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Prediction of Glycosaminoglycan Synthesis in Intervertebral Disc under Mechanical Loading

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Abstract

The loss of glycosaminoglycan (GAG) content is a major biochemical change during intervertebral disc (IVD) degeneration. Abnormal mechanical loading is one of the major factors causing disc degeneration. In this study, a multiscale mathematical model was developed to quantify the effect of mechanical loading on GAG synthesis. This model was based on a recently developed cell volume dependent GAG synthesis theory that predicts the variation of GAG synthesis rate of a cell under the influence of mechanical stimuli, and the biphasic theory that describes the deformation of IVD under mechanical loading. The GAG synthesis (at the cell level) was coupled with the mechanical loading (at the tissue level) via a cell-matrix unit approach which established a relationship between the variation of cell dilatation and the local tissue dilatation. This multiscale mathematical model was used to predict the effect of static load (creep load) on GAG synthesis in bovine tail discs. The predicted results are in the range of experimental results. This model was also used to investigate the effect of static (0.2 MPa) and diurnal loads (0.1/0.3 MPa and 0.15/0.25 MPa in 12/12 hours shift with an average of 0.2 MPa over a cycle) on GAG synthesis. It was found that static load and diurnal loads have different effects on GAG synthesis in a diurnal cycle, and the diurnal load effects depend on the amplitude of the load. The model is important to understand the effect of mechanical loading at the tissue level on GAG synthesis at the cellular level, as well as to optimize the mechanical loading in growing engineered tissue.

Keywords

intervertebral disc; glycosaminoglycan synthesis; mechanical stimuli; mechanobiology; multiscale mathematical model; finite element analysis

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Conflict of Interest

None of the authors have any financial or personal relationships with other people or organizations that could have inappropriately influenced or biased the work.

Introduction

The initiation of intervertebral disc (IVD) degeneration is much earlier than that of other musculoskeletal tissues (Urban and Roberts, 2003). The most significant biochemical change in IVD degeneration is the loss of glycosaminoglycan (GAG) content (Lyons et al., 1981). The loss of GAG directly compromises the disc mechanical roles in the spinal system (Adams et al., 1996; Lyons et al., 1981; Urban and McMullin, 1988; Zhu et al., 2014). This is because the fixed negatively charged groups on the GAG generate a higher osmolarity within the tissue than that outside the tissue. The imbalance of osmolarity results in Donnan osmotic pressure within the tissue which contributes significantly to the load-bearing capabilities of discs (Urban and Maroudas, 1981). Abnormal mechanical loading has been thought to be a major factor that initiates the disc degeneration (Adams and Roughley, 2006; Stokes and Iatridis, 2004; Urban and Roberts, 2003). It has been suggested that cell-mediated remodeling may be the main pathway of disc degeneration initiated by abnormal mechanical loading, aside from the structure failure directly caused by mechanical loading (Setton and Chen, 2006).

During daily activities, the disc is subjected to compression, bending, torsion, and shear, and the combination of the above. The GAG synthesis within the disc has been shown to be significantly affected by mechanical loading (Chan et al., 2011; Ohshima et al., 1995; Setton and Chen, 2004). Many in-vivo and in-vitro experimental studies on the discs reveal that the effects of mechanical loading on GAG synthesis depend on the loading regimes (static or dynamical loading), magnitude, amplitude, duration, and frequency [see reviews, (Chan et al., 2011; Setton and Chen, 2004)].

The mechanism as to how the mechanical loads alter the cell biosynthetic behaviors is still unknown. This is because the mechanical loading on the tissue can induce many stimuli to the cell, including cell volume change, high level of stresses and strains arising from the deformation of extracellular matrix (ECM), high magnitude of hydrostatic pressure, shear stress caused by interstitial fluid flow, electrokinetic effects caused by the interactions between fixed charge groups and mobile charged ions, the change of nutrient level, and others (Bibby et al., 2001; Setton and Chen, 2006; Urban, 2002). All of these stimuli could be a potential regulator for cell synthetic activities in the disc.

Recently, we have developed a cell volume dependent GAG synthesis theory to characterize the cell biosynthetic behavior in response to mechanical stimuli (Gao et al., 2015). The theory is based on the hypothesis that the change of cell volume is a regulator for downstream posttranslational biosynthesis (Guilak et al., 1995; O'Connor et al., 2014; Wong et al., 1997). The deviation of cell volume from its optimal state was considered as a mechanical stimulus, and it was correlated with the reduction (or decrease) of GAG synthesis rate from its maximum value. This theory has been shown to be able to predict GAG synthesis rate of isolated intervertebral disc cells under different osmotic loading conditions (Gao et al., 2015). However, there is no mathematical model available to quantify the variation of GAG synthesis rate within the disc in response to the externally applied mechanical load. The difficulty of developing such a mathematical model lies in the fact that the tissue deformation and the GAG synthesis are two events at different scale levels.

Therefore, the objective of this study was to develop a multiscale mathematical model to predict GAG synthesis within disc tissue under mechanical loading. In this study, the mathematical model was first developed and validated with experimental results, then used to investigate the GAG synthesis within the disc subjected to static and diurnal compressions.

Multiscale Mathematical Model

The multiscale mathematical model was based on cell volume dependent GAG synthesis theory (at the cell level) (Ohshima et al., 1995) and biphasic theory for cartilaginous tissues (at the tissue level) (Mow et al., 1980). In this study, it was assumed that the newly synthesized GAG binds to the solid matrix. Thus, the mass balance equation for GAG can be written as (Ateshian, 2007; Gu et al., 2014; Gu et al., 1998; Lai et al., 1991),

$$\frac{\partial C^{GAG}}{\partial t} + \nabla \cdot (\mathbf{v}^s C^{GAG}) = R^{GAG} \rho^{cell} - T^{GAG} \quad (10)$$

where c^{GAG} is the concentration of GAG (per tissue volume), $\mathbf{v}^s$ is the velocity of the solid matrix, R^{GAG} is GAG synthesis rate of each single cell, ρ^{cell} is the cell density (per tissue volume), and T^{GAG} is the breakdown rate of GAG (per tissue volume). Cell density changes with tissue deformation, that is $\rho^{cell} = \rho_r^{cell} / J$, where ρ_r^{cell} is the cell density at reference state, and J is the volume ratio of tissue. T^{GAG} was assumed to be zero in this study because the turnover time for GAG in the IVD is about 20 years which is much longer than the duration of interest in this study (Roughley, 2004).

A cell volume dependent GAG synthesis theory was adopted to model the GAG synthetic behavior of a single cell in response to mechanical stimuli (Gao et al., 2015). In this theory, it was assumed that the relative change of GAG synthesis rate (i.e., R^{GAG}) is proportional to the relative cell volume change (i.e., dilatation), and there is an optimal cell volume corresponding to the maximum GAG synthesis rate, mathematically,

$$\frac{R^{GAG}}{R_0^{GAG}} = 1 - \beta \left| \frac{V^{cell} - V_0^{cell}}{V_0^{cell}} \right|, \quad (2)$$

where R_0^{GAG} is the maximum GAG synthesis rate, V and R_0^{cell} are the cell volumes at current and optimal state, respectively, and β is a positive parameter characterizing the effect of cell volume change on GAG synthesis rate and it was assumed to be independent of cell volume in this study. The value of β was found to be 2.41 for isolated bovine intervertebral disc cells (Gao et al., 2015), and this value was used in this study. Combining eqs. (1) and (2) yields,

$$\frac{\partial C^{GAG}}{\partial t} + \nabla \cdot (\mathbf{v}^s C^{GAG}) = Q \left(1 - \beta \left| \frac{V^{cell} - V_0^{cell}}{V_0^{cell}} \right| \right), \quad (3)$$

where $Q (= \rho^{cell} R_0^{GAG})$ is the maximum synthesis rate per tissue volume.

In order to evaluate the change of cell volume within the matrix caused by the mechanical loading on the tissue level, a cell-matrix unit approach was adopted, in which a spherical cell is encapsulated in a larger sphere of the ECM, see Fig. 1. It was assumed that the cell-matrix composite is subjected to a uniformly distributed normal stress at the outer boundary, and the cell and matrix are attached perfectly. Therefore, the relationship between the cell

dilatation, $e^c = (V^{cell} - V_r^{cell}) / V_r^{cell}$, where V_r^{cell} is the cell volume at the reference state], and the composite dilatation, $e (= J - 1)$, can be established through this unit approach. In this study, we assumed the total cells occupy 1% of the total tissue volume (Bibby et al., 2001; Urban et al., 2000), thus the ratio between cell radius (r^c) and composite radius (r) is $0.215 (= 3 \sqrt{0.01})$. The cell was modeled as a single phase linear elastic material and the matrix was modeled as a biphasic material.

The deformation of IVD under mechanical loading was modeled based on the biphasic theory (Mow et al., 1980). The mass balance equation for the mixture can be written as,

$$\nabla \cdot (\phi^s \mathbf{v}^s + \phi^w \mathbf{v}^w) = 0, \quad (4)$$

where ϕ^a ($a = s, w$) denotes for the volume fraction and $\mathbf{v}^a$ for the velocity of solid and fluid phases, respectively.

The transport of the interstitial fluid within the extracellular solid matrix is characterized by Darcy's law, that is,

$$\mathbf{J}^w = \phi^w (\mathbf{v}^w - \mathbf{v}^s) = k \nabla p, \quad (5)$$

where $\mathbf{J}^f$ is the fluid flux, and k is the hydraulic permeability which depends on water volume fraction (Gu et al., 2003),

$$k = a \left(\frac{\phi^w}{1 - \phi^w} \right)^b, \quad (6)$$

where a and b are two parameters.

The stress of the mixture is contributed by both solid and fluid phases, thus, the linear momentum balance equation for the mixture can be written as (Mow et al., 1980),

$$\nabla \cdot (-p\mathbf{I} + \boldsymbol{\sigma}^s) = 0, \quad (7)$$

where p is the fluid pressure, $\mathbf{I}$ is the second order identity tensor, and $\boldsymbol{\sigma}^s$ is the stress arising from the deformation of solid phase. Under a finite deformation, the stress of the solid

matrix can be evaluated through, $\boldsymbol{\sigma}^s = \frac{2}{J} \mathbf{F} \frac{\partial \psi}{\partial \mathbf{C}} \mathbf{F}^T$, where $\mathbf{F}$ is the deformation gradient tensor, $J = \det(\mathbf{F})$, and $\mathbf{C} = \mathbf{F}^T \mathbf{F}$. A strain energy density function for hyperelastic compressible materials was adopted for modeling the disc tissue (Ehlers and Eipper, 1999), which states,

$$\psi = \frac{\mu}{2} (I_1 - 3) - \mu \ln J + \lambda (\phi_r^w)^2 \left[\frac{J-1}{\phi_r^w} - \ln \left(\frac{J-1}{\phi_r^w} + 1 \right) \right], \quad (8)$$

where λ and μ are two Lamé constants, I_1 is the first invariant of $\mathbf{C}$, and ϕ_r^w is the fluid volume fraction at the reference state and it is related to the fluid volume fraction at current state through, $\phi^w = (\phi_r^w + J - 1) / J$.

Numerical Examples

This multiscale mathematical model was applied to study GAG synthesis in the live bovine tail IVD (Ohshima et al., 1995). The IVD was modeled as concentric cylinders with 6.0 mm in height. The core is nucleus pulposus (NP) with 5.5 mm in radius, and the outer layer is annulus fibrosus (AF) with 5.5 mm in radial thickness. The AF was divided into inner AF and outer AF with equal radial thickness, i.e., 2.75 mm each, see Fig. 1 (Ohshima et al., 1995). Only one-eighth of the disc was used in the numerical simulation because of the axial-symmetry of the disc geometry (Fig. 1). Finite element method was used to discretize the mathematical model following the approach in (Gao et al., 2016; Sun et al., 1999), and it was implemented in COMSOL 5.1. A mesh with 2560 quadratic hexahedral elements were used in the simulation (Fig. 1). The biphasic material properties of the tissue and cell used in this study were listed in Table 1.

Several assumptions were made in the numerical model in order to compare our model prediction with experimental results by Ohshima et al.'s study (Ohshima et al., 1995): (1) the strain state under in-vivo loading condition was assumed to be an optimal condition for GAG synthesis in which its synthesis rate reaches a maximum value (Bayliss et al., 1986; Ishihara et al., 1997; Schneiderman et al., 1986), (2) the variation of maximum GAG synthesis rate in the axial direction was assumed to be negligible, (3) the variation of GAG content had no effect on mechanical properties, and (4) the maximum GAG synthesis rate per tissue volume (i.e., Q in eq. (3)) was assumed to be uniform in the NP and linearly varied from inner AF to outer AF as suggested by experimental findings (Bayliss et al., 1988; Bayliss et al., 1986; Ohshima et al., 1993).

In Ohshima et al.'s study, the discs were undergone with unconfined compression creep tests with a constant load of 4.9 N, 49.0 N, 98.0 N, or 147.0 N for 8 hours, and GAG content synthesized (amount of incorporated ^{35}S -sulfate) per tissue weight during the eight hours

creep process was measured. In order to compare predicted results and experimental results, the unit of GAG content in mole per tissue volume was converted to be in mole per tissue weight by scaling tissue mass density (ρ^{tissue}) of 1.04 g/cm³ for NP and 1.10 g/cm³ for AF (Gu et al., 1996).

The in-vivo strain state (i.e., the optimal state) in the bovine tail disc was determined by adjusting in-vitro compressive loads on the disc until the simulated overall water content in NP reached the value in vivo (i.e., $\phi^w = 0.86$) at the end of 8-hour creep test. The cell dilatation (with respect to the cell volume in the optimal state) was calculated based on the local tissue dilatation. The spatial distributions of maximum GAG synthesis rate per unit tissue volume (Q) in eq. (3) was determined by curve-fitting the distributions of the synthesized GAG content in the disc subjected to the 49.0 N compressive load in 8 hours (only the medial to dorsal experimental results were used in the curve-fitting due to the axial-symmetry assumption) (Ohshima et al., 1995). The model validation was conducted by comparing model predictions of GAG content distribution in the disc with those measured at 4.9 N, 98.0 N, and 147.0 N compressive loads.

The effects of different mechanical loading conditions on the GAG synthesis were investigated with this model, including one static compression (0.2 MPa) and two dynamic compressions with diurnal loads (0.15/0.25 MPa and 0.1/0.3 MPa in 12/12 hours shift with an average value of 0.2 MPa over a cycle). For the dynamic loading cases, the disc was compressed for two diurnal cycles under each loading condition, the first cycle was a preconditioning cycle and the GAG content synthesized during the second cycle was used to investigate the effects of different loading regimes on GAG synthesis.

Results

The relationship between cell dilatation and the dilatation of cell-matrix composite were determined with the cell-matrix unit approach, see Fig. 1. The relationships were similar for both the NP and AF regions. The cell dilatation was about two times larger than the dilatation of the cell-matrix composite (Fig. 2).

The creep processes in bovine tail IVD under various compressive loads (from 0 to 150 N) were simulated. The water content averaged over the NP region was evaluated at the end of 8-hour creep process, which decreased from 0.89 to 0.82 with increasing the compressive load, see Fig. 3. The predicted water contents under various compressive loads were in agreement with the experimentally measured results (Ohshima et al., 1995). At the end of 8-hour creep process, the strain in the disc under 58.8 N was chosen as the in-vivo loading condition (i.e., optimal state) because the water content averaged over the NP region at this condition equaled to the in-vivo water content ($\phi^w = 0.86$).

The spatial distribution of maximum GAG synthesis rate was obtained by curve-fitting ($R^2=0.94$) the measured GAG content (per tissue weight) from the experimental results under 49.0 N compressive load, see Fig. 4. Note that only the experimental results from medial to dorsal were used in curve-fitting because it was assumed that the bovine tail disc is axial-symmetric in this study. Then, the GAG content synthesized during the 8-hour creep

process under 4.9 N, 98.0 N, and 147.0 N were predicted with the multiscale mathematical model. The predictions were in the range of experimental results, see Fig. 5. The predicted GAG synthesis rate averaged over NP, inner AF, and outer AF were shown in Fig. 6, respectively. The GAG synthesis rate in discs under 4.9 N and 49.0 N increased with time while the rate in the discs under 98.0 N and 147.0 N first increased then decreased with time (Fig. 6).

Spatial distributions of GAG content synthesized under static and diurnal loading conditions were predicted to be similar, with highest GAG content at the interface of AF and NP and decreasing toward the central NP and outer AF. The GAG content synthesized in the NP during a diurnal cycle with 0.1/0.3 MPa and 0.15/0.25 MPa diurnal loads was about 5% lower and 4% higher than the GAG content synthesized with a 0.2 MPa static load, respectively (Fig. 7). The GAG contents synthesized in the AF during a diurnal cycle were similar to that with a 0.2 MPa static load and 0.1/0.3 MPa diurnal loads, which was about 12% lower than the synthesized GAG content under 0.15/0.25 MPa diurnal load, see Fig. 7.

Discussions

The primary goal of this study was to develop a multiscale mathematical model to predict the effect of mechanical loading on the GAG synthesis within the IVD. This multiscale mathematical model is based on the cell volume dependent GAG synthesis theory (Gao et al., 2015) that predicts the variation of GAG synthesis rate of a cell under the influence of a mechanical stimulus, and the biphasic theory (Mow et al., 1980) that predicts the tissue deformation of IVD under mechanical loading. These two theories describe two different (biological and mechanical) phenomena at different scale levels (cell level and tissue level) were coupled together with a cell-matrix unit approach which establishes a relationship between GAG synthesis rate and local tissue dilatation.

In the cell volume dependent GAG synthesis theory, the variation of GAG synthesis rate is correlated with the variation of cell volume. The mechanism by which disc cell volume change is correlated with the GAG synthesis rate is currently unclear. This unknown mechanism is related to the positive parameter β in Eq. 2, which was assumed to be independent of cell volume in this study. The model prediction is sensitive to the value of β as GAG synthesis rate is proportional to its value (see Eq. 2). The value of β has been found to be 2.41 for isolated bovine NP cells under osmotic loading (Gao et al., 2015), and this value was also found to work for both bovine NP and AF cells resided within the ECM in this studies.

Both solid matrix of NP and AF were modeled as isotropic hyperelastic materials in this study. The water contents predicted with the biphasic theory are within the range of experimental measurements (Fig. 3), which indicates that the biphasic finite element model is able to predict the tissue volumetric strain (i.e., tissue dilatation). It is important to note that the effect of GAG content change on ECM material properties must be taken into consideration when this multiscale mathematical model is used to predict GAG synthesis over a long period of time. This is because the GAG has a remarked contribution to the

effective mechanical properties of connective tissues (Canal Guterl et al., 2010; Lai et al., 1991).

The ratio of cell dilatation to tissue dilatation was evaluated with finite element method based on an idealized morphology in this study, but the ratio is comparable with that evaluated with finite element method based on a real cell morphology (Cao et al., 2011). In this study, the ratio was evaluated at a static (or equilibrium) state with the cell-matrix unit, which is different from creep and diurnal loading conditions. However, this difference in loading condition will have a very limited influence on the prediction of GAG synthesis. This is because the characteristic time (or diffusion time, $t = h^2 / [(\lambda + 2\mu)k]$, where h is the characteristic length of the unit) for the cell-matrix unit is on the magnitude of seconds, which is much smaller than the characteristic time of creep and diurnal loading (Gao and Gu, 2014). In the above analysis, the mechanical and transport properties of the matrix instead of equivalent properties of the cell-matrix unit were used in the estimation because the cell volume fraction is only about 1%. Note that this relationship cannot be used if the IVD is under dynamical loading with frequency higher than 0.01 Hz, because the diffusion time is comparable with the period of dynamical loading, thus the fluid flow will have a significant effect on the deformation of cell (Kim et al., 2008; Soltz and Ateshian, 2000).

The maximum GAG synthesis rates fitted by the multiscale mathematical model averaged over NP, inner AF, and outer AF were about 3.0, 2.6, and 1.7×10^{-9} mol/ g wet wt./hour, respectively (Fig. 4), which are comparable with GAG synthesis rates measured in vitro with tissue samples from human IVD and bovine tail IVD (Bayliss et al., 1988; Oshima et al., 1993). The content of GAG synthesized during the creep processes predicted by the multiscale mathematical model were in the range of experimental measurements (Fig. 5). The model is also capable of predicting the higher GAG content at the interface between AF and NP, which is consistent with experimental findings (Oshima et al., 1995), see Figures 4a and 5. The synthesized GAG content depends on both maximum GAG synthesis rate (Q) and the cell dilatation (or the tissue dilatation) as shown in eq. (3). Since the maximum GAG synthesis rate was assumed to be uniform in the NP (Fig. 4b), the higher synthesized GAG content at the interface between AF and NP is due to the inhomogeneous volumetric strain in the NP. This inhomogeneous volumetric strain is caused by the constrained effect of the AF.

The GAG synthesis rate predicted by the multiscale mathematical model was shown to depend on loading magnitude, loading duration, and the anatomic region (Fig. 6), which is in accordance with the experimental findings (Chan et al., 2011; Lotz, 2004; Setton and Chen, 2004, 2006; Stokes and Iatridis, 2004). The temporal variation of GAG synthesis rate depends on the tissue strain. The synthesis rate increases when the tissue strain approaching the strain at the optimal state, and it decreases when the tissue strain deviates from the strain at the optimal state. The GAG synthesis rate in the outer AF was found to be the lowest compared with that in NP and inner AF, which is consistent with experimental findings (Horner et al., 2002).

Several assumptions have been made in the model development and numerical simulation, which may affect the prediction. Firstly, we assumed that the mechanical properties are

nonlinear and isotropic for AF, which differs from its anisotropic nature. This assumption would affect the predicted cell dilatation and, subsequently, the GAG content in the AF. Secondly, we assumed the disc is perfectly axial-symmetric in all aspects (e.g., geometry, material properties, and cellular metabolic activities) in the numerical simulation, and only the experimental results from median to dorsal disc were used in the curve-fitting, thus the model prediction matches better with experimental results at the dorsal AF than that at the ventral AF (Figs. 4a and 5). Thirdly, we assumed the newly synthesized GAG binds to the extracellular matrix without considering the diffusion of GAG within the disc and release of GAG to the culture medium (Korecki et al., 2007). This assumption may not affect predicted GAG content significantly because the GAG diffusivity in the tissue is very low [about $5 \times 10^{-8} \text{ mm}^2/\text{s}$ (Nims et al., 2014)] and its diffusion length (0.05 mm) in 8 hours is much smaller than the length scale of the disc. Fourthly, we assumed the maximum GAG synthesis rate linearly varies from inner AF to outer AF, which may differ from that in the real situation and affect the predicted results. This assumption may also contribute to the discrepancy between model prediction and experimental result in the AF (Figs. 4a and 5).

It was found that static loading and diurnal loading have different effects on the content of synthesized GAG (within the same time period), and the effects of diurnal loading depend on the amplitude of the load (Fig. 7). The difference in synthesized GAG content between 0.2 MPa static load and 0.1/0.3 MPa diurnal loads is small (primarily in the NP), which agrees with experimental findings (Korecki et al., 2007). The higher GAG content synthesized with 0.15/0.25 MPa diurnal loads indicates that mechanical loading plays an important role in maintaining ECM integrity.

In summary, a multiscale mathematical model was developed to predict the GAG synthetic activity within IVD under mechanical loading. The robustness of the model was demonstrated by the agreement between predicted results and experimental results. The model is important to understand the effect of mechanical loading at the tissue level on GAG synthesis at the cellular level. This model is also important for designing loading strategies to grow tissue in tissue engineering.

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Fig. 1. Schematics of (a) cell-matrix unit; (b) bovine tail IVD. Scale unit in mm.

Fig. 2.
Relationship of the dilatations between cell-matrix composite (e) and cell (e^c).

Fig. 3.
Effect of mechanical loading on the water content averaged over NP.

Fig. 4.

(a) Curve-fitting of experimental results of the synthesized GAG content under 49.0 N compressive load. R^2 was calculated with the experimental results from medial to dorsal.

(b) Maximum GAG synthesis rate (per tissue weight) yielded from curve-fitting in (a). GAG content refers to the amount of newly synthesized GAG per tissue weight over a period of 8 hours.

Fig. 5.

Model predictions of synthesized GAG content over 8-hour creep process under (a) 4.9 N; (b) 98.0 N; (c) 147 N compressive load. GAG content refers to the amount of newly synthesized GAG per tissue weight over a period of 8 hours.

Fig. 6. Model prediction of GAG synthesis rate under (a) 4.9 N; (b) 49.0 N; (c) 98.0 N; (d) 147.0 N compressive loads.

Fig. 7.

Comparison of synthesized GAG contents under diurnal and static loading conditions. GAG content refers to the amount of newly synthesized GAG per tissue weight over the second loading cycle (i.e., 24-48 hours).

Table 1

Material properties for finite element analysis.

	NP [#]	AF [#]	Cell [*]
λ	0.04 MPa	0.14 MPa	1.29 kPa
μ	0.13 MPa	0.30 MPa	0.32 kPa
ϕ_r^w	0.89	0.76	N/A
k	$a = 0.04 \times 10^{16} \text{m}^4/(\text{N}\cdot\text{s})$ $b = 2.283$	$a = 0.18 \times 10^{16} \text{m}^4/(\text{N}\cdot\text{s})$ $b = 3.125$	N/A

[#] The ECM of NP and AF were modeled as biphasic material (Farrell and Riches, 2013; Iatridis and ap Gwynn, 2004; Ohshima et al., 1995; Perie et al., 2005).

^{*} The cell was modeled as a linear elastic material (Cao et al., 2011).