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Synthesis rates and binding kinetics of matrix products in engineered cartilage constructs using chondrocyte-seeded agarose gels

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ABSTRACT

Large-sized cartilage constructs suffer from inhomogeneous extracellular matrix deposition due to insufficient nutrient availability. Computational models of nutrient consumption and tissue growth can be utilized as an efficient alternative to experimental trials to optimize the culture of large constructs; models require system-specific growth and consumption parameters. To inform models of the [bovine chondrocyte] – [agarose gel] system, total synthesis rate (matrix accumulation rate + matrix release rate) and matrix retention fractions of glycosaminoglycans (GAG), collagen, and cartilage oligomeric matrix protein (COMP) were measured either in the presence (continuous or transient) or absence of TGF-β3 supplementation. TGF-β3's influences on pyridinoline content and mechanical properties were also measured. Reversible binding kinetic parameters were characterized using computational models. Based on our recent nutrient supplementation work, we measured glucose consumption and critical glucose concentration for tissue growth to computationally simulate the culture of a human patella-sized tissue construct, reproducing the experiment of Hung et al. (2003). Transient TGF-β3 produced the highest GAG synthesis rate, highest GAG retention ratio, and the highest binding affinity; collagen synthesis was elevated in TGF-β3 supplementation groups over control, with the highest binding affinity observed in the transient supplementation group; both COMP synthesis and retention were lower than those for GAG and collagen. These results informed the modeling of GAG deposition within a large patella construct; this computational example was similar to the previous experimental results without further adjustments to modeling parameters. These results suggest that these nutrient consumption and matrix synthesis models are an attractive alternative for optimizing the culture of large-sized constructs.

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1. Introduction

Osteoarthritis (OA) is a debilitating disease causing significant pain and immobility as the cartilage of the diarthrodial joints degrades to expose the underlying bone. The low cellularity and avascular nature of adult articular cartilage contribute to the limited capacity of the tissue to heal from minor injuries and defects, eventually developing into symptomatic OA (Stockwell, 1979). Cartilage tissue engineering (CTE) is a promising strategy targeting the replacement of defective native cartilage with mechanically and biochemically similar engineered cartilage. In CTE, systems usually consist of a cell species embedded in a biocompatible polymeric scaffold (Langer and Vacanti, 1993). The scaffold maintains the cells in a three-dimensional environment with access to a nutrient rich culture media and retains the extracellular matrix (ECM) products synthesized by the cells. In particular, agarose is a well-characterized, bio-inert scaffold that shows great promise in CTE systems as chondrocytes elaborate a functional ECM when cultured in the 3D agarose environment, reaching native levels of glycosaminoglycans (Benya and Shaffer, 1982; Buschmann et al., 1992; Byers et al., 2008; Lima et al., 2007).

A remaining challenge in CTE is the development of clinically relevant-sized tissue constructs, which are required to repair the large surface defects ($>5\ cm^2$) present in symptomatic OA (Hung et al., 2003, 2004; Moisio et al., 2009). Engineered constructs of this size suffer from inhomogeneous ECM deposition as the transport of critical nutrients to the interior of the construct is hindered by consumption by peripheral cells. Lacking a homogenous and structural ECM, these constructs are unable to support physiologic loads and are therefore unlikely to function successfully upon implantation. In an effort to reduce ECM heterogeneity, strategies for enhancing nutrient transport have been employed such as direct media perfusion, dynamic mechanical loading, and

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the introduction of nutrient channels into the tissue (Bian et al., 2009; Buckley et al., 2009; Davisson et al., 2002a,b; Lima et al., 2007; Mauck et al., 2000). However, quantitative predictive methods for optimizing the culture of large-sized constructs remain unexplored.

An overarching aim of our research is to develop and implement continuum growth models to optimize the culture of large-sized constructs with sufficient nutrient availability for ECM deposition throughout the construct's interior. Such models may prove insightful and efficient in analyzing how nutrient distribution and ECM deposition are affected by culture conditions (Nikolaev et al., 2010; Obradovic et al., 2000; Sengers et al., 2004b, 2005; Zhou et al., 2008). System-specific CTE modeling requires knowledge of: (1) the nutrient(s) essential for cell viability and concentrations of the nutrient (s) necessary for tissue growth, (2) consumption rates of the nutrient (s), (3) matrix constituents critical to mechanical integrity, and (4) synthesis rates and binding kinetics for essential ECM constituents. In cartilage, proteoglycans (PGs) and type II collagen are the major ECM structural components. PGs, predominantly consisting of aggregated glycosaminoglycans (GAG), contribute to the compressive stiffness while the fibrillar collagen network contributes to the tensile behavior (Jurvelin et al., 1988; Kempson et al., 1973). Pyridinoline cross-links between collagen fibrils act to retain and strengthen the PG-collagen network (Eyre and Wu, 2005; Williamson et al., 2003a,b). In addition, cartilage oligomeric matrix protein (COMP) is a high molecular weight (~500 kDa) matrix protein that may play a structural role in cartilage given its concentration (0.4%ww) in the tissue and binding affinity for both PGs and type II collagen (Chen et al., 2007; DiCesare et al., 1996; Hedbom et al., 1992; Rosenberg et al., 1998; Roughley, 2001).

Previously, we have identified that tissue growth is diminished at a glucose concentration of 7.5 mM as compared to 25 mM in the [bovine chondrocyte] – [agarose gel] system (Cigan et al., 2013). The present study therefore focuses on the remaining tasks of experimentally measuring ECM synthesis and retention to determine the matrix binding kinetics, measuring the rate of glucose consumption for this system, and refining the glucose concentration threshold required for ECM synthesis. The synthesis rates, retention fractions, and binding constants of GAG, collagen, and COMP were examined under either continuous, temporary (2 week), or no transforming growth factor β3 (TGF-β3) supplementation (Byers et al., 2008; Lima et al., 2007). Pyridinoline content was also assessed under the influence of TGF-β3. Biochemical composition was correlated to the mechanical properties to investigate the ECM's influence in the growth of this culture system. To preclude the confounding influence of heterogeneous nutrient availability present in large constructs, the experimental work of this study was performed with small constructs. To illustrate the utility of these parameters, we modeled the growth of a human patella-sized construct replicating the experiment by Hung et al. (2003), by incorporating glucose transport from a periodically refreshed bath, cellular glucose consumption, matrix synthesis based on glucose availability, and binding and release of matrix products.

2. Materials and methods

Cell isolation, construct culture, mechanical characterization and biochemical analysis follow standard methods employed in our previous studies and are summarized in the Supplementary material. Briefly, juvenile primary bovine chondrocytes were encapsulated in 2% agarose at a density of 30×10^6 cells/ml. Constructs (∅4 mm × 2.3 mm thick) were cultured in chemically defined chondrogenic media. Media were supplemented with 10 ng/ml TGF-β3 (R&D Systems, Minneapolis, MN) for either the entire culture period (β3+ group) or for only the first 14 days of culture (β3− group). A control was cultured without TGF-β3 supplementation. Constructs were removed and characterized after 14, 28, and 45 days ($n=4$ per group, time point).

2.1. Synthesis rates and retention fractions

For each synthesized ECM constituent (GAG, collagen, COMP) two distinct rates were measured: (A) the constituent scaffold accumulation rate and (B) the constituent media release rate. The scaffold mass accumulation rate, m_c, was the slope of the linear regression of the mass of matrix within each construct (normalized to the construct day 0 reference volume) over the culture period (typical data set and linear regression shown in Fig. 1A). The media mass release rate, m_m, was the slope of the linear regression of the mass of matrix released into the media (normalized to the construct day 0 volume) over the culture period (typical data set and linear regression shown in Fig. 1B). The linear regressions for the β3+ and control groups were calculated over the entire 45-day culture period (days 0–45) and the regressions for the β3− group were calculated after discontinuing TGF-β3 supplementation (days 14–45). The total synthesis rate was the sum of m_c and m_m. The retention ratio, R_c, of each matrix constituent was calculated by dividing m_c by the total synthesis rate:

$$R_c = \frac{m_c}{m_c + m_m} \tag{1}$$

The uncertainties of both the total synthesis rates and retention ratios were calculated according to standard uncertainty analysis from the standard deviations associated with the linear regression fits.

2.2. Binding parameters

The reversible binding kinetics of each matrix constituent were assessed based on the following assumptions: the synthesis rate was based on experimental results; each constituent was synthesized in soluble form and bound reversibly according to the law of mass action; when in soluble form, matrix products underwent Fickian transport with a diffusivity negligibly altered by matrix deposition; soluble matrix products diffused out of the construct into a well-mixed bath; there were no nutrient limitations in these small constructs; electric charge effects were neglected; construct swelling as a result of matrix growth was also neglected. All model simulations were performed in the open-source finite element code FEBio (www.febio.org), customized for this application (Ateshian, 2007; Ateshian et al., 2013; Maas et al., 2012). Models consisted of a construct (∅4 mm × 2.3 mm thick, cell density: 30×10^6 cells/ml) in a 0.5 ml bath, similar to the day 0 experimental conditions (Fig. S1A in Supplementary material). The governing equation for soluble matrix product was the mass balance relation in the

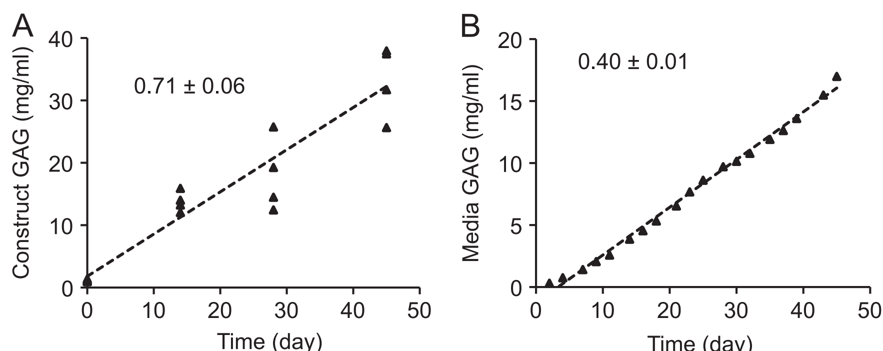

Fig. 1. Representative synthesis profiles for (A) matrix accumulation in the construct (normalized to day 0 reference volume) and (B) cumulative matrix release in the media (normalized to day 0 reference volume). Synthesis rates were calculated from the slope of the linear regression.

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