IGF Uptake with Competitive Binding in Articular Cartilage

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1. Abstract

Experiments on the transport of radiolabeled Insulin-like Growth Factors (IGF-I and -II) into bovine articular cartilage show differential uptake depending on the relative proportion of IGF-I and -II. In this study, we present a mathematical model describing both the transport and competition of IGF-I and -II for binding sites represented by two functional groupings of IGF binding proteins (IGFBPs). The first grouping has approximately similar binding affinity to both IGF-I and -II (i.e. IGFBPs 1-5), whereas the second group has significantly higher binding preference for IGF-II compared to IGF-I (i.e. IGFBP-6). Using nonlinear least squares, it is shown that the experimental equilibrium competitive binding results can be described using a reversible Langmuir sorption isotherm involving two dominant IGFBP functional groups.

After coupling the sorption model with a poromechanical continuum model, parametric studies are carried out to investigate the effect of model changes including IGF boundary conditions and the ratios of the two IGFBP functional groups. The results show that ignoring competitive binding leads to a significant overestimation of total IGF-I uptake, but an underestimation the rate of 'free' (physiologically active) IGF-I within the cartilage. An increase of first group of IGFBPs (i.e. IGFBPs 1-5) as has been reported for osteoarthritis, is observed to hinder the bioavailability of free IGF-I in cartilage, even though the total IGF-I uptake is enhanced. Furthermore, the combination of dynamic compression and competitive binding is seen to enhance the IGF-I uptake within cartilage, but this enhancement is overestimated if competitive binding is neglected.

Key Words: IGF-I; IGF-II; Binding proteins; Transport; cartilage; dynamic loading; Competitive binding

2. Introduction

Insulin-like growth factors (IGF-I and -II) play an important role in stimulating the synthesis of cartilage's extracellular matrix (ECM)[1], counteracting 'catabolic agents' that result in tissue degradation[2, 3]. As experiments have shown that chondrocytes synthesize little or no IGF-I under the culture conditions[1], it is reasonable to assume that the majority of IGF-I in cartilage is supplied from synovial fluid. However, IGFs must be transported into the cartilage from synovial fluid through the processes of diffusion and advection, before they can exert their physiological effect. Further mechanisms for controlling the effects of IGF-1 on chondrocytes include alterations in the concentrations of IGF-I and -II, and alterations in the concentrations of binding proteins. Disturbance of any one of the above elements may induce a dysregulation of the mechanisms involved in the local control of joint tissue integrity. The central aim of this paper is to construct a quantitative model of cartilage behaviour capturing the interplay between these elements. We begin by developing a model to describe a set of experiments on IGF transport into cartilage, and then proceed to a parametric study exploring the effect of variations in IGF and IGFBP, as well as investigating the effect of cyclic compression on transport processes through the cartilage.

The experiment of Bhakta et al[4] on binding of radiolabeled IGFs to bovine articular cartilage showed differential uptake depending on the combination of IGFs present. Bhakta et al studied the effect of graded levels of unlabeled IGFs competing with radiolabeled ^{125}I -IGFs ($\sim 0.003\text{nM}$) in adult bovine articular cartilage discs. The measurements by Bhakta et al[4], were purposely performed at 4°C (binding) or $8\text{--}16^\circ\text{C}$ (transport) to minimize the contributions of cell-mediated activity and thereby to focus on binding of IGFs to IGFBPs. The discs (3 mm diameter x $400\text{ }\mu\text{m}$ depth) were equilibrated in solutions for 48 hours containing both radiolabelled ^{125}I -

IGFs and unlabeled IGFs. The radioactive counts in each disc were normalized by the tissue water weight (cpm/mg) and by the initial radioactive counts per volume (cpm/ml) of the equilibrating solution. The results revealed that when graded levels of unlabeled IGF-I and -II are in competition with radiolabeled ^{125}I -IGF-II entering cartilage discs, a significant differential uptake of radiolabeled ^{125}I -IGF-II was observed. Bhakta et al postulated that the observed difference in uptake was due to difference in binding of IGFBPs for IGF-I and -II. To test this hypothesis, a numerical model describing IGF-I and -II competitively binding to multiple binding proteins is required.

There are relatively few studies that experimentally and theoretically investigate the influence of IGFBPs on IGF-I transport within the cartilage. Garcia et al (2003) [5] studied the transport and binding of IGF-I through articular cartilage. Their experiment followed the methodology used by Bhakta et al[4], and their theoretical results provided strong evidence that IGFBPs regulate the transport of IGF-I through cartilage. Moreover, they presented a simple reversible equilibrium binding theoretical model to quantify the roles that diffusion and binding play in the transport of IGF-I within cartilage tissue. However, without extending this model to include competitive binding of growth factors to IGFBPs, the distinctive results in Bhakta et al's experiments[4] cannot be reproduced. Therefore, the first objective of this study is to develop a relatively sophisticated model with the aim of closely reproducing Bhakta et al's experimental results[4].

A family of six IGF binding proteins (IGFBPs) regulate IGF activity[2, 6-8]. In vivo, IGF-I and -II competitively bind to these six IGFBPs simultaneously, but with differential affinities. The measurements of the interaction kinetics between IGFs and their binding proteins in solutions showed that IGFBPs 1-5 have a similar binding preference for IGF-I and -II

(although IGFBP-2 has a slight IGF-II binding preference), whereas the IGFBP-6 differs from other binding proteins in that it displays a 20 to 100 fold higher affinity for IGF-II than IGF-I [7, 9-12]. To constrain the complete set of possible interactions, as an approximation we study the interaction of the six IGFBPs with the IGF-I and -II by considering two IGFBP functional groupings. That is, one group has similar binding affinity for IGF-I and II (i.e. IGFBPs 1-5), whereas the second group has a significantly higher binding preference for IGF-II compared to IGF-I (i.e. IGFBP-6).

The second objective of this study is to conduct a series of computational experiments by varying boundary conditions of IGFs and the ratio of two IGFBP functional groups according to various cartilage conditions. In diseased cartilage, such as osteoarthritis and rheumatoid arthritis as examples, the level of IGF-I in arthritic synovial fluid is found to be increased relative to normal cartilage[13]. It has also been noticed that there is a significant increase in content of IGFBPs in diseased cartilage, especially IGFBP-3[14]. Furthermore, IGFBP content in cartilage has been shown to differ from species to species. For example, although large amount of IGFBP-6 was identified in bovine cartilage[15], but it has been reported that the level of IGFBP-6 was too low to detect in human cartilage[14]. All these changes may have a significant impact on IGF-I uptake by the cartilage, and so are worthy of further investigation.

Physiological relevant mechanical loading has been theoretically demonstrated to enhance the IGF transport through advective fluid flow[16, 17]. Our previous study[18] demonstrated that IGFBPs are essential for enhancing the uptake of IGF into cartilage. It is likely though, that under physiological conditions, the presence of IGF-II in synovial fluid and the competitive binding of IGF-I and -II to their IGFBPs will modify the advective transport

behavior of IGFs. The current study further investigates the combined effect of cyclic loading and competitive binding on IGF uptake into cartilage tissue.

3. Governing Equations

To describe solute transport in a deforming cartilage, a porous media model is often employed[17, 19-21]. The porous media continuum approach treats the articular cartilage as a combination of solid, fluid and solute phases. The volume fraction of each phase is

$$\phi^\alpha = \frac{V^\alpha}{V} \quad (3.1)$$

where V^α is the volume of α phase, and V the total sum of individual phase volumes. The superscripts s, f and w indicate the solid, fluid and solute phase in the cartilage, respectively. As the volume of solute phase is relatively small compared with the solid and fluid phases, it can be assumed that $\phi^s + \phi^f \approx 1$. With Eq. (3.1), the concentration of solute relative to α phase (c^α) can be expressed as

$$c^\alpha = \frac{\bar{c}^\alpha}{\phi^\alpha} \quad (3.2)$$

where $\bar{c}^\alpha$ is volume-based solute concentration.

The total concentration of IGF-I and -II ($\bar{c}_j$) in cartilage is assumed to be composed of mobile (free) solute ($\bar{c}_j^f$) and its immobile complexes ($\bar{c}_{ji}^s$) attached to two functional groups of IGFBPs. That is

$$\bar{c}_j = \bar{c}_j^f + \sum_{i=1}^2 \bar{c}_{ji}^s, \quad j = 1, 2 \quad (3.3)$$

where subscript i refers to each group of IGFBPs, and subscript $j=1$ refers to IGF-I and $j=2$ to IGF-II.

Conservation of mass

Conservation of mass of free IGF-I and -II is expressed as

$$\frac{\partial(\phi^f c_j^f)}{\partial t} + \nabla \bullet \mathbf{J}_j^f = -\sum_{i=1}^2 s_{ji} \quad j = 1, 2 \quad (3.4)$$

where s_{ji} are source sink terms for IGF-I and -II respectively, representing their interaction with each functional group of IGFBPs attached to the solid phase. The contribution of the IGF receptors are not considered in the model due to the relatively low total receptor concentration and binding affinities in comparison with that of IGFBPs [5, 22].

The mass fluxes ($\mathbf{J}_j^f$) of free IGF-I and -II can be expressed as

$$\mathbf{J}_j^f = -\phi^f D_j \nabla c_j^f + \phi^f \mathbf{v}^f c_j^f \quad j = 1, 2 \quad (3.5)$$

where $\mathbf{v}^f$ is the true fluid velocity relative to a fixed representative element volume (REV). D_j are the effective diffusion coefficients for IGF-I and -II in the uniform cartilage.

The conservation of mass of the complexed IGF-I and -II attached to the two functional groups on the solid phase is described by,

$$\frac{\partial[(1-\phi^f)c_{ji}^s]}{\partial t} + \nabla \bullet \mathbf{J}_{ji}^s = s_{ji} \quad j = 1, 2 \quad (3.6)$$

where $\mathbf{J}_{ji}^s$ are the mass fluxes of IGF-I and -II in solid phase. Assuming that solute transport in the solid phase via diffusion is negligible, then,

$$\mathbf{J}_{ji}^s = (1-\phi^f) \mathbf{v}^s c_{ji}^s \quad j = 1, 2 \quad (3.7)$$

where $\mathbf{v}^s$ is the velocity of the solid phase relative to the REV.

Summing Eq. (3.4) and (3.6), leads to

$$\frac{\partial(\phi^f c_j^f)}{\partial t} + \nabla \bullet \mathbf{J}_j^f + \frac{\partial}{\partial t} \left[(1 - \phi^f) \sum_{i=1}^2 c_{ji}^s \right] + \nabla \bullet \left(\sum_{i=1}^2 \mathbf{J}_{ji}^s \right) = 0 \quad j = 1, 2 \quad (3.8)$$

Substituting $\mathbf{J}_j^f$, $\mathbf{J}_{ji}^s$ in Eq. (3.5) and (3.7) into (3.8), leads to

$$\begin{aligned} \frac{\partial(\phi^f c_j^f)}{\partial t} + \nabla \bullet \left(-\phi^f D_j \nabla c_j^f + \phi^f \mathbf{v}^f c_j^f \right) \\ + \frac{\partial}{\partial t} \left[(1 - \phi^f) \sum_{i=1}^2 c_{ji}^s \right] + \nabla \bullet \left[\sum_{i=1}^2 (1 - \phi^f) \mathbf{v}^s c_{ji}^s \right] = 0 \quad j = 1, 2 \end{aligned} \quad (3.9)$$

which describes solute transport by diffusion and advection in a deforming porous media.

The conservation of mass of solid and fluid phase in the REV leads to a governing equation linking the cartilage matrix deformation to interstitial fluid motion[16, 18].

$$\nabla \bullet (\mathbf{v}^s - \kappa \nabla p) = 0 \quad (3.10)$$

where $\mathbf{v}^s$ is solid phase velocity, κ the hydraulic permeability tensor, and p the interstitial fluid pressure. Using Eq. (3.10), we can simplify the Eq. (3.9) after some algebraic manipulations[18]

$$\begin{aligned} \phi^f \frac{\partial c_j^f}{\partial t} + (1 - \phi^f) \sum_{i=1}^2 \frac{\partial c_{ji}^s}{\partial t} - \phi^f D_j \nabla^2 c_j^f + [\phi^f \mathbf{v}^s - \kappa \nabla p - \nabla(\phi^f D_j)] \bullet \nabla c_j^f \\ + [(1 - \phi^f) \mathbf{v}^s] \bullet \nabla \left(\sum_{i=1}^2 c_{ji}^s \right) = 0 \quad j = 1, 2 \end{aligned} \quad (3.11)$$

Eq. (3.11) is the governing transport equation for IGF-I and -II. The mechanical quantities, such as fluid pressure, displacement and velocity can be determined using methods presented in our previous studies[16, 18].

The Law of mass action

The chemical equations describing the binding of IGFs to their binding proteins are

where IGFBP_{*i*} (*i* = 1 to 2) represents two functional groups of IGFBPs; k_{+ji} is the association rate constant of solute_{*j*} (IGF-I and -II) with each two IGFBP functional groups; and k_{-ji} the dissociation rate constant of IGF-I and -II with each IGFBP functional group.

Assuming no net change of IGFBPs on the timescale of transport experiment, the chemical reactions involving IGFs and two groups of IGFBs are described mathematically using the Law of Mass Action[23-25]. That is, it is assumed that the reaction rate is directly proportional to the product of the effective volume-based concentrations of each participating molecule. These assumptions lead to the following simultaneous equations.

$$\frac{d\bar{c}_{BPi}^s}{dt} = \sum_{j=1}^2 (k_{-ji}\bar{c}_{ji}^s - k_{+ji}\bar{c}_{BPi}^s\bar{c}_j^f) \quad i = 1, 2 \quad (3.13a)$$

$$\frac{d\bar{c}_{li}^s}{dt} = k_{+li}\bar{c}_1^f\bar{c}_{BPi}^s - k_{-li}\bar{c}_{li}^s \quad i = 1, 2 \quad (3.13b)$$

$$\frac{d\bar{c}_{2i}^s}{dt} = k_{+2i}\bar{c}_2^f\bar{c}_{BPi}^s - k_{-2i}\bar{c}_{2i}^s \quad i = 1, 2 \quad (3.13c)$$

By adding Eq. (3.13a), (3.13b) and (3.13c), we obtain

$$\frac{d(\bar{c}_{BPi}^s + \bar{c}_{li}^s + \bar{c}_{2i}^s)}{dt} = 0 \quad i = 1, 2 \quad (3.14)$$

Thus, $\bar{c}_{BPi}^s(t) + \bar{c}_{li}^s(t) + \bar{c}_{2i}^s(t) = m_i$. The integration constants m_i can be obtained from the initial condition,

$$\bar{c}_{BPi}^s(t) + \bar{c}_{li}^s(t) + \bar{c}_{2i}^s(t) = \bar{c}_{BPi}^s(0) + \bar{c}_{li}^s(0) + \bar{c}_{2i}^s(0) \quad i = 1, 2 \quad (3.15)$$

Letting $\bar{c}_{BPi}^s(t=0) = \bar{c}_{BPi0}^s$, $\bar{c}_{li}^s(t=0) = \bar{c}_{li0}^s$ and $\bar{c}_{2i}^s(t=0) = \bar{c}_{2i0}^s$ and substituting Eq. (3.15) into (3.13b) and (3.13c), we obtain

$$\frac{1}{k_{+li}} \frac{d\bar{c}_{li}^s}{dt} = \bar{c}_1^f (\bar{c}_{BPi0}^s + \bar{c}_{li0}^s + \bar{c}_{2i0}^s - \bar{c}_{2i}^s) - (K_{Dli} + \bar{c}_1^f) \bar{c}_{li}^s \quad i = 1, 2 \quad (3.16)$$

$$\frac{1}{k_{+2i}} \frac{d\bar{c}_{2i}^s}{dt} = \bar{c}_2^f (\bar{c}_{BPi0}^s + \bar{c}_{1i0}^s + \bar{c}_{2i0}^s - \bar{c}_{1i}^s) - (K_{D2i} + \bar{c}_2^f) \bar{c}_{2i}^s \quad i = 1, 2 \quad (3.17)$$

where

$$K_{D1i} = \frac{k_{-1i}}{k_{+1i}}, \quad K_{D2i} = \frac{k_{-2i}}{k_{+2i}} \quad i = 1, 2 \quad (3.18)$$

are the dissociation constants for the binding reactions. Eq. (3.16) and (3.17) are the governing equations describing the time-dependent competitive binding behavior of IGF-I and -II. The equilibrium competitive binding equations can be obtained from Eq. (3.16) and (3.17) by setting

$$\frac{d\bar{c}_{1i}^s}{dt} = 0 \quad \text{and} \quad \frac{d\bar{c}_{2i}^s}{dt} = 0. \quad \text{We find,}$$

$$\bar{c}_1^f (\bar{c}_{BPi0}^s + \bar{c}_{1i0}^s + \bar{c}_{2i0}^s - \bar{c}_{2i}^s) - (K_{D1i} + \bar{c}_1^f) \bar{c}_{1i}^s = 0 \quad i = 1, 2 \quad (3.19)$$

$$\bar{c}_2^f (\bar{c}_{BPi0}^s + \bar{c}_{1i0}^s + \bar{c}_{2i0}^s - \bar{c}_{1i}^s) - (K_{D2i} + \bar{c}_2^f) \bar{c}_{2i}^s = 0 \quad i = 1, 2 \quad (3.20)$$

Solving Eq. (3.19) and (3.20) for the ‘complexed’ IGF-I and -II, leads to expressions

$$\bar{c}_{1i}^s = \frac{\bar{c}_{BPi0}^s K_{D2i} \bar{c}_1^f}{K_{D11} (K_{D21} + \bar{c}_2^f) + K_{D21} \bar{c}_1^f} \quad i = 1, 2 \quad (3.21)$$

$$\bar{c}_{2i}^s = \frac{\bar{c}_{BPi0}^s K_{D1i} \bar{c}_2^f}{K_{D11} (K_{D21} + \bar{c}_2^f) + K_{D21} \bar{c}_1^f} \quad i = 1, 2 \quad (3.22)$$

These turn out to be a pair of competitive Langmuir sorption isotherms[24]. The total growth factor uptake ratio (R_{ui}) can be expressed as the sum of mobile (free) solute uptake (R_{ui}^f) and bound (complexed) solute uptake (R_{ui}^s), viz,

$$R_{ui} = R_{ui}^f + R_{ui}^s \quad i = 1, 2 \quad (3.23)$$

where

$$R_{ui}^f = \frac{\bar{c}_i^f}{\bar{c}_{i0}}, \quad R_{ui}^s = \sum_{j=1}^2 \frac{\bar{c}_{ij}^s}{\bar{c}_{i0}} \quad i = 1, 2 \quad (3.24)$$

and $\bar{c}_{i0}$ are IGF-I and -II concentration in synovial fluid at the outer surface of cartilage respectively. At steady-state, $\bar{c}_i^f = \bar{c}_{i0}$ resulting in $R_{ui}^f = 1$.

Substituting Eq. (3.21) and (3.22) into (3.23), we find a suitable expression for comparison with the experimental results of Bhakta et al[4], viz,

$$\text{IGF-I uptake ratio} \quad R_{u1} = 1 + \sum_{i=1}^2 \frac{\bar{c}_{BPi0}^s K_{D2i}}{K_{D11} (K_{D21} + \bar{c}_2^f) + K_{D21} \bar{c}_1^f} \quad (3.25)$$

$$\text{IGF-II uptake ratio} \quad R_{u2} = 1 + \sum_{i=1}^2 \frac{\bar{c}_{BPi0}^s K_{D1i}}{K_{D11} (K_{D21} + \bar{c}_2^f) + K_{D21} \bar{c}_1^f} \quad (3.26)$$

Equations (3.25) and (3.26) are the growth factor uptake ratios for the steady-state condition. Using the experimental data (shown in Figure 1), we have used a nonlinear least squares and curve-fitting feature in MATLAB's optimization toolbox[26] to determine the six unknown model parameters, namely, K_{D11} , K_{D12} , K_{D21} , K_{D22} , c_{BP10} and c_{BP20} .

4. Results and Discussion

The values of parameters estimated by the optimization process are shown in Table 1. The estimates of K_{D12} and K_{D22} indicate that IGF-II has a 39-fold greater affinity for IGFBP-6 than for IGF-I. This is consistent with previous experimental studies showing IGFBP-6 has 20-100 fold higher binding affinity for IGF-II than IGF-I, while the estimates of K_{D11} and K_{D12} are also consistent with the estimates for IGFs and IGFBPs 1-5 obtained in previous experimental studies[7, 9-12]). Our results also estimate a total binding protein density of 145nM for IGF-I and -II, with around 70% of IGFBPs in bovine cartilage being IGFBP-6. These findings agree with Morales et al's investigation of IGFBP in bovine cartilage[15], which found the total binding protein density ranges from 30~150nM, and that IGFBP-6 is the major IGFBP species in bovine cartilage.

We conclude that the proposed model can reproduce the main features of distinctive competitive binding phenomena of IGFs observed experimentally, and is consistent with published literature. It should be noted that the parameters obtained are from an optimization based on an experiment performed at 4°C, as such it is expected that parameters (e.g. binding affinities) would likely vary with temperature in a coordinated fashion. The parameters are now incorporated in the poroelastic transport model, and the composite model is then employed to predict the IGF uptake under a variety of conditions.

Theoretical prediction of growth factor transport in cartilage

Consistent with previous experimental and theoretical protocols for the study of solute transport in cartilage[16-18], we first study the radial solute transport into a cylindrical cartilage disk under free diffusion, and later the solute transport within a cartilage disk under unconfined cyclic compression is also explored. The governing equations in cylindrical coordinates[18] are solved numerically using the commercial Finite Element software FEMLAB[27]. Parameters given in Table 1-2 are adopted to enable direct comparison with our previous works[16, 18]. To describe the time-dependent competitive binding behavior of IGF-I and -II, the input of association rate constants k_{+1i} and k_{+2i} in Eq. (3.16) and (3.17) can be estimated from the experiments of Bach et al, 1993[28] and Headey et al, 2004[12] (around $3 \times 10^5 \text{ s}^{-1} \text{ M}^{-1}$). The applied strain protocol is discretized into finite time steps, and at each time step, the solid phase displacement u_r , solid phase velocity v_r and interstitial fluid pressure p are calculated. The solutions for v_r and p are then employed to solve for the solute concentrations (c_i^f and c_{ji}^s) in the transport equations.

Since the solute concentration is generally non-uniform in the radial direction, it is useful to define the average total IGF uptake ratio ($\bar{R}_{ui}$) as the sum of average free IGF uptake ratio

($\bar{R}_{ui}^f$) and average complexed IGF uptake ratio ($\bar{R}_{ui}^s$). These parameters provide a measure of IGF concentration in the cartilage tissue, relative to the bath concentration, and are defined through equations (4.1) and (4.2)

$$\bar{R}_{ui} = \bar{R}_{ui}^f + \bar{R}_{ui}^s \quad i = 1, 2 \quad (4.1)$$

where

$$\bar{R}_{ui}^f = \frac{1}{\bar{c}_{i0}} \left(\frac{\int_0^{r_0} 2\pi r \bar{c}_i^f dr}{\int_0^{r_0} 2\pi r dr} \right) \text{ and } \bar{R}_{ui}^s = \frac{1}{\bar{c}_{i0}} \left[\sum_{j=1}^2 \left(\frac{\int_0^{r_0} 2\pi r \bar{c}_{ij}^s dr}{\int_0^{r_0} 2\pi r dr} \right) \right] \quad i = 1, 2 \quad (4.2)$$

Note, the complexed IGF uptake ratio quantifies the concentration of IGF bound to binding proteins within the cartilage relative to the concentration of free IGF in the synovial fluid.

Due to the avascular nature of cartilage, IGFs must diffuse or be advected into the cartilage to interact with chondrocytes. The major source of circulating IGF-I is from liver, whereas IGF-II in cartilage is likely contributed by many tissues including brain, bone and muscle.[29]. The immediate source of the IGFs found in articular cartilage is believed to be from synovial fluid [22]. In normal human synovial fluid, the concentration of IGF-I is much lower than that of IGF-II[13]. By setting boundary conditions to that observed in human synovial fluid[13, 22, 30], we see that competitive binding leads to a relatively higher free IGF-I uptake within the cartilage compared to predictions based on a model that neglects competitive binding by IGF-II (around 6%) as shown in Figure 2a. As experimental studies[1, 31] suggested that the biosynthesis of matrix molecules can be rapidly “switched on” when IGF-I concentration reaches a certain threshold, the predicted increase in free IGF-I, though small, may have a significant effect on cartilage biosynthesis. In contrast, ignoring competitive binding results in an overestimation of complexed IGF-I uptake (Figure 2b), and prolongs the time for the complexed IGF-I to reach equilibrium. IGF-I actions are principally determined by the capacity

of free (unbound) IGF-I to interact with IGF-I receptors[32]. The results of Figure 2a reveals that the competitive binding may play an important role in increasing the bioavailability of free IGF-I to receptors.

These findings may be explained qualitatively in the following way. The presence of IGF-II in cartilage effectively inhibits the binding of IGF-I to the first group of IGFBPs (i.e. IGFBPs 1-5) by competing for the limited numbers of binding sites. IGF-I then is free to move into the cartilage without needing to “fill up” vacant binding sites. Thus, ligand competition for binding sites results in an increase in the rate of free IGF-I uptake.

The parametric studies

In human OA cartilage, it has been found that IGF-I concentration in synovial fluid, as well as the IGFBP content in cartilage, differ from normal physiological state[13, 14]. The effect of these changes on free IGF-I uptake within the cartilage is relatively difficult to quantify in experiments, either in vitro or in vivo. For this reason, the purpose of current parametric study is to conduct a series of ‘mathematical experiments’ to understand the bioavailability of free IGF-I under various cartilage conditions.

Both free IGF-I and -II influence cartilage biosynthesis. However, IGF-II is less potent than IGF-I, for example, the effects of 100ng/ml IGF-II on cartilage biosynthesis was found only about 60% of those for 5 ng/ml IGF-I[1]. According to experimental reports [13], there is a significant increase in the total IGF-I level in human synovial fluid in both osteoarthritis and rheumatoid arthritis patients in comparison with normal cartilage (e.g. increasing from 20 to 80ng/ml[13]), but no change in the level of IGF-II. In Figure 3, the IGF-I concentration (c_{I0}) at cartilage boundary is varied to explore the possible IGF-I transport behavior for OA (i.e.

diseased) conditions. The results of this analysis indicate that the rate of free IGF-I uptake is relatively higher in the diseased condition (Figure 3a). As for the complexed IGFs, the model predicts a decrease of fixed complexed IGF-I uptake (Figure 3b). In OA cartilage, a disruption of the homeostasis occurs between matrix synthesis and degradation, favouring the progressive loss of matrix molecules (e.g. GAG). Although the numerical results indicate an increased rate of free IGF-I uptake within the cartilage due to the increased content in the synovial fluid, the enhanced anabolic action of IGF-I presumably still fails to counterbalance the degradation process. However, changes in binding proteins in diseased cartilage can further complicate this picture, as we shall now demonstrate.

In arthritic conditions, variations of IGFBP concentration may also modulate the growth factor uptake. Studies have demonstrated that OA cartilage produces increased concentrations of IGFBPs, especially IGFBP-2, -3, -4 and -5. Indeed, the production of IGFBP-3 can be 3 fold higher than that in normal cartilage[33, 34]. Therefore there could be a commensurate increase of the concentration of all IGFBPs in diseased conditions, particularly the first functional group of IGFBP (e. g. IGFBPs 1-5). In Figure 4, the IGF-I concentration as a function of time for various concentrations of the first functional group of IGFBP (c_{BP1}) is shown. With an increase in c_{BP1} , there is a significant increase of complexed IGF-I (Figure 4b) but a substantial decrease in the rate of free IGF-I uptake (Figure 4a). These results show that although an increased amount of IGF-I in arthritic synovial fluid could enhance the bioavailability of IGF-I within the cartilage (Figure 3), a simultaneous elevation of the c_{BP1} in diseased cartilage may lead to a decrease of free IGF-I concentration. This numerical prediction is supported by experimental studies[33, 35] suggesting that the synthesis of IGFBPs limits the presence of free IGF-I in the cartilage tissue, thus inhibiting its anabolic action. Hence the current study underlines the

importance of considering the cartilage as a system, with multiple interacting components. It should be mentioned here that to simplify the problem, this study ignores the possible IGF-independent effects of IGFBPs suggested by Mohan et al, 2002[29]. Clearly additional systems need to be included in the model, and so the predictions made here must necessarily be considered provisional.

The content of IGFBPs in cartilage varies between species. Although previous work had identified large amounts of IGFBP-6 in bovine cartilage extracts using quantitative Western ligand blots[15], IGFBP-6 has not been detected in human cartilage so far using the same method. However, a recent study by Aigner et al (2006)[36] detected IGFBP-6 mRNA expression in human cartilage through a large gene expression profiling study using a complementary DNA array covering more than 4,000 genes. Their study revealed a 0.43 fold change of IGFBP-6 gene expression in OA compared with normal cartilage. Therefore, the effect of IGFB-6 on IGF-I transport and its availability is worthy of further investigation. Therefore in Figure 5 we show model predictions of IGF-I uptake in cartilage in the presence or absence of IGFBP-6. It can be seen that the presence of IGFBP-6 increases the level of complexed IGF-I in cartilage but has a relatively little influence on free IGF-I, especially in comparison to that of IGFBPs 1-5 (Figure 4a). The theoretical results presented here indicate that the variation in IGFBP-6 content in cartilage between species may not affect the bioavailability of free IGF-I. The questions then are, what exactly is the functional role IGFBP-6 in articular cartilage, and why does it vary between species? Our future studies will address these questions.

Diffusion with cyclic deformation

The model developed here can also be used to explore the combined effects of cyclic compression and competitive binding on IGF uptake. Here the dynamic compression is applied under strain control, assuming ε_0 is the peak-to-peak strain amplitude and f is the loading frequency, then

$$\varepsilon_z(t) = \frac{\varepsilon_0}{2} [1 - \cos(2\pi f t)] \quad (4.3)$$

Our previous study[18] examined the effect of solute binding on IGF-I transport in a cyclically deformed cartilage. It was demonstrated that IGFBPs enhanced IGF-I uptake by the cartilage. To further understand the effect of competitive binding in combination with cyclic loading, the uptake of IGF-I in cartilage subjected to 5 hour 0.1 Hz and 6% strain dynamic compression is considered. Figure 6 shows cyclic loading significantly enhances both free and complexed IGF-I uptake, however, it is noted that the enhancement is considerably exaggerated if the competitive binding is ignored in the model.

5. Conclusion

In this study, we have developed a poromechanical continuum transport model coupled with the second order competitive binding model of IGFs, and employed this model to describe growth factor uptake by cartilage. The results demonstrated that by modeling the IGFBP as two functional groups, the distinctive experimental binding outcomes of Bhakta et al's experiment[4] can be described using reversible competitive Langmuir sorption models. Moreover, this sorption model suggests that the availability of free IGF-I in the cartilage is mainly regulated by IGFBPs 1-5, while IGFBP-6 appears to have little influence due to its relatively low binding affinity to IGF-I.

The model parameters, such as IGF concentration at cartilage surface, and ratio of two functional IGFBP groups, are then examined so as to investigate the growth factor transport behavior for various cartilage conditions. A summary of main findings are:

- The numerical results show that ignoring competitive binding results in an underestimation of the rate of free IGF-I uptake, but an overestimation of complexed IGF uptake, and prolongs the time for total IGF-I to reach equilibrium. The biological implication of these findings is that competitive binding may increase the bioavailability of IGF-I by reducing the transport inhibiting effect of IGFBPs, while still provide a store of IGFs.
- The increase of IGF-I level at cartilage surface leads to a greater rate of free IGF-I uptake in the cartilage, however, the increase of the first IGFBP functional group (i.e. IGFBPs 1-5) significantly decreases the rate of free IGF-I uptake.
- Dynamic compression enhances the rate of free IGF-I uptake; however, this enhancement is exaggerated if the competitive binding is ignored.

Articular cartilage is a complex tissue, and so should be considered from a systems perspective. Although IGFs and IGFBPs have been widely studied, it still remains unclear exactly how their presence in cartilage contribute to ECM synthesis. In diseased conditions, large modules like IGFBPs and their complexes (with IGFs), may also permeate the cartilage from synovial fluid due to the damage of ECM. Future research will focus on the contribution of IGFBPs, as well as their complexes with IGFs, to the level of free IGFs in normal and diseased cartilage tissue.

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Table 1 Binding parameters computed from fitting Equations (34) and (35) to the data of Bhakta et al (2000)[4]

Parameter	Calculated value	Previous experimentally reported range
Dissociation constant for IGF-I and IGFBPs 1-5 (K_{D11})	4.8 nM	0.5-10 nM [7, 8, 25, 28]
Dissociation constant for IGF-II and IGFBPs 1-5 (K_{D21})	5.2 nM	0.8-10 nM [7, 12, 37]
Dissociation constant for IGF-I and IGFBPs 6 (K_{D12})	222.1 nM	(20-70) x K_{D22} [4]
Dissociation constant for IGF-II and IGFBPs 6 (K_{D22})	5.7 nM	0.6-10 nM [12, 38, 39]
IGFBPs 1-5 concentration ($\bar{c}_{BP10}^s$)	45 nM	$\bar{c}_{BP10}^s + \bar{c}_{BP20}^s = 30-150\text{nM}$ [15]
IGFBP-6 concentration ($\bar{c}_{BP20}^s$)	101 nM	

Table 2: Range of parameters used throughout this study

Parameter	Value	Ref
IGF-I diffusion coefficient (D_I)	$4.1 \times 10^{-11} \text{ m}^2/\text{s}$	[4]
IGF-II diffusion coefficient (D_2)	$4.1 \times 10^{-11} \text{ m}^2/\text{s}$	[4]
Fluid phase volumetric fraction (ϕ^f)	0.8	[40]
Aggregate elastic modulus (H_A)	0.27 MPa	[40]
Hydraulic permeability (κ_r)	$1.16 \times 10^{-15} \text{ m}^4/\text{Ns}$	[41, 42]
Free (unbound) IGF-I concentration in synovial fluid (c_{I0})	0.1nM	[13, 30]
Free (unbound) IGF-II concentration in synovial fluid (c_{II0})	1nM

(b)

Figure 1 Comparison of numerical solution to Bhakta et al's experimental data[4]. (a) Competition of unlabeled IGF-I and -II with ^{125}I -IGF-I. (b) Competition of unlabeled IGF-I and -II with ^{125}I -IGF-II.

Figure 2 Comparison of IGF-I uptake ratio as a function of time in the presence ($c_{I0} = 0.1\text{nM}$, $c_{H0} = 1\text{nM}$) or in the absence of competitor ($c_{I0} = 0.1\text{nM}$, $c_{H0} = 0$) in normal cartilage. (a) Free IGF-I; (b) Complexed IGF-I.

Figure 3 Comparison of time-dependent average IGF-I uptake ratio in the cartilage under various boundary IGF-I concentrations ($c_{IIO}=1$ nM, $c_{BP1} = 45$ nM; $c_{BP2} = 101$ nM). (a) Free IGF-I; (b) Complexed IGF-I.

Figure 4 Comparison of time-dependent average IGF-I uptake ratio, under various ratio of two functional IGFBP groups ($c_{I0} = 0.1$ nM, $c_{II0} = 1$ nM, $c_{BP1} = 45$ nM; $c_{BP2} = 101$ nM). (a) Free IGF-I; (b) Complexed IGF-I.

Figure 5 Comparison of time-dependent average IGF-I uptake ratio in cartilage under various ratio of two functional IGFBP groups ($c_{I0} = 0.1$ nM, $c_{II0} = 1$ nM, $c_{BP1} = 45$ nM; $c_{BP2} = 101$ nM). (a) Free IGF-I; (b) Complexed IGF-I.

Figure 6 Percent increase in IGF-I uptake ratio as a function of time in normal condition, in the absence or in the presence of IGF-II competition, after 1 hour dynamic compression (0.1 Hz, 6% strain). (a) Free IGF-I; (b) Complexed IGF-I.