, depending on the frequency and magnitude of applied stress [15,16]. Static compression, in contrast, generally suppresses synthesis of proteoglycans and protein [4,10,15]. In addition, chondrocytes respond to changes in pH and osmolarity of the interstitial fluid, which may be related to the tissue's fixed charge density (FCD) and water content [10,17]. Compaction of the ECM during compression brings charged groups on GAG and collagen molecules closer together, resulting in a local increase in the fixed charge density. The local increase in FCD influences local pH and osmolarity, which in turn, may trigger a biosynthetic response through cell-surface ion channels or changes in cell volume. Similarly, cells may be sensitive to changes in ECM composition or material properties through integrins or other cell-surface attachment molecules.

The aim of this study was to develop mathematical models that describe the accumulation of GAG and collagen and the degradation of a polymeric scaffold in an engineered cartilage construct. The models are based on hypothesized mechanisms governing these processes and are intended to guide future studies in the biophysics of cell metabolism and cell–matrix interactions. The presented models also provide a framework to reduce the many influencing variables affecting engineered constructs into a few comparable parameters. The combination of the ECM accumulation and scaffold degradation models are combined to predict changes in overall tissue mass and composition with time. In the future, these models may be extended to structure–function relationships to estimate the mechanical properties of maturing engineered cartilage constructs.

Materials and methods

Model formulation

A first-generation matrix accumulation model based on product inhibition is proposed and can be stated mathematically as

$$\frac{d[ECM]}{dt} \propto [ECM]_{SS} - [ECM], \quad (1)$$

where the rate of net synthesis of a matrix molecule, $d[ECM]/dt$, is proportional to the difference between a steady state (SS) concentration of that molecule ($[ECM]_{SS}$) and its current concentration. A rate constant, k, is substituted to form an equation,

$$\frac{d[ECM]}{dt} = k\{[ECM]_{SS} - [ECM]\}, \quad (2)$$

which upon rearrangement and integration,

$$\int \frac{d[ECM]}{[ECM]_{SS} - [ECM]} = k \int dt, \quad (3)$$

yields the following first-order model equation for matrix molecule accumulation:

$$[ECM](t) = [ECM]_{SS}(1 - e^{-kt}). \quad (4)$$

Once k is substituted with one over a characteristic time constant, τ, the final ECM model equation is

$$[ECM](t) = [ECM]_{SS}(1 - e^{-t/\tau}), \quad (5)$$

where $[ECM]_{SS}$ and τ are adjustable parameters and may be dependent on system factors including cell type and seeding density, presence of growth factors, scaffold geometry and chemistry, culture conditions, and mechanical or other physicochemical parameters. Each matrix component (collagen, GAG, etc.) is assumed to accumulate independently.

A variety of scaffold materials for use in engineered tissues have been investigated [18–20]. Hydrogels and poly(α-hydroxy esters) are some of the most rigorously researched polymers since they exhibit long-term biocompatibility and have degradation kinetics that can be tailored to compliment neo-tissue growth kinetics [21]. For the purposes of this study, the scaffold degradation model describes the behavior of some poly(α-hydroxy esters). Polyglycolic acid, polylactic acid, and their copolymers have been shown to support chondrocyte attachment and neo-tissue formation in vitro and in vivo [18,22–25]. These polymers undergo hydrolysis in which chain scission occurs at the ester bonds, releasing monomers of glycolic or lactic acid [21]. Under conditions in which diffusion is not greatly limited by scaffold geometry, porosity, or culture conditions, the rate of monomer release (and thus mass loss) is proportional to the amount of scaffold material remaining [26]. In this way, scaffold degradation obeys first-order decay kinetics:

$$\frac{d[Scaffold]}{dt} \propto [Scaffold]. \quad (6)$$

A rate constant, k, is introduced to form an equation,

$$\frac{d[Scaffold]}{dt} = -k[Scaffold], \quad (7)$$

which upon rearrangement and integration,

$$\int \frac{d[Scaffold]}{[Scaffold]} = -k \int dt, \quad (8)$$

yields the following first-order model equation:

$$[Scaffold](t) = [Scaffold]_0 e^{-kt}. \quad (9)$$

Again, k is replaced by $1/\tau$ for the final scaffold model equation:

$$[Scaffold](t) = [Scaffold]_0 e^{-t/\tau}. \quad (10)$$

The initial scaffold mass, $[Scaffold]_0$, is known, leaving τ as the only adjustable parameter in this model. An important assumption is that degradation is independent of neo-tissue formation and cell activity. It is also significant that this model is designed to describe the behavior of systems in which there are no major barriers to diffusion of water into the scaffold or monomers out of the scaffold, since these phenomena may affect polymer degradation kinetics.

The processes of scaffold degradation and matrix accumulation occur simultaneously. As the scaffold degrades, new tissue is deposited in its place. To assess the maturity of an engineered construct, it is useful to model the total mass as a function of culture time. Given the previously described assumption of independence between degradation and accumulation, a preliminary approach to modeling the total construct mass is the simple addition of the scaffold degradation and matrix accumulation models. This approach is hypothesized to be capable of describing the total engineered cartilage construct mass as a function of time, where construct mass, $M(t)$ is given as

$$M(t) = \text{cell mass} + [GAS]_{SS}(1 - e^{-t/\tau_{GAG}}) + [Collagen]_{SS}(1 - e^{-t/\tau_{Collagen}}) + [Scaffold]_0 e^{-t/\tau_{Scaffold}}. \quad (11)$$

Important assumptions for this model include (1) cell mass is constant and (2) matrix accumulation and scaffold degradation are mutually independent processes. Mathematically, the behavior of the total construct mass is governed by the competing degradation and accumulation models.

Experimental validation methods

Twenty-four cell–polymer constructs and 24 polymer-only scaffolds were prepared as previously described [28]. Briefly, bovine articular chondrocytes were harvested from the femoropatellar and glenohumoral grooves of a 2- to 3-month-old calf (A. Arena Brothers, Hopkinton, MA), within 6 h of slaughter. Cells were liberated via a 0.3% collagenase digestion overnight at 37 °C on a horizontal shaker, counted, and stored in medium (Ham's F-12, 10% heat-inacti-

vated fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B) at 37 °C for <48 h. Scaffolds were made from nonwoven polyglycolic acid fleece (Albany International Research, Mansfield, MA). A 1/2-inch-diameter machinist's punch was used to cut 48 circular patches approximately 1 mm thick [28]. The patches were immersed in a 1% w/v poly-L-lactic acid solution in methylene chloride for 10 s and allowed to dry for at least 10 min. The scaffolds were weighed, sterilized in ethanol for 30 min, and allowed to dry in a desiccator for at least 24 h prior to cell seeding. Twenty-four scaffolds were prewet and incubated in medium for at least 6 h prior to seeding. The scaffolds were then incubated with a suspension of 2.5 million cells in 1 mL of medium for 12 h at 37 °C on a horizontal shaker. Once seeded, the constructs (cells + scaffold) were transferred to 12-well plates for culture under standard incubation conditions in 3 mL medium, with medium changes every 2–3 days. Unseeded scaffolds were prewet and incubated under identical conditions. Twenty-four constructs were cultured for 1, 2, 4, 6, 8, and 10 weeks ($n = 3$–4 per time point) in vitro. Concomitantly, 24 polymer-only scaffolds ($n = 4$ per time point) were incubated in medium to measure degradation kinetics.

At the end of construct culture and scaffold incubation, wet and dry weights were obtained, and the constructs were digested in papain (0.125 mg/mL) for biochemical analysis. Sulfated-GAG content was measured via the dimethylmethylene blue spectrophotometric method [29]. Collagen content was measured indirectly through a spectrophotometric hydroxyproline assay [30] using a Hy-pro:collagen ratio of 1:10. DNA content, an indicator of cell number and viability (7.7 pg DNA/cell), was measured by the Hoechst 33258 dye method [31]. Finally, the GAG, collagen, and scaffold masses were normalized to construct dry weight for modeling and analysis.

Model fitting and statistical methods

Data on matrix accumulation and scaffold degradation were gathered from previously published reports and from the results of the described experiment. These data sets provided a means to validate the model application to engineered cartilage and representative scaffold materials. For the published data, image analysis software (Photoshop v5; Adobe Systems, San Jose, CA) was used to determine mean and standard deviations directly from graphical plots or, when available, directly from tables.

The accumulation and degradation models were fit to average data for each culture time by finding least squares estimates of the time constants (τ_{Collagen}, τ_{GAG}, τ_{Scaffold}) and steady state matrix molecule concentrations ($[\text{Collagen}]_{\text{SS}}$, $[\text{GAG}]_{\text{SS}}$) via an unweighted, quasi-Newton convergence method with spreadsheet software (Excel, Microsoft, Redmond, WA). Initial conditions were assumed: $\tau = 1$; $[\text{ECM}]_{\text{SS}} = 1$. The quasi-Newton algorithm is considered a fast and reliable method of regressing up to as many as 100 parameters [27]. Strength of model fit was assessed via the coefficient of determination (R^2) and root mean square (RMS) deviation, where $\text{RMS} = \sqrt{(\sum(x - \bar{x}))/n}$. Models were considered strong with $R^2 \geqslant 0.80$, while RMS deviation of <50% of the mean were considered reasonably accurate. For analysis of time dependency, an analysis of variance (ANOVA) and Tukey's post hoc test for multiple comparisons were applied (where $p < 0.05$ was considered statistically significant) between culture time groups (SAS Institute, Cary, NC). Graphical data are shown as means ± standard deviation (SD).

Results

The matrix accumulation model was fit to 13 published data sets from a variety of scaffold systems and culture conditions. Nine data sets represent the bulk of available data on the temporal accumulation of GAG and/or collagen. In general, the model described the data for GAG and collagen contents well (Table 1), with coefficients of determination (R^2) ranging from 0.77 to 0.99 and generally low RMS deviations (4.6–37%). The data are reported in several different units, though each matrix molecule mass measurement is normalized to construct volume, mass, or cell count. The strength of model fits and reasonably high accuracies found with these published data sets served as preliminary validation of the model.

The ANOVA and post hoc tests for multiple comparisons revealed the statistical ($p < 0.05$) influence of culture time on cell number (as indicated by DNA content) and ECM accumulation. However, the slight increasing trend in the DNA data (Fig. 2), indicates near-constant cell number with culture time. The resulting matrix accumulation behavior observed in this study (Figs. 3 and 4) is qualitatively similar to that reported by other investigators. The newly synthesized matrix included GAG and collagen in nearly physiologic proportions (~3:1 collagen:GAG). The accumulation model (Eq. (5)) was fit to the data for GAG and collagen contents with strong goodness of fit ($R^2 = 0.95$ and 0.80, respectively) and reasonable accuracy (RMS deviation = 13.5 and 19.8%, respectively).

The degradation model (Eq. (10)) was fit to the results from this study and to five published scaffold data sets indicating dependencies on specific PGA or PGA/PLA chemistry with a variety of initial porosities

Table 1
Results of matrix accumulation model (Eq. (5)) as fit to published and original data

Scaffold	Cell Type	Reference	Matrix component	$[ECM]_{SS}$	τ_{ECM} (Days)	R^2	RMS Dev. %
Agarose	BAC[a]	[2]	GAG	17.2 mg/mL	17.1	0.96	6.6
	Mouse Chondrocytes	[56]	GAG	2.0 mg/mL	24.7	0.77	21.7
PGA	BAC	[57]	GAG	11.7% dw	18.6	0.97	5.4
			Collagen	50.4% dw	131.5	0.98	6.9
	BAC	[58]	GAG	6.8% ww	31.3	0.99	5.9
			Collagen II	3.7% ww	15.9	0.98	6.8
	BAC	[59]	GAG	1.6% ww	22.2	0.99	4.6
			Collagen	2.4% ww	16.7	0.98	9
	BAC	[60]	Collagen	3.5% ww	22.3	0.92	8.1
Alginate	BAC	[61]	GAG	118.3 μg/μg DNA	86.4	0.89	29.0
			Collagen	27.5 μg/μg DNA	17.9	0.77	37.0
	BAC	[4]	Collagen	15.7 μg/μg DNA	22.4	0.89	24.4
Self-assembling peptide	BAC	[62]	GAG	5.9 mg/mL	7.4	0.96	6.1
PGA/PLA	BAC	This study	GAG	6.1% dw	187	0.95	13.5
			Collagen	6.5% dw	18.9	0.80	19.8

[a] Bovine articular cartilage (BAC).

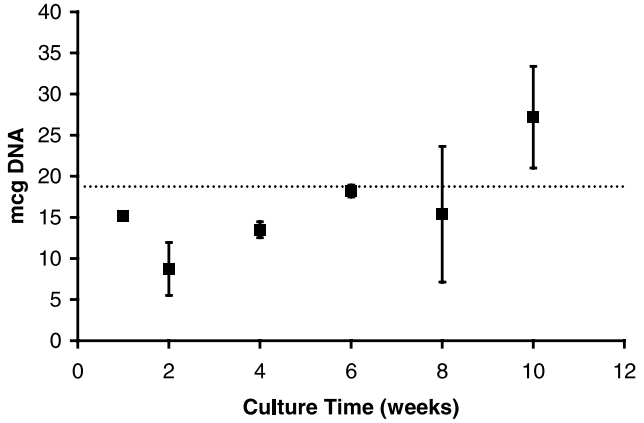

Fig. 2. DNA content of engineered cartilage constructs with time in culture. Dashed line indicates target DNA content from initial cell seeding density. Data reported as means ± standard deviation (sample sizes of $n = 3$–4 per data point).

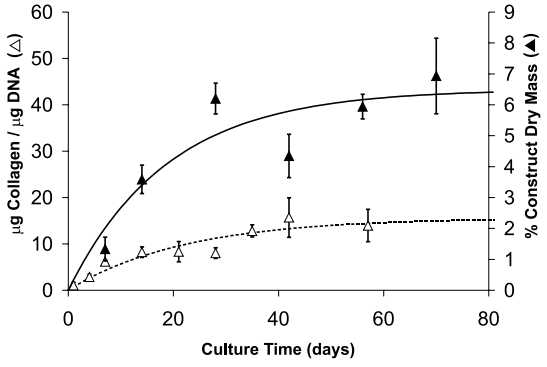

Fig. 3. Normalized collagen accumulation over culture time in engineered cartilage constructs. The model curves describe data measured in this study (▲) and by Ragan et al. [4] (△). Data points are reported as mean with standard deviation error bars (sample sizes of $n = 3$–4 per data point were measured for this study).

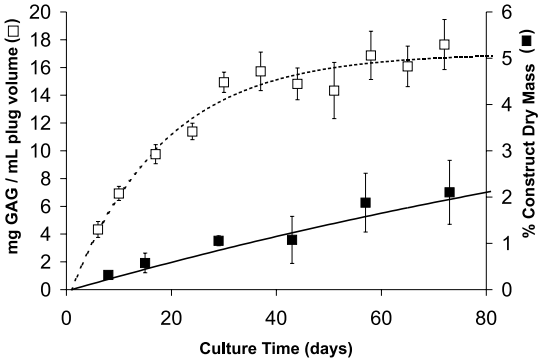

Fig. 4. Normalized GAG accumulation over culture time in engineered cartilage constructs. Modeled curves describe data measured in this study (■) and by Buschmann et al. [2] (□). Data points are reported as means ± standard deviation (sample sizes of $n = 3$ to 4 per data point for this study).

Table 2
Results of scaffold degradation model (Eq. (10)) as fit to published and original data

Scaffold material	Initial scaffold porosity	Reference	$\tau_{Scaffold}$ (days)	R^2	RMS dev %
PGA	97%	[22]	56.1	0.94	13.8
		[58]	24.3	0.99	4.8
Fiber-bonded PGA/PLA	97%	This study	28.1	0.90	27.3
50:50 PLGA	92%	[63]	70.0	0.89	10.1
	73%	[64]	175.1	0.87	18.8
75:25 PLGA	0%	[65]	114.6	0.75	27.5

(Table 2). In general, the degradation model described the data well with coefficients of determination between 0.75 and 0.99 and generally low root mean

square deviations (4.8–27.5%). Overall, the best fits were to data from higher-porosity scaffolds. For scaffolds of relatively low porosity, the model fits were characterized by lower coefficients of determination and higher deviations. The degradation behavior from this study (Fig. 5) was modeled ($R^2 = 0.95$) with moderate accuracy (25% RMS deviation).

The predictive power of the matrix accumulation and scaffold degradation models was evaluated by estimating total construct mass as a function of culture time. Dry mass measurements in this study were independent of the GAG, collagen, and scaffold degradation data measurements, so a comparison of predicted and measured construct masses provided insight into the predictive power of the models. The total mass calculation is an additive combination of the matrix accumulation model (fit to data on GAG and collagen contents), the scaffold degradation model (fit to data on mass of scaffold), and a constant cell mass term (determined to be ~0.21 mg, using 10^{-11} g dry mass/chondrocyte). In general, the total mass model (Eq. (11)) described the data well, with a coefficient of determination of 0.81. A root mean square deviation of 31% indicates that the total mass of an engineered tissue construct can be predicted with reasonable accuracy using data on GAG, collagen, and scaffold contents. The relative contributions of each of the components within the combined model indicate the dominance of the scaffold state during early time points (Fig. 6).

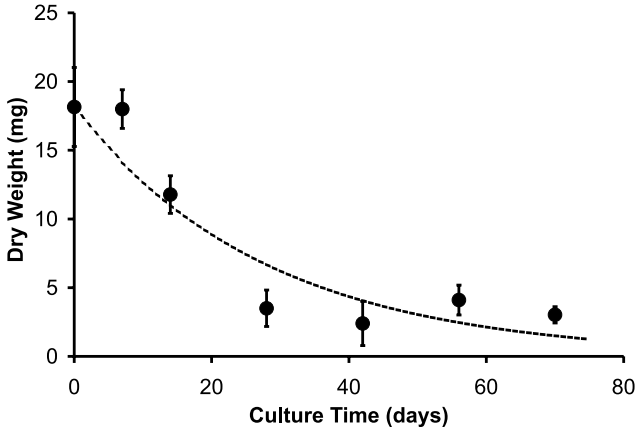

Fig. 5. Time-dependent PGA/PLA scaffold degradation as measured in this study, with the best-fit model curve shown. Data are reported as means ± standard deviation ($n = 4$ samples per data point).

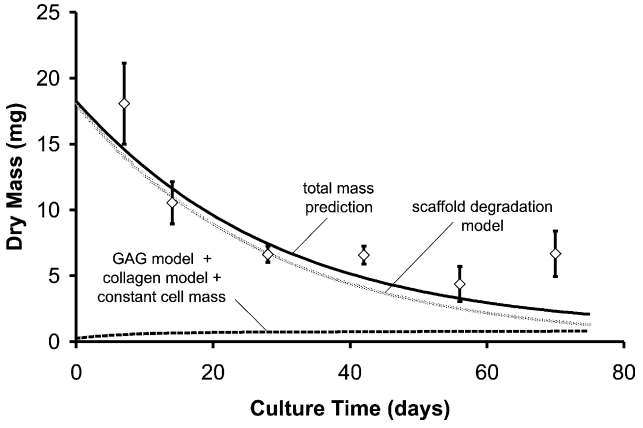

Fig. 6. Total tissue engineered cartilage mass as measured directly from the constructs prepared in this study and predicted using the combined model (Eq. (11)). The contributions of the cell mass and the ECM components (dashed line) are shown relative to the scaffold component (gray line). Data points are reported as means ± standard deviation ($n = 3$–4 samples per data point).

Discussion

A generalized accumulation and degradation model for the description of engineered cartilage is presented. The matrix accumulation model was validated by fitting to 13 published data sets on GAG and collagen, and by experimental data generated in this study. The significance of the models lies in their potential to guide future studies exploring the biophysics of cell metabolism and cell–matrix interactions. Additionally, the consequences of product inhibition kinetics on matrix assembly have been addressed in the context of tissue engineering.

Overall, the model accurately described accumulation of both GAG and collagen (Table 1). The scaffold degradation model was validated by fitting to five published data sets on poly(α-hydroxy ester) degradation kinetics and to data generated with our scaffold. The model was judged to be appropriate for describing the kinetics of poly(α-hydroxy ester) degradation, although it was less useful in lower-porosity systems (Table 2), which often demonstrate an initial lag phase indicative of a transport-limited response. Finally, the models were applied to estimate the total construct mass as a function of time; the total mass calculations described the observed evolution of construct mass well and with reasonable accuracy. It may be noted that of the three time-dependent components described in Eq. (11), polymer degradation dominates the construct mass at initial time points. However, the polymer degradation curve tends toward zero mass at later times, while the total construct mass levels off, indicating the longer-term ECM mass influence.

Although abundant empirical data for engineered cartilage cultured in vivo and in vitro have been reported, a mechanistic approach to interpreting and modeling such data is necessary for its proper use in the rational design of tissue constructs. Galban and Locke [32–34] have proposed several models to describe cell growth, and these models are based on fundamental equations of mass transfer. There are few published

models of collagen deposition or total construct mass for engineered cartilage. With respect to modeling proteoglycan synthesis in a cartilage repair device, one phenomenological model has been proposed [15], and one mechanistic model based on mass transport to chondrocytes has been proposed [35]. In reporting the latter model, which is most relevant to this work, Obradovic et al. [35] hypothesize that regulation of GAG synthesis is governed by oxygen delivery to cells and can be described by equations of mass transfer. The model predicts that as new tissue is formed, permeability of the construct drops and oxygen becomes less available to the cells. The model accounts for spatial variations in oxygen concentration within a cell–scaffold construct (due to local differences in permeability and diffusivity) and can be used to estimate the temporal changes in quantity and location of GAGs within a construct. Importantly, the model is consistent with the product-inhibition behavior predicted by the current model and with the current data.

The mechanistic link between oxygen tension and matrix molecule production remains unclear since the assumption that GAG and collagen production are coupled has not been rigorously tested. Decreasing oxygen tension decreases both collagen and GAG production in bovine articular chondrocytes in monolayer and in cell polymer constructs in vitro [36,37], supporting the assumption of coupled GAG–collagen production. It has also been shown, however, that decreasing oxygen tension downregulates collagen type II gene expression while having little effect on expression of aggrecan in bovine articular chondrocytes in alginate beads [38]. In addition, the differences in characteristic time constants, τ_{ECM}, for paired collagen and GAG data shown here (Table 1) suggest that production of these molecules is not coupled. Oxygen tension plays an important part in governing chondrocyte metabolism, but the individual mechanisms regulating production of collagen and GAG in native and engineered cartilage should be investigated further.

Cell surface proteins contribute to another potential feedback mechanism for regulating chondrocyte metabolism. One class of proteins, the integrins, can form focal adhesions with extracellular matrix molecules such as collagen, fibronectin, and laminin. Integrins have been associated with mechanotransduction and intracellular phosphorylation cascades that alter expression of aggrecan, matrix metalloproteinases (MMPs), and cytokines such as transforming growth factor β [39–42]. Another class of surface proteins, CD44s, binds hyaluronate (HA) and localizes proteoglycans in the pericellular space; CD44–HA binding has been shown to modulate proliferation, HA internalization, and expression of aggrecan and MMPs in chondrocytes [43–47]. From the anchorin class of cell surface proteins, annexins V and A5 have been shown to play an important role in binding collagen and regulating the uptake of calcium by chondrocytes [48,49]. Intracellular signaling pathways are still unclear, but there is growing evidence that suggests that these matrix-binding cell surface proteins allow chondrocytes to "sense" features of their surrounding ECM. Additionally, signal transduction mediated by these proteins can trigger specific metabolic pathways leading to synthesis of matrix molecules, production of degradative enzymes, and secretion of cytokines.

The models proposed here, although simplified, represent a new approach to investigate the mechanisms underlying matrix biosynthesis. There are several potential mediators of a product-inhibition mechanism governing chondrocyte metabolism. Cell sensitivity to tissue pH, osmolarity, and fixed charge density has been demonstrated; in addition, matrix-binding cell surface proteins and cytokine receptors have been implicated in intracellular signaling pathways regulating chondrocyte metabolism. Alternatively, the accumulation of matrix molecules may be governed by mass transfer phenomena. Future models will parametrically relate the kinetics of in vitro neo-tissue formation to events occurring at the cell and molecular levels, potentially allowing for enhanced design and optimization of engineered tissues.

Models describing construct composition may be used with known structure–function relationships to estimate the mechanical properties of developing tissue constructs. Williamson et al. [50] report the statistically significant dependence of native cartilage aggregate modulus on GAG and collagen content and provide an empirical model. Theoretical models for relating tissue mechanics and transport to biochemical composition have also been proposed. Buschmann and Grodzinsky [51] describe the application of Poisson–Boltzman electrostatics theory to the derivation of tissue swelling pressure from GAG concentration. In addition, Quinn et al. [52] treat GAG aggregates as an array of cylinders in a modified classical fluid dynamics model to predict hydraulic permeability; for a free-swelling case, GAG solid volume fraction is the only independent variable in this model. Such models emphasize the dependence of native and neo-tissue mechanical and transport properties on parameters describing biochemical composition.

The resulting parameters summarized in Tables 1 and 2 represent the models' utility in reducing the many influential variables into a few comparable properties. These results also reveal the influence of a variety of factors not controlled in this study. At the scaffold level, these variables represent both structural properties such as mass, porosity, permeability, geometry, and convective state and molecular properties including cross-links and molecular weight. Factors influencing cell adhesion, proliferation, and differentiation, which in turn control

ECM synthesis, include a number of previously noted properties such as cell type and donor characteristics, seeding density, nutrient and waste transport (diffusion, convection), vascularity, static versus dynamic culture, surface chemistry and quality, any available growth factors, and the overall biochemical, biomechanical, and bioelectrical environment. As the performance of the implanted biomaterials and associated cellular activity is dependent upon these factors, future efforts will be made to quantify the influence of specific variables. Most of the noted variables can be defined from the initial state of the engineered tissue and will ultimately influence the characteristic time constants and the steady state matrix content. Experiments that utilize a statistical methodology in which screening for significant parameters alternates with factorial experiments to optimize critical ECM–scaffold parameters will be designed [53,54].

The generalized composition–kinetics models described in this work will be combined with or used to define structure–function models at different organizational hierarchies (cell, tissue, organ). Results of this effort could then describe and predict time-dependent changes in engineered tissue mechanical properties. The ongoing development of the mechanistic model will use a combinatorial approach carried out in a sequential series examining the additional factors [55] to guide the design of functional engineered tissues.

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