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Analyzing the Effects of Mechanical and Osmotic Loading on Glycosaminoglycan Synthesis Rate in Cartilaginous Tissues

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Abstract

The glycosaminoglycan (GAG) plays an important role in cartilaginous tissues to support and transmit mechanical loads. Many extracellular biophysical stimuli could affect GAG synthesis by cells. It has been hypothesized that the change of cell volume is a primary mechanism for cells to perceive the stimuli. Experimental studies have shown that the maximum synthesis rate of GAG is achieved at an optimal cell volume, larger or smaller than this level the GAG synthesis rate decreases. Based on the hypothesis and experimental findings in the literature, we proposed a mathematical model to quantitatively describe the cell volume dependent GAG synthesis rate in the cartilaginous tissues. Using this model, we investigated the effects of osmotic loading and mechanical loading on GAG synthesis rate. It is found our proposed mathematical model is able to well describe the change of GAG synthesis rate in isolated cells or in cartilage with variations of the osmotic loading or mechanical loading. This model is important for evaluating the GAG synthesis activity within cartilaginous tissues as well as understanding the role of mechanical loading in tissue growth or degeneration. It is also important for designing a bioreactor system with proper extracellular environment or mechanical loading for growing tissue at the maximum synthesis rate of the extracellular matrix.

Keywords

biophysical stimuli; GAG production; cell volume; mechanobiology; biomechanics; mathematical model

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Conflict of interest statement

No financial support or benefits have been or will be received from any commercial source related directly or indirectly to the scientific work reported in this manuscript.

1. Introduction

The glycosaminoglycan (GAG) plays an important role in cartilaginous tissues to support and transmit mechanical loads, and the decrease of GAG content is a sign of tissue degeneration (Antoniou et al., 1996; Maroudas, 1979). The negatively charged groups on the GAG impart a high extracellular osmolarity and hence high swelling pressure to the extracellular matrix (ECM) (Lai et al., 1991; Maroudas, 1979). GAG acts in concert with collagen to give cartilage the capacity to support and transmit mechanical loads (Maroudas, 1976). The GAG is produced and maintained by cells which live in a very complex mechano-electrochemical environment within the tissue (Maroudas, 1979; Setton and Chen, 2004; Snowden and Maroudas, 1972; Urban, 2002). A change in cell shape or volume has been known as a mechanism to perceive the alteration of extracellular environment by mammalian cells (Benzeev, 1991; Folkman and Moscona, 1978; Sarkadi and Parker, 1991). Currently, it has been found that direct membrane stretch has little or no influence on chondrocyte metabolic activities (O'Connor et al., 2014), which indicates cell volume change may play a role in regulating the synthesis activities. It was hypothesized that the change of cell volume could be a factor in mediating mechanotransduction in cartilaginous tissues (Guilak et al., 1995; Wong et al., 1997).

Osmotic loading and mechanical loading have been shown to affect the synthesis of GAG in vitro in both explant and cell culture experiments [e.g., (Bush and Hall, 2001; Gray et al., 1988; Gray et al., 1989; Guilak et al., 1994; Johnson et al., 2014; Maroudas and Evans, 1974; O'Connor et al., 2014; Sampat et al., 2013; Schneiderman et al., 1986; Urban et al., 1993)]. For example, the extracellular osmolarity has been found to have a dose-dependent effect on GAG synthesis rate by isolated cells (Ishihara et al., 1997; Negoro et al., 2008); the cells in the NP explant exhibit a higher synthesis rate of GAG at the osmolarity level close to the in situ value (van Dijk et al., 2011; van Dijk et al., 2013); the GAG synthesis rate has been observed to decrease in osmotic loaded articular cartilages (Bayliss et al., 1986; Schneiderman et al., 1986); a static compression has been shown to suppress GAG synthesis in articular cartilage (Kim et al., 1994; Schneiderman et al., 1986), whereas the GAG synthesis rate in bovine tail disc was found to be elevated if the static loading is less than 5 kg (Ohshima et al., 1995). However, there is no adequate theoretical model in the literature for the quantitative analysis and prediction of the effect of mechanical loading and/or osmotic loading on the synthesis rate of GAG in the cartilaginous tissues. Thus, the objective of this study was to develop a mathematical model to quantitatively investigate GAG synthesis rate by cells in cartilaginous tissues (or in a culture) under the mechanical and/or osmotic loading.

2. Model Development

It has been found that cells of cartilaginous tissues exhibit maximum synthesis rate (R_0 , mole or mass per unit cell per unit time) of GAG at an in vivo state (refer to the optimal state, denoted by subscript 0) as mentioned in Introduction. Ishihara et al. experimentally revealed that the change of GAG synthesis rate (R_s) is proportional to the relative change of a cell volume (Ishihara et al., 1997). Based on their findings, in this study, we proposed a mathematical model for the relationship between GAG synthesis rate and cell volume:

$$\frac{R - R_0}{R_0} = -\alpha \left| \frac{V - V_0}{V_0} \right|, \quad (1)$$

where V_0 and V are the cell volumes at the optimal state and current state, respectively, and α is a positive parameter characterizing the effect of cell volume change on GAG synthesis rate. Curve-fitting of eq. (1) to the experimental results (Ishihara et al., 1997) of GAG synthesis rate versus the volume changes of the cells from bovine nucleus pulposus (NP), yielded $\alpha = 2.41$ ($R^2=0.94$), see Fig. 1a.

The alteration of the cell volume can be attributed to its passive or active responses to biophysical stimuli. In this study, we focused only on the passive response of cell volume to osmotic loading and/or mechanical loading.

2.1. Effects of osmotic loading

It has been shown that the passive volumetric response of isolated chondrocytes or chondrocyte-like cells from the intervertebral disc (IVD) to osmotic loading in a culture medium can be characterized by Boyle van't Hoff equation (Guilak et al., 2002; Nobel, 1969),

$$\frac{V - V_0}{V_0} = \beta \left(\frac{\pi_0}{\pi} - 1 \right), \quad (2)$$

where π_0 and π are the osmolarities at the optimal state and current state, respectively. The osmolarity can be estimated by the sum of the concentration of all dissolved particles (or solutes, denoted by i), that is, $\pi = \sum_i c^i$, where c^i is the concentration of solute i per fluid volume. The parameter β in eq. (2) is positive and its value is equal to the hydration of the cell at the optimal state (Ateshian et al., 2006). Substituting eq. (2) into eq. (1), it yields:

$$\frac{R}{R_0} = \left(1 - \gamma \left| \frac{\pi_0}{\pi} - 1 \right| \right), \quad (3)$$

where $\gamma = \alpha\beta$. Curve-fitting to the experimental results (Ishihara et al., 1997) on GAG synthesis rate versus extracellular osmolarity, yielded $\gamma = 1.487$ and $\pi_0 = 404$ mOsm ($R^2=0.91$), see Fig. 1b. Thus, the value of parameter β for bovine NP cells is 0.62, which is close to the value (~ 0.6) for mesenchymal stem cells (Sampat et al., 2013).

2.2. Effects of mechanical loading

The Boyle van't Hoff equation [i.e., eq. (1)] can characterize the volume change of an isolated cell under osmotic loading, but it is no longer valid for the cells in the tissue because the cells are encapsulated by the extracellular matrix (ECM) which provides external resistance to the deformation of the cell. In order to estimate the cell volume change as a function of tissue deformation, let us consider a simple case where a spherical cell is encapsulated in a larger sphere of the ECM, see Fig. 2. The radii of a cell and matrix at the optimal state are r_c and r_m , respectively. The cell-matrix composite (Fig. 2) is assumed to be subjected to a normal stress uniformly distributed at the matrix outer boundary, and the cell

be attached to the matrix perfectly. At equilibrium state (without fluid flow), the deformation of the cell-matrix composite can be estimated by a linear elasticity theory. The equation of equilibrium in the spherical coordinate system is

$$\frac{d}{dr} \left[\frac{1}{r^2} \frac{d}{dr} (r^2 u_r) \right] = 0, \quad (4)$$

where r is the radial coordinate, and u_r is the displacement in the radial direction. The relative volume change (i.e., dilatation) of the cell or composite can be obtained through calculating the change of the radius of the cell or composite using eq. (4). Thus, the dilatation of the cell, e_c [$e_c = (V - V_0)/V_0$, where V_0 is again the volume of the cell at the optimal state for GAG synthesis], is related to the dilatation of the composite (e), through

$$e_c = f(e) = \left[1 - \frac{E_m}{E_c} \frac{2(1 - 2\nu_c)(1 - \chi^3)(\sqrt[3]{1+e} - 1)}{2(1 - 2\nu_m)(\chi^3 - \zeta) + (1 - \zeta)(1 + \nu_m)\chi^3} \right]^3 - 1, \quad (5)$$

where E_m and E_c are the Young's moduli of the matrix and cell, respectively, ν_m and ν_c are the Poisson's ratios of the matrix and the cell, respectively, $\chi = r_c/r_m$ (i.e., the ratio of radii), and

$$\zeta = \frac{E_c [2\chi^3(1 - 2\nu_m) + 1 + \nu_m] + 2(1 - \chi^3)(1 - 2\nu_c)E_m}{3(1 - \nu_m)E_c}. \quad (6)$$

From eqs. (1) and (5), the effect of local tissue deformation on GAG synthesis rate can be modeled as

$$\frac{R}{R_0} = 1 - \alpha|e_c| = 1 - \alpha|f(e)|. \quad (7)$$

The Young's modulus for cartilage ECM and chondrocyte are 1000 kPa and 0.35 kPa, respectively, and the Poisson's ration for ECM and chondrocyte are 0.04 and 0.43, respectively (Kim et al., 2008). The variation of the cell volume as a function of the composite dilatation is shown in Fig. 3. Schneiderman et al. studied the effects of mechanical compression on GAG synthesis in human adult femoral head cartilage (Schneiderman et al., 1986). Based on experimental findings of (Schneiderman et al., 1986) and (Kim et al., 1994), the maximum GAG synthesis rate in cartilage is achieved when no external load is applied to the explant; thus, we assumed that the in situ cell volume at this state is V_0 . The volume fraction of chondrocytes in cartilage is assumed as 1% (i.e., $\chi^3 = 1\%$) (Stockwell, 1979), according to eq. (5), $|e_c| = |f(e)| \approx 1.52|e|$, in the range of physiological deformations, see Fig. 3. Curve-fitting to experimental results of (Schneiderman et al., 1986) on GAG synthesis rate versus tissue dilatation yielded $\alpha = 2.42$ ($R^2 = 0.84$), see Fig. 4.

3. Discussion

We proposed a mathematical model on cell volume dependent GAG synthesis rate [eq. (1)]. This model is based on the hypothesis that the change of cell volume is a primary

mechanism for regulating the cell metabolic activities in cartilaginous tissues, and on the experimental findings that the GAG synthesis rate is sensitive to the cell volume and its maximum value is reached at an optimal cell volume (Ishihara et al., 1997; Negoro et al., 2008). In our model, the value of parameter α is assumed to be constant (i.e., independent of cell volume change). Although the physical meaning of the parameter α is not clear at cellular and molecular levels, it is interesting to note that the value of parameter α for isolated bovine NP cells in the culture medium under osmotic loading ($\alpha=2.41$) is very close to the value for human chondrocytes in femoral head cartilage explant under mechanical loading ($\alpha=2.42$). This is because the cell volume can be changed by osmotic loading or mechanical loading. It has been shown theoretically and experimentally that these two types of loading are equivalent if they impart to the cell with the same deformation (Lai et al., 1998; Schneiderman et al., 1986). Thus, it is the change in cell volume rather than the loading type that regulates the GAG synthesis. It is not a surprise to see the effect of osmotic loading on GAG synthesis [eq. (3)] for cells in the culture medium and the effect of static mechanical loading [eq. (7)] on GAG synthesis for cells in the explant can be delineated by a single model [eq. (1)], with the same value for parameter α . This finding indicates that one may obtain the value of α using isolated cells with an osmotic loading experiment to predict the GAG synthesis rate for cells in the IVD or articular cartilage under mechanical loading.

Since the relative volume change of the cells in the tissue is related to the local deformation of the tissue, an optimal loading condition exists in the tissue for cells to exhibit the maximum synthesis activities. For cartilage explants, the unloading condition has been reported to be the optimal loading condition (Kim et al., 1994; Schneiderman et al., 1986), whereas a compressed condition may be the optimal loading condition for IVDs in vitro (Ohshima et al., 1995).

We developed an analytical relationship for relative volume changes between cell and tissue by an approximation method [i.e., eq. (5)]. It predicts that the ratio of relative cell volume change to tissue dilatation is in the range of 1.42 to 1.55 for cartilage with 1% – 10% (Stockwell, 1979) of cell volume fraction and under less than 20% deformation (Fig. 3). The ratio, with 1% of cell volume fraction, changes less than 1% when the Young's modulus of ECM is increased or decreased by 5 times (Fig. 5a), whereas it changes by -4% to 7% (relative to the value at Poisson ratio equal to 0.04) when the Poisson's ratio varies in the physiological range from 0.0 to 0.1 (Athanasίου et al., 1991) (Fig. 5b). This ratio has been found to be ~1.0 to ~1.2 for articular cartilage experimentally (Guilak et al., 1995), and ~2 numerically by modeling matrix and cell as biphasic materials (Guilak and Mow, 2000). Our result is consistent with these previous findings in the literature, which indicates our eq. (5) is reasonably accurate for estimating the relative cell volume change in the tissue at equilibrium state.

Notice that eq. (5) may not be used to estimate the relative cell volume change in the tissue under dynamic loading conditions because of the fluid pressurization effect (Lee et al., 1981; Soltz and Ateshian, 2000). This fluid pressurization effect will increase the stiffness of the tissue under dynamic loading, and may effectively reduce the tissue deformation. Thus, the deformation of a cartilage explant is smaller at a higher compressive loading frequency than that at a lower loading frequency, provided that the loading amplitude remains the same.

Under this loading condition, the cell within the explant will experience smaller deformation at a higher frequency than that at a lower frequency. According to eq. (1), the GAG synthesis rate will be higher at a higher loading frequency. This analysis is consistent with the experimental findings on GAG synthesis rate reported in the literature (Sah et al., 1989).

In summary, a new mathematical model has been developed for GAG synthesis rate in cartilaginous tissues and it is able to well describe the change of GAG synthesis rate with variations of the osmotic loading or mechanical loading. One can estimate the volume change of cells in tissue by calculating tissue dilatation (relative to the optimal configuration). Based on this model, the loading applied to a cartilage explant should be reduced if the modulus of the matrix or scaffold is small at an initial stage of a repair or regeneration process in order to maintain cell volume at its optimum condition. One can also use this model to design a bioreactor system with proper extracellular environment or mechanical loading for growing tissue at maximum rate of extracellular matrix synthesis. Incorporating this mathematical model and our previous developed cell-activity-coupled, mechano-electrochemical theory for cartilaginous tissues (Gu et al., 2014; Zhu et al., 2014), we can quantitatively investigate the role of mechanical loading in tissue growth or degeneration in cartilaginous tissues.

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Fig. 1. (a) GAG synthesis rate versus relative volume change of cells; (b) GAG synthesis rate versus extracellular osmolarity. The synthesis rates were normalized by the rate at 280 mOsm (R_{280}) (Ishihara et al., 1997).

Fig. 2.
Schematic of a cell-matrix composite used for estimating the relative cell volume change.

Fig. 3. Relationship between composite dilatation (e) and relative cell volume change (e_c), χ^3 represents the cell volume fraction in the tissue.

Fig. 4.

GAG synthesis rate in human femoral head cartilage explant versus tissue dilatation. The dilatation was converted from hydration (Lai et al., 1991).

Fig. 5. The effects of the (a) Young's modulus, and (b) Poisson's ratio of ECM on the ratio of relative cell volume change to tissue dilatation for $\chi^3=1\%$.