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Quantitative Analysis of Exogenous IGF-1 Administration of Intervertebral Disc Through Intradiscal Injection

C.-Y. Huang¹, F. Travascio², and W.Y. Gu²

¹Stem Cell and Mechanobiology Lab, Dept of Biomedical Engineering, University of Miami, Coral Gables, Florida 33146, USA

²Tissue Biomechanics Lab, Dept of Mechanical and Aerospace Engineering, University of Miami, Coral Gables, Florida 33146, USA

Abstract

Exogenous administration of IGF-1 has been proposed as a therapy for disc degeneration. The objectives of this study were to develop a numerical model for quantitatively analyzing exogenous administration of IGF-1 into the intervertebral disc (IVD) via intradiscal injection and to investigate the effects of IGF-1 administration on distribution of glucose and oxygen in the IVD. In this study, *the reversible binding reaction between IGF-1 and IGF binding proteins was incorporated into the mechano-electrochemical mixture model.* The model was used to numerically analyze transport of IGF-1, glucose, oxygen and lactate in the IVD after IGF-1 administration. The enhancement of IGF-1 on lactate production was also taken into account in the theoretical model. The numerical analyses using finite element method demonstrated that the binding reactions significantly affect the time-dependent distribution of IGF-1 in the IVD. *It was found that the region affected by IGF-1 was smaller and the duration of the therapeutic IGF-1 level was longer in the degenerated disc with a higher concentration of IGF binding proteins.* It was also found that the IGF-1 injection can reduce glucose concentration and increase lactate accumulation (i.e., lower pH) in the IVD and these influences were regulated by the IGF-1 binding reactions. This study indicated the complexity of intradiscal administration of growth factors, which needs to be fully analyzed in order to achieve a successful outcome. The new theoretical model developed in this study can serve as a powerful tool in analyzing and designing the optimal treatments of growth factors for disc degeneration.

Keywords

intervertebral disc; IGF-1; disc degeneration; growth factor therapy

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Corresponding Author: WY Gu, Ph.D., Department of Mechanical and Aerospace Engineering, College of Engineering, University of Miami, P.O. Box 248294, Coral Gables, FL 33124-0624, USA, Telephone: (305) 284-8553, Fax: (305) 284-2580, wgu@miami.edu.

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.

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INTRODUCTION

The homeostasis of the intervertebral disc (IVD) is regulated by a delicate balance between catabolic and anabolic cellular processes. Unbalanced metabolic activity leads to the degeneration of the IVD tissue (Masuda and An, 2004b). Several growth factors are known to up-regulate the anabolic activity of disc cells (e.g., TGF- β , IGF, BMPs, etc.) (Masuda and An, 2004a). In particular, it has been reported that IGF-1 stimulates the production of proteoglycans (PG) composing the extracellular matrix (ECM) of the IVD (Osada et al., 1996; Thompson et al., 1991). Therefore, the exogenous administration of IGF-1 has been proposed as a therapy for disc degeneration (Osada et al., 1996; Thompson et al., 1991). Since the response of IVD cells to IGF-1 stimulation is dose-dependent (Osada et al., 1996; Thompson et al., 1991), knowledge and control of the distribution of this molecule in the IVD is crucial for the success of the therapy. However, molecular transport in the IVD is very complex and involves a series of coupled mechano-electrochemical events. In particular, transport of IGF-1 is regulated by reversible binding reactions with binding sites (mainly IGFBP-3) present on the ECM of cartilaginous tissues (Bhakta et al., 2000; Garcia et al., 2003; Osada et al., 1996). In order to optimize the administration of IGF-1 for a successful therapy, analyzing the complex molecular transport is necessary and requires the aid of a numerical tool.

Administration of IGF-1 has been shown to promote cell proliferation and ECM production (Osada et al., 1996; Pratsinis and Kletsas, 2007b), which in turn increases demand for nutrients. Thus, after injection, tissue regions infused by exogenous IGF-1 might require higher nutritional supply. Since the IVD is the largest avascular tissue in the body, increasing nutritional demand may further worsen the condition of malnutrition within the IVD, which could be detrimental to cell viability. The investigation on the effect of IGF-1 administration on nutritional supply would provide crucial information for optimizing this therapeutic strategy for disc degeneration. Therefore, the objectives of this study were to develop a numerical model for quantitatively analyzing exogenous administration of IGF-1 of the IVD via intradiscal injection *and to test the following hypotheses*:

1. Binding reactions affect the effectiveness of IGF-1 treatment in the degenerated IVD by intradiscal injection;
2. Intradiscal IGF-1 injection may not improve nutritional conditions in the degenerated IVD.

THEORETICAL MODEL

The theoretical model developed in this study was based on the mechano-electrochemical mixture theory (Lai et al., 1991; Gu et al., 1998; Ateshian, 2007) and an extension of the theoretical framework reported in our previous study (Huang and Gu, 2008). The IVD tissue was considered as a mixture of an elastic, porous, permeable, negatively charged solid phase with IGF binding proteins, an interstitial fluid phase, and six solute phases (i.e., sodium and chloride ions, oxygen, glucose, lactate, and IGF-1). *In the IVD, an immobile IGF bound complex (BC) was assumed to be formed by and reversibly dissociated into a mobile IGF-1 molecule (I) and an immobile IGF binding protein molecule (BP). This reversible binding of IGF-1 and binding proteins was described using a simple binding (Langmuir) model (Zhang et al., 2007):*

where k_{on} and k_{off} are essentially the rates of association (binding) and dissociation (unbinding) of the constituents, respectively. *It was assumed that the rate of generation of*

the bound complex is proportional to the concentrations of IGF-1 and IGF binding protein molecules, while the rate of dissociation of immobile bound complex is proportional to its concentration. Using the law of mass action, the relation presented in Eq. (1) can be described as:

$$\frac{dc^{BC}}{dt} = k_{on}c^I c^{BP} - k_{off}c^{BC}, \quad (2)$$

$$\frac{dc^I}{dt} = -k_{on}c^I c^{BP} + k_{off}c^{BC}, \quad (3)$$

$$\frac{dc^{BP}}{dt} = -k_{on}c^I c^{BP} + k_{off}c^{BC}, \quad (4)$$

where c^{BC} is the concentration of the immobile bound complex formed by IGF-1 and binding proteins (per unit interstitial water volume), c^I is the concentration of IGF-1 (per unit interstitial water volume), c^{BP} is the concentration of free binding proteins (per unit interstitial water volume). Note that it follows from Eqs (2) and (4) that

$$\frac{dc^{BP}}{dt} + \frac{dc^{BC}}{dt} = 0. \quad (5)$$

Eq. (5) indicates that, at any time, the sum of the concentrations of the immobile bound complex and the free binding proteins is constant. By integrating Eq. (5), the concentration of the free binding proteins (c^{BP}) present in the ECM of the tissue can be expressed as:

$$c^{BP}(t) = c_o^{BP} + c_o^{BC} - c^{BC}(t) = c_t^{BP} - c^{BC}(t), \quad (6)$$

where c_o^{BP} and c_o^{BC} are the initial concentrations of free binding proteins and bound complex (at $t = 0$), respectively, and $c_t^{BP} (= c_o^{BP} + c_o^{BC})$ is the total concentration of binding proteins. Assuming that IGF-1 is not consumed or produced by IVD cells, the mass balance equations for IGF-1, binding proteins and, the immobile bound complex in the mechano-electrochemical mixture model (Lai et al., 1991; Gu et al., 1998; Ateshian, 2007; Huang and Gu, 2008) were derived based on Eqs. (2)–(5), and given as:

$$\partial(\phi^w c^I)/\partial t + \nabla \cdot (\mathbf{J}^I + \phi^w c^I \mathbf{v}^s) = -\phi^w Q^{bind}, \quad (7)$$

$$\partial(\phi^w c^{BC})/\partial t + \nabla \cdot (\phi^w c^{BC} \mathbf{v}^s) = \phi^w Q^{bind}, \quad (8)$$

$$Q^{bind} = k_{on}(c_t^{BP} - c^{BC})c^I - k_{off}c^{BC}, \quad (9)$$

where $\mathbf{J}^I$ is the molar flux of IGF-1 relative to the solid phase, ϕ^w is the tissue porosity (volume fraction of water), $\mathbf{v}^s$ is the velocity of solid matrix, and Q^{bind} is the source (or sink) resulted from binding reactions. The total concentration of binding proteins can be expressed as a function of tissue deformation:

$$c_t^{BP} = \frac{c_{t_0}^{BP}}{1 + e/\phi^w} \quad (10)$$

where e is the relative volume change of the tissue (equal to the dilation for an infinitesimal strain case) and $c_{t_0}^{BP}$ is the total concentration of binding proteins at reference configuration (i.e., no deformation). The metabolic rates of sodium and chloride ions were assumed to be zero (i.e., $Q^+ = Q^- = 0$). The oxygen consumption rates of IVD cells were pH dependent and described in our previous study (Huang and Gu, 2008). Based on the most recent findings (Guehring et al., 2009; Bibby et al., 2005), the lactate production rate of IVD cells was described as:

$$Q^{Lactate} = \exp(-2.47 + 0.93 \times pH + 0.16 \times c^{O_2} - 0.0058 \times c^{O_2^2}) \rho^{cell} c^{glucose} / (K_M + c^{glucose}), \quad (11)$$

where c^{O_2} and $c^{glucose}$ is the concentration of oxygen (the unit: kPa) and glucose (the unit: mM) and ρ^{cell} is the cell density of tissue, and $K_M (=0.19 \text{ mM})$ is the Michaelis-Menten constant (Guehring et al., 2009). The ratio between lactate production and glucose consumption was assumed to be 2.0 (Guehring et al., 2009; Bibby et al., 2005). The relationship between pH and lactate concentration reported in previous studies (Soukane et al., 2005; Huang and Gu, 2008; Bibby et al., 2005) was used to determine pH within the IVD. The tension-compression nonlinearity of the solid matrix in the annulus fibrosus (AF) region was modeled using the conewise linear elasticity (CLE) (Soltz and Ateshian, 2000; Huang et al., 2001; Curnier et al., 1995), while the solid matrix in the nucleus pulposus (NP) region was assumed to be an isotropic material. Strain-dependent properties (fixed charge density, solute diffusivities, hydraulic permeability, and volume fraction of water) were taken into account in the simulation (Lai et al., 1991; Gu et al., 2003a; Gu et al., 2004; Yao and Gu, 2004). More details of the theoretical formulation can be found in our previous studies (Huang and Gu, 2008; Huang and Gu, 2007; Yao and Gu, 2004; Yao and Gu, 2006; Yao and Gu, 2007).

FINITE ELEMENT ANALYSIS

Human lumbar IVD was schematized as a 2D axisymmetric object consisting of two anatomical regions, the NP and the AF, with dimensions and properties similar to those reported in our previous study (Huang and Gu, 2008) (Figure 1). *All numerical simulations were performed on degenerated disc and the data were compared to those for healthy disc in some cases. The solute exchange area of the cartilaginous endplate (CEP) above the NP was 50% and 25% for healthy and degenerated discs, respectively (Huang and Gu, 2008). The CEP above the AF was impermeable (no solute exchange).* Due to the lack of experimental data, IGF-1 transport parameters in the disc were assumed to be similar to those reported in previous studies for articular cartilage (Garcia et al., 2003; Zhang et al., 2007). In the simulations, the IVD was initially in equilibrium with prescribed boundary conditions without IGF-1 injection and then a saline solution containing 1 $\mu\text{g/ml}$ IGF-1 was injected at the center of the NP, via a 22 gauge needle (outer diameter = 0.72 mm). A total of 100 μl IGF-1 solution was injected with a constant flow rate of 10 $\mu\text{l/min}$. Injected solutions containing different concentrations of oxygen and glucose were also examined. The diffusion coefficient of IGF-1 in water solution is $3.23 \times 10^{-11} \text{ m}^2/\text{s}$ (Bonassar et al., 2000; Schneiderman et al., 1995) and its hydrodynamic radius is 1.72 nm (Kuang et al., 2009). Clearance of IGF-1 was assumed to occur through the vascular network surrounding the disc at the CEPs and around the periannular surface of the disc. In this study, only the interaction between IGF-1 and IGFBP-3 binding protein was taken into account, since IGFBP-3 is the most abundant binding protein and IGF-1 is mostly found in a complex with

IGFBP-3 (Collett-Solberg and Cohen, 2000). The rates of association and dissociation of IGF-I and IGFBP-3 used in this study were $k_{on} = 3.67 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, and $k_{off} = 0.001 \text{ s}^{-1}$ (Cassino, 2002). Previous studies suggested that the concentration of IGFBP-3 in bovine cartilage varies from 30 to 150 nM (Morales, 1997). In this study, the initial concentrations of IGFBP-3 were within the same range of variation. In-vitro studies indicated that the enhancement of proteoglycan production occurs when IGF-1 concentration is above the threshold value of 1 ng/ml (Osada et al., 1996; Pratsinis and Kletsas, 2007a). The percentage of the effective volume in the NP region characterized by free (i.e., unbound) IGF-1 concentration higher than 1 ng/ml was evaluated at two different concentrations (i.e., 1.5 to 15 nM) of IGFBP-3 in the IVD. Previous studies also showed that IGF-1 increased energy-demanded cellular activities such as cell proliferation and cellular ECM production (Osada et al., 1996; Pratsinis and Kletsas, 2007c). Since glycolysis is the main pathway of energy production in the IVD cells, it was assumed that lactate production of IVD cells was enhanced by IGF-1 and the relationship between the lactate production rates with and without IGF-1 influence was described as

$$Q^{Lactate*} = \lambda Q^{Lactate} \quad (10)$$

where $Q^{Lactate*}$ is the lactate production rates under IGF-1 influence and λ is a dimensionless scalar factor which is a function of IGF-1 concentration only. In the study of Osada et al (Osada et al., 1996; Pratsinis and Kletsas, 2007d), no effect on proteoglycan production was found when NP cells were treated at the IGF-1 level lower than 1 ng/ml; however, the treatment at the IGF-1 levels of 1 ng/ml, 10 ng/ml and 100 ng/ml increased the proteoglycan production of NP cells by 72%, 182%, and 378%, respectively (determined from the Fig. 1 in the study of Osada et al (1996), while no further increment was found for the IGF-1 concentration higher than 100 ng/ml. By assuming the same effect of IGF-1 on lactate production of IVD cells, λ can be derived based on the data described above and expressed as

$$\lambda = \begin{cases} 1 & \text{when } c^I < 1 \text{ ng/ml} \\ 1.714 \times c^{I 0.2227} & \text{when } 1 \text{ ng/ml} \leq c^I \leq 100 \text{ ng/ml} \\ 4.78 & \text{when } 100 \text{ ng/ml} \leq c^I \end{cases} \quad (11)$$

The boundary conditions for the finite element analyses were listed in Table 1. The parameters used in the numerical simulations for healthy disc were the same as those used in our previous study (Huang and Gu, 2008). To simulate disc degeneration, the following changes in material properties were assumed for the degenerated disc in comparison to the healthy disc: (1) the water content decreased 8% and 9% in the NP and AF regions, respectively; (2) the elastic moduli of NP and AF tissues were increased 100% except the tensile modulus of AF tissue which remained unchanged (Ebara et al., 1996; Iatridis et al., 1997; Iatridis et al., 1999); and (3) the fixed charge density decreased 15% and 38% in the NP and AF regions, respectively. Tissue diffusivity and hydraulic permeability in the NP and AF regions for the degenerated disc were estimated based on the constitutive equations described in our previous study (Huang and Gu, 2008). The specific parameters used in the numerical analyses for the degenerated disc were listed in Table 2. Since previous studies have shown that viability of disc cells began to decrease when glucose levels fell below 0.5 mM (Bibby and Urban, 2004; Bibby, 2002; Guehring et al., 2009; Horner and Urban, 2001), the region of the IVD containing glucose concentration less than 0.5 mM was defined as the endangered volume. The governing equations with the initial and boundary conditions were solved using a finite element method package (COMSOL® 3.5, COMSOL Inc., Burlington,

MA). A mesh of 5404 quadratic Lagrange triangular elements was used in the simulations. The convergence criterion for the solution was the relative error tolerance of less than 10^{-3} .

RESULTS

Distribution of IGF-1 after intradiscal injection *in degenerated discs*

After injection of IGF-1 solution containing no glucose, lactate, or oxygen at the center of NP region, *IGF-1 transports through the IVD by diffusion and convection. The distribution of IGF-1 depended on the initial concentration of binding proteins (Figures 2 and 3).* Compared to the IVD without binding proteins, IGF-1 spread slower in the IVD containing binding proteins after injection (Figures 2a and 3a) since part of injected IGF-1 molecules were bound to binding proteins to form complexes (Figures 2b and 3b). The binding reaction reduces the concentration of free IGF-1, leading to a decrease in IGF-1 diffusion. The effective volume (defined as the volume containing free IGF-1 greater than 1 ng/ml) was time-dependent and regulated by the concentration of binding proteins (Figure 4a). The effective volume of NP was inversely related to the concentration of binding proteins (Figure 4a). However, the duration of the therapeutic level of IGF-1 in the NP region increased with the concentration of binding proteins (Figure 4a). Furthermore, *it was also found that the duration of the therapeutic IGF-1 level in the degenerated IVD was longer compared to that in the healthy disc (Figure 4b).*

Distribution of glucose, lactate and pH after intradiscal injection *in degenerated discs*

Injection of IGF-1 decreased glucose concentration in the NP region (Figures 5a). Such decreases were regulated by the concentration of binding protein (Figures 5a). *Following IGF-1 injection, a low glucose level close to zero remained in the injection site of the degenerated disc for about 10 days before starting to recover (Figures 5a).* The recovery of glucose level in the center of NP region was slower in the IVD with the higher concentration of binding proteins (Figure 5). In addition, the reduction in the glucose level of the outer NP region ($r > 5\text{mm}$) was less in the IVD with 15 nM of IGF binding proteins than that without IGF binding proteins (Figure 6a) *due to slow diffusion of IGF-1 as described in the previous section (Figure 3b).* Therefore, the endangered volume (defined as the volume containing glucose less than 0.5 mM) was regulated by IGF binding proteins (Figure 6b). The IVD with low concentration of IGF binding proteins exhibited a higher endangered volume with a short duration of IGF-1 influence (Figure 6b). However, the glucose level stayed above 0.5 mM in the healthy disc after IGF-1 injection. Similarly, IGF-1 injection increased lactate concentration, resulting in a decrease in pH (Figures 5b&c). These effects were dependent on the concentration of binding proteins (Figures 5b&c).

Injection of IGF-1 solution with glucose would prevent glucose concentration from reducing to zero at the injection site (Figure 7), but the effect of glucose injection diminished within 24 hours (Figure 7a) due to small injection amount (e.g., 100 μl). Increasing oxygen concentration in the IGF-1 injection had a similar effect (Figure 7b).

DISCUSSION

This study quantitatively analyzed intradiscal IGF-1 injection and its effects on the distributions of pH, glucose, and lactate in the IVD. A finite element model, based on the mixture theory for charged hydrated soft tissues, was developed to describe the coupled convective, diffusive, and reactive IGF-1 transport in the disc. This study was the first to demonstrate the effects of binding reaction on distribution of IGF-1 after intradiscal IGF-1 injection and the influence of IGF-1 on distributions of nutrients and metabolites in the IVD.

The analysis performed in this study showed that binding reaction significantly affected the time-dependent distribution of IGF-1 in the IVD *and mediated the effective volume of IGF-1, verifying our first hypothesis*. The binding reactions *reduce both the amount of available (i.e., free) IGF-1 and the rate of IGF-1 diffusion (i.e., reducing the effective volume) in the IVD* (Figure 4a). The binding reactions also serve as a “reservoir” for IGF-1, leading to an increase in the duration of the therapeutic level of IGF-1 (Figure 4a). Therefore, in order to optimize the dosage and the time sequence of IGF-1 administration in the IVD, binding phenomena must be taken into account and the amount of binding proteins needs to be characterized.

This study also *verified our second hypothesis by demonstrating* that the IGF-1 injection can significantly decrease the concentration of glucose near the injection site *due to an increase in the glucose consumption by IVD cells*. Such reduction in glucose level may be *detrimental to disc cells as demonstrated by the size of endangered volume* (Figure 6b) in which the glucose level is harmful (<0.5 mM). Although IGF-1 may enhance the extracellular matrix production and proliferation of IVD cells, increase in cellular metabolism by IGF-1 may not improve the existing condition of malnutrition in the avascular IVD.

The other potential problem of IGF-1 administration is lactate accumulation. It was found that IGF-1 injection increased lactate concentration in the NP region due to *IGF-1 enhanced glucose consumption* as described above, resulting in lower pH. Previous studies demonstrated that pH lower than 6.8 tended to decrease viability of IVD cells while the effects of low pH on cell viability were more pronounced at low glucose levels (Bibby and Urban, 2004; Guehring et al., 2009). Therefore, *IGF-1 injection may not be beneficial to disc cells if it lowers both pH and glucose concentration at the same time as shown by the simulations* (Figure 5). However, the assumption for the relationship between the increase in glucose consumption and the IGF-1 concentration made in this study needs to be experimentally verified in order to provide more precise theoretical predictions.

This study demonstrated the capability of a newly developed theoretical model to predict the distributions of IGF-1, nutrients, and metabolites, and physical signals such as fluid pressure, stress, and strain in the IVD. The new theoretical model can also be extended for the analysis of administration of different growth factors, and, thus, serve as a powerful tool in analyzing and designing the optimal treatments of growth factors for disc degeneration *in the future*. However, *the numerical simulations performed in this study were simplified by several assumptions based on the availability of information in the literature*. In order to provide more accurate prediction, the assumptions made in this study need to be verified, especially for *reversible IGF-1 binding reactions* and the effect of IGF-1 on glucose consumption. *In addition, other important factors, which have a strong influence on the distributions of IGF-1 and nutrients, also need to be taken into account in the theoretical model, such as alterations of cellularity, cell metabolism of IGF-1, and solute diffusivity with disc degeneration. In particular, cellular consumption and production of IGF-1 were not included in this study because no supportive data were available in the literature. These simplifications may affect the outcomes of our analysis.*

In summary, this study demonstrated the effects of binding reactions on IGF-1 distribution and the influence of IGF-1 on the distributions of nutrients and metabolites in the IVD after IGF injection. A new theoretical model developed in this study is capable of predicting distributions of IGF-1, nutrients, and metabolites, and physical signals such as fluid pressure, stress and strain, and thus can serve as a powerful tool in analyzing and designing the optimal treatments of growth factors for disc degeneration. The theoretical analyses showed that the binding reactions of IGF-1 exhibited significant influences on the IGF-1 effective volume and the distributions of IGF-1, glucose, and lactate. These findings indicate

the complexity of intradiscal administration of growth factors, which needs to be fully analyzed in order to achieve a successful outcome.

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Figure 1.
Schematic of 2-D axisymmetric model of IVD for the finite element analysis.

(a) Distribution of IGF-1 (b) Distribution of bound complex **Figure 2.**

Distribution of (a) free IGF-1 and (b) bound complex in the *degenerated IVDs* (25% *CEP permeability*) containing 0 nM and 15 nM binding proteins 14 days after IGF-1 injection. The data were normalized by the IGF-1 injection concentration (i.e., 1 $\mu\text{g/ml}$).

Figure 3. Effect of binding proteins on distributions of (a) free IGF-1 and (b) bound complex along $z=0$ in the NP region of the degenerated IVDs (25% CEP permeability) at 14 days after IGF-1 injection. The data were normalized by the IGF-1 injection concentration (i.e., $1\mu\text{g/ml}$).

Figure 4.

(a) Effect of binding proteins on the effective volume in the *degenerated IVDs* (25% CEP permeability). (b) Effect of *disc degeneration* and CEP permeability on the effective volume in the IVD containing 15 nM binding proteins.

Figure 5. Effect of IGF-1 treatment on (a) glucose concentration, (b) lactate concentration, and (c) pH at the injection site ($r=0.36$ mm and $z=0$) in the *degenerated IVDs* (25% CEP permeability) with different concentrations of IGF binding proteins. The data of glucose and lactate concentrations were normalized by the boundary conditions at the CEP above the NP region (i.e., glucose: 4 mM; lactate: 0.8 mM).

Figure 6. Effect of binding proteins on (a) glucose concentration distribution along $z=0$ at 14 days after IGF-1 injection and (b) the endangered volume in the NP region of the degenerated IVDs (25% CEP permeability). The data of glucose concentration were normalized by the glucose concentration at the CEP above the NP region (i.e., 4 mM).

Figure 7. Transient responses of (a) glucose and (b) oxygen concentrations to the injection of the IGF-1 solution containing different glucose and oxygen concentrations at the injection site ($r = 0.36 \text{ mm}$ and $z=0$) in the degenerated IVDs (25% CEP permeability; 15 nM binding proteins). The data of glucose and oxygen concentrations were normalized by the boundary conditions at the CEP above the NP region (i.e., glucose: 4 mM; oxygen: 5.1 kPa)

Table 1 Boundary conditions of the IVD used in the finite element analysis[§]

Injection site	$J^{lactate} = 0, u_r = u_z = 0$ During the injection, $J^{Water} = 2 \mu\text{l/min or } 10 \mu\text{l/min}, J^{O_2} = c_i^{O_2} J^{Water}, J^{glucose} = c_i^{glucose}$ $J^{Water}, J^I = c_i^I J^{Water}, J^{Na^+} = c_i^{Na^+} J^{Water}, J^{Cl^-} = c_i^{Cl^-} J^{Water}$ where $c_i^{O_2} = 0$ or 10.2 kPa, $c_i^{glucose} = 0, 4\text{mM}, 8\text{mM or } 20 \text{ mM}, c_i^I = 1 \mu\text{g/n}, c_i^{Na^+} = c_i^{Cl^-} = 0.15M$ After injection, $J^{Na^+} = J^{Cl^-} = J^{O_2} = J^{glucose} = J^I = 0$
At the AF edge	$c^{O_2} = 5.8 \text{ kPa}, c^{lactate} = 0.9 \text{ mM}, c^{glucose} = 5 \text{ mM}, c^I = 0, c_i^{Na^+} = c_i^{Cl^-} = 0.15M$ $\sigma_r = \sigma_{rz} = 0$
At the endplate ($z=h_0$)	$\begin{cases} \text{if impermeable, } J_z^{Na^+} = J_z^{Cl^-} = J_z^{O_2} = J_z^{lactate} = J_z^{glucose} = J_z^I = 0 \\ \text{if permeable, } c^{O_2} = 5.1 \text{ kPa}, c^{lactate} = 0.8 \text{ mM}, c^{glucose} = 4 \text{ mM}, c^I = 0, \\ c_i^{Na^+} = c_i^{Cl^-} = 0.15M \end{cases}$ $u_r = u_z = 0$
At the mid-height ($z=0$)	$J_z^{Water} = J_z^{Na^+} = J_z^{Cl^-} = J_z^{O_2} = J_z^{lactate} = J_z^{glucose} = J_z^I = 0$ $u_z = 0, \sigma_{rz} = 0$
At the disc center ($r=0$)	$J_r^{Water} = J_r^{Na^+} = J_r^{Cl^-} = J_r^{O_2} = J_r^{lactate} = J_r^{glucose} = J_r^I = 0$ $u_z = 0, \sigma_{zr} = 0$

[§] u_i is the displacement in i -direction and σ_{ij} is the stress in j direction on the surface with a normal along the direction i .

Table 2

The parameters of the degenerated disc used in the numerical analysis

Region	Water content	Parameters of the CLE model (MPa)	Fixed charge density (mol/m ³)	Parameters for the constitutive equation of diffusivity ^a	Parameters for the constitutive equation of hydraulic permeability ^b
AF	68%	$H_{+A} = 2.52; H_{-A} = 0.38; \lambda_2 = 0.28; \mu = 0.05$	170	$A = 1.29$ $B = 0.375$	$a = 0.00339 \text{ nm}^2$ $n = 3.24$
NP	78%	$H_{+A} = H_{-A} = 0.28; \lambda_2 = 0.1; \mu = 0.02$	125	$A = 1.25$ $B = 0.681$	$a = 0.00044 \text{ nm}^2$ $n = 7.1929$

^a Tissue diffusivity (D_0^α) of solute α was estimated using the constitutive equation: $D_0^\alpha = \exp \left(-A \left(\frac{r_s^\alpha}{\sqrt{k\eta}} \right)^B \right)$ (Gu et al., 2004) where D_0^α is the solute diffusivity of solute α in aqueous solution ($D_0^{O_2} = 3 \times 10^{-9} \text{ m}^2/\text{s}$; $D_0^{lactate} = 1.28 \times 10^{-9} \text{ m}^2/\text{s}$, $D_0^{glucose} = 9.2 \times 10^{-10} \text{ m}^2/\text{s}$, $D_0^{GF-1} = 3.23 \times 10^{-11} \text{ m}^2/\text{s}$), r_s^α is the hydrodynamic radius of solute α ($r_s^{O_2} = 0.1 \text{ nm}$; $r_s^{lactate} = 0.255 \text{ nm}$; $r_s^{glucose} = 0.3 \text{ nm}$; $r_s^{GF-1} = 1.72 \text{ nm}$) k is the hydraulic permeability, η is the viscosity of water, and A and B are the material constants. (Huang and Gu, 2008)

^b Hydraulic permeability (k) was estimated using the constitutive equation: $k = \frac{a}{\eta} \left(\frac{\phi^{iv}}{1 - \phi^{iv}} \right)^n$ (Gu et al., 2003b) where a and n are material constants.