Strain-Dependent Oxygen Diffusivity in Bovine Annulus Fibrosus

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The intervertebral disk (IVD) is the largest avascular structure in the human body. Transport of small molecules in IVD is mainly through diffusion from the endplates and the peripheral blood vessels surrounding IVD. Studies have investigated the structure, chemical components, and water content in IVD, but to our knowledge no study has investigated the effect of mechanical loading on oxygen transport in IVD. The objective of this study was to determine the strain-dependent behavior of oxygen diffusivity in IVD tissue. A one-dimensional steady-state diffusion experiment was designed and performed to determine the oxygen diffusivity in bovine annulus fibrosus (AF). The oxygen diffusivity was calculated using equation derived from Fick's law. A total of 20 AF specimens ($d=6$ mm, $h\sim0.5$ mm) from bovine coccygeal IVD were used to determine oxygen diffusivity at three levels of compressive strain. The average oxygen diffusivity (mean $\pm$ SD) of bovine AF in the axial direction was $1.43\pm0.242\times10^{-5}$ cm^2/s ($n=20$) at $4.68\pm1.67\%$ compressive strain level, $1.05\pm0.282\times10^{-5}$ cm^2/s ($n=20$) at $14.2\pm1.50\%$ strain level, and $7.71\pm1.63\times10^{-6}$ cm^2/s ($n=20$) at $23.7\pm1.34\%$ strain level. There was a significant decrease in oxygen diffusivity with increasing level of compressive strain (ANOVA, $p<0.05$). Oxygen diffusivity of bovine AF in the axial direction has been determined. The mechanical loading has a significant effect on oxygen transport in IVD tissues. This study is important in understanding nutritional transport in IVD tissues and related disk degeneration.
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Keywords: compression, nutrition, transport, intervertebral disk, diffusion coefficient, spine

1 Introduction

Statistics show that low back pain affects up to 75% of the adult population during some time in their lives. Studies aimed at quantifying the effects of low back pain on productivity and profitability have estimated the combined cost of back pain-related medical care and disability compensation to reach upwards of billions of dollars annually in the United States alone [1]. While the exact cause of low back pain is still poorly understood, scientists and physicians have reached a popular assumption that its cause can be primarily traced to the degeneration of the intervertebral disk [2–5].

The intervertebral disk, or IVD, is the largest avascular structure in the human body. Due to its avascular feature, nutrition supply into IVD is mainly through the diffusion of small solutes from the peripheral blood vessels [6–8]. Poor nutrition is believed to be an important factor leading to the onset of disk degeneration [9–13]. While many studies have aimed at analyzing the effects of mechanical loading on water content, chemical composition, and nutritional levels in the IVD [10,12,14], to our knowledge, no study has investigated the effect of mechanical compression on oxygen diffusivity in the IVD tissue. Therefore, the objective of this study was to investigate the effects of mechanical loading on oxygen transport in IVD tissue by determining oxygen diffusivity in bovine annulus fibrosus (AF) under three levels of compressive strain (5%, 15%, and 25%).

2 Materials and Methods

Previous studies have shown that the composition, mechanical properties, and synthesis rates of bovine coccygeal disks are similar to those for human IVD [15,16]. In order to meet our objective of investigating the strain-dependent behavior of oxygen diffusivity using a tissue similar to human IVD, bovine coccygeal IVD were used in this study as they are easily obtainable at a low cost. A total of three fresh bovine tails (~6 months old) were obtained from a local supermarket. After carefully removing the excess tissue and ligaments surrounding the disks, a total of 5 coccygeal IVD (S2-3 and S3-4) were harvested. A total of 20 axial AF samples were prepared using a corneal trephine (Biomedical Research Instruments, Inc., Silver Spring, MD) and sledge microtome (Model SM2400, Leica Instruments, Nussloch, Germany) with freezing stage (Model BFS-30, Physitemp Instruments, Inc., Clifton, NJ). The cylindrical samples were 6 mm in diameter and approximately 0.5 mm in thickness. Note that the thickness of each specimen was measured using a custom-designed current sensing micrometer (an accuracy of ±3 μm) [17] and was used for calculating the level of compressive strain (i.e., engineering strain) in the testing chamber. Each of the 20 specimens was tested to measure oxygen diffusivity at three different levels of nominal compressive strain (5%, 15%, and 25%).

In order to accurately measure the strain-independent diffusivity of oxygen in IVD tissue, an acrylic diffusion chamber was custom-designed and built (Fig. 1). The diffusion chamber consisted of two compartments divided by a specimen holder located in the middle. Internal volumes of upstream and downstream compartments were 0.24 ml and 0.2 ml, respectively. Prior to the start of the experiment, de-oxygenated phosphate buffered saline (PBS) solution was prepared by introducing nitrogen gas into a 2 ml PBS solution for 1 h [18].

The specimen was compressed between two porous plates with 50–90 μm pore size and 50% porosity, and sealed with an o-ring. The amount of compression was controlled by the size of the spacer. The AF specimen was first confined to 5% nominal compressive strain, and the downstream compartment was filled with the de-oxygenated PBS solution while the upstream compartment was filled with air-saturated PBS. To maintain a constant oxygen concentration upstream, the PBS solution in it was replaced peri-

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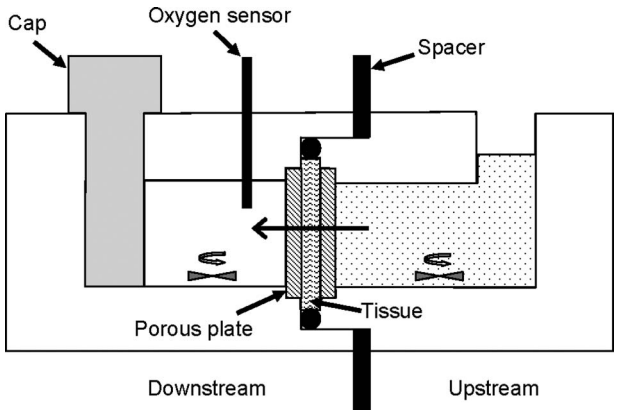

Fig. 1 Schematic of the custom-designed diffusion apparatus. Oxygen diffusion occurs from upstream chamber (right), across the tissue specimen, and into the downstream chamber (left), where the oxygen concentration is measured using an oxygen sensor.

odically (about every 2 min) with fresh solution. Real-time oxygen concentration downstream was recorded using an oxygen sensor system (Ocean Optics Inc., Dunedin, FL).

From Fick's law and conservation of mass, we can derive the following differential equation:

$$\frac{V_{\text{down}} dC_{\text{down}}}{Adt} = D\frac{K(C_{\text{up}} - C_{\text{down}})}{h} \tag{1}$$

where D is the diffusivity, C_{up} is the oxygen concentration in the upstream compartment, C_{down} is the concentration of oxygen in the downstream compartment, V_{down} is the volume of solution in the downstream compartment, A is the diffusion area, and h is the thickness of the sample after compression. K is the partition coefficient; in this study, it was assumed to be unity since oxygen is a small molecule. In arriving at Eq. (1), a linear distribution of concentration within the specimen has been assumed. The oxygen diffusivity may be calculated by [19,20]

$$D = \ln\left[\frac{C_{\text{up}} - C_{\text{down}}(t_o)}{C_{\text{up}} - C_{\text{down}}(t)}\right]\frac{V_{\text{down}} h}{A(t - t_o)} \tag{2}$$

where $C_{\text{down}}(t_o)$ and $C_{\text{down}}(t)$ are the concentrations of oxygen in the downstream chamber at times t_o (initial time) and t, respectively. To ensure that concentration distribution within the tissue is linear, oxygen diffusivity was calculated from the measured data using the final 15 minutes of the experiment (i.e., $t_o = 45$ min and $t - t_o = 15$ min). The experiment was repeated for 15% and 25% (nominal) compressive strains.

3 Results

A sample of raw experimental data showing the change in the oxygen concentration in the downstream chamber with elapsed time is shown in Fig. 2. Our results, shown in Fig. 3, indicate that the oxygen diffusivity in AF decreased with increasing compressive strain. To estimate the value of diffusivity at zero-strain, a linear regression line was used to fit the experiment data. It was found that the oxygen diffusivity in AF at 0% compression was 1.56×10^{-5} cm^2/s (Fig. 3). The average oxygen diffusivity (mean $\pm$ SD) of bovine AF in the axial direction was $1.43 \pm 0.242 \times 10^{-5}$ cm^2/s ($n = 20$) at 4.68 ± 1.67% compressive strain level, $1.05 \pm 0.282 \times 10^{-5}$ cm^2/s ($n = 20$) at 14.2 ± 1.50% strain level, and $7.71 \pm 1.63 \times 10^{-6}$ cm^2/s ($n = 20$) at 23.7 ± 1.34% strain level. Measurements were carried out at room temperature ($22.2\,^\circ\text{C} \pm 0.45\,^\circ\text{C}$). The mean height of the specimens under zero compression condition was 0.525 ± 0.009 mm.

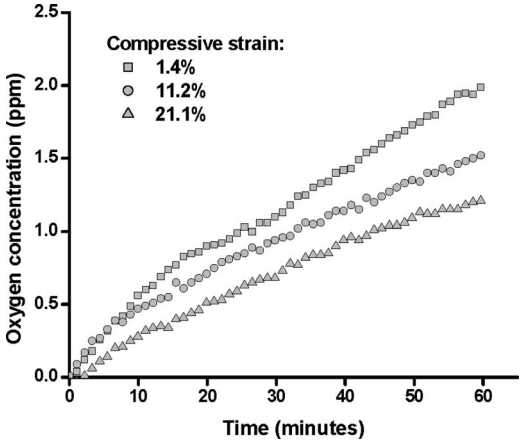

Fig. 2 An example of raw experimental data showing the change in the oxygen concentration in the downstream chamber with elapsed time. For this particular specimen, the actual levels of compression were 1.4%, 11.2%, and 21.1%. The diffusivity was calculated using Eq. (2). Note the decrease in the slope with increasing compressive strain, indicating the strain-dependent behavior of oxygen diffusivity.

A one-way analysis of variance (ANOVA) test was used to analyze the data and showed that oxygen diffusivity was significantly affected by the level of compression ($P < 0.05$). A Student–Newman–Keuls post hoc test showed a significant difference between oxygen diffusivity in compressive strain groups ($P < 0.05$).

4 Discussion

There are few studies in the literature that have focused on oxygen diffusivity of IVD and cartilaginous tissues [19,21–23]. Previous studies have reported the strain-dependent diffusivity behavior of different solutes in articular cartilage and IVD tissues [20,24–29], but no study has investigated the strain-dependent behavior of oxygen diffusivity in IVD.

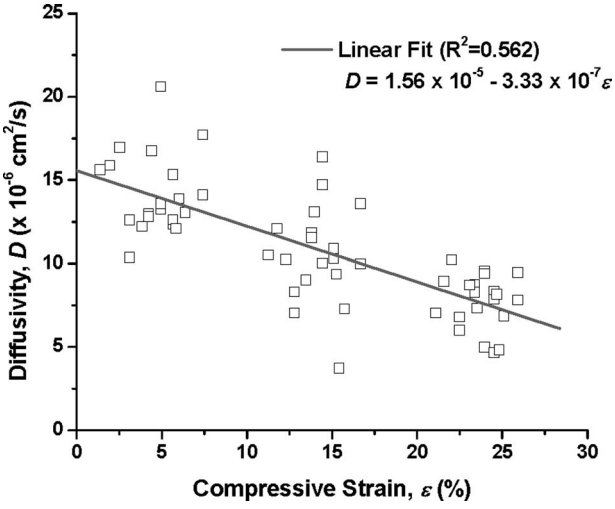

Fig. 3 Variation in diffusivity of oxygen with applied strain at room temperature ($22.2\,^\circ\text{C} \pm 0.45\,^\circ\text{C}$). A linear regression ($R^2 = 0.562$, $n = 60$) was used to estimate the diffusivity at zero stain. In the linear regression, D is the diffusivity and ε is the applied compression (%). From this, the oxygen diffusivity in bovine AF at 0% compression (i.e., $\varepsilon = 0$) was determined to be 1.56×10^{-5} cm^2/s.

Our previous study [30] showed that the diffusivity (D) of small and macromolecules in gel and cartilaginous tissue could be estimated by the following model:

$$\frac{D}{D_o} = \exp\left[-\alpha\left(\frac{r_s}{\sqrt{\kappa}}\right)^{\beta}\right] \tag{3}$$

where r_s is the solute Stokes radius, κ is the tissue Darcy permeability, α and β are the two positive parameters that depend on the structure of the tissue, and D_o is the solute diffusivity in water at the same temperature. While the values of both D and D_o are sensitive to temperature, the value of the relative diffusivity (D/D_o) should not be, assuming the structure and composition of the tissue do not change with temperature. One previous study showed that the relative oxygen diffusivity (D/D_o) for porcine AF was between 0.3 and 0.6 [22], and the other studies showed that the relative oxygen diffusivities for bovine and avian articular cartilage were ~0.71 ($\sim 2.2\times10^{-5}$ cm^2/s in tissue at 37°C) and ~0.66 ($\sim 2.0\times10^{-5}$ cm^2/s in tissue at 35°C), respectively [19,21]. Using the value (2.2×10^{-5} cm^2/s) of oxygen diffusivity in water at 22°C [31], and our experimental data, it was found that the relative oxygen diffusivity for AF at zero compression was 0.71, which is in agreement with the result reported for bovine articular cartilage [19].

The results from our experiment showed that oxygen diffusivity decreased as the compressive strain increased. IVD tissue may be considered as a porous material with various sizes of pores, which are filled with water. The overall porosity of tissue can be measured by the value of water volume fraction. The average size of the pores in the tissue can be estimated by the square root of its Darcy permeability [30,32], which is at nanometer scale for cartilaginous tissues [32]. For these tissues, the Darcy permeability (or average pore size) is related to the volume fraction of water (or water content) (e.g., Ref. [32]). When a tissue is compressed, fluid exudation leads to a reduction in tissue water content. For bovine coccygeal AF, our previous study showed that water content was 76% at zero compression, and was estimated to decrease to 75%, 72%, and 68% under 5%, 15%, and 25% compressive strains, respectively [20]. A decrease in the water content of IVD tissue results in decreased pore size of the tissue [10,12,14,20,29,33–36]. Because the main factor governing the relative diffusivity in cartilaginous tissues is the ratio of solute size to the pore size of the tissue [30,37], see Eq. (3), compression would contribute to a decrease in the diffusivity of oxygen in the tissue. Recent studies have also shown the existence of microtubes, which are small (~10 μm in diameter) tubular structures that extend along the direction of collagen fiber bundles, in bovine and murine AF [20,36,38]. These microtubes have been suggested as a pathway for solute transport through AF tissue [20,38]. We believe that, when compression is applied to the tissue, tissue compaction may also cause a reduction in the size of the microtubes which, in turn, leads to a decrease in the solute diffusivity in the tissue. Therefore, the strain-dependence of oxygen diffusivity in bovine AF can likely be attributed to the decreased tissue porosity and pore size, at both the nano- and microlevels, caused by compression.

The current study only investigated the strain-dependent oxygen diffusivity in the axial direction in AF tissue. Previous studies have shown that transport in IVD tissues is anisotropic (i.e., direction-dependent) [20,28,38–40]. For instance, our earlier studies have shown that, for bovine AF tissue, diffusivity in the axial direction is approximately 1.5 times that in the radial direction [20,38]. Therefore, further investigation into the effects of compression on oxygen diffusivity in other directions (e.g., radial and circumferential) of AF, similar to our previous study on strain-dependent glucose diffusivity in bovine AF [20], is needed to better understand the anisotropy of oxygen transport in IVD. This information is important to the development of numerical models for nutritional transport in IVD tissues.

In summary, this study measured the oxygen diffusivity of bovine AF in the axial direction and was the first study to report strain-dependent oxygen diffusivity in AF. The results in the current study show that mechanical loading affects the oxygen diffusivity in bovine IVD tissue, decreasing as the compression increases. The findings from this study provide an additional insight into the nutrition transport in IVD under mechanical loading and highlight the importance of approaching and understanding mechanisms of nutrient transport in IVD tissue.

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References

[1] NIH, 1997, "Research on Low Back Pain and Common Spinal Disorders," NIH Guide Vol. No. 26(16).
[2] Eyre, D. R., Benya, P., Buckwalter, J., Caterson, B., Heinegard, D., Oegema, T., Pearce, R., Pope, M., and Urban, J., 1989, "Intervertebral Disk: Basic Science Perspectives," *New Perspectives on Low Back Pain*, J. W. Frymoyer and S. L. Gordon, eds., American Academy of Orthopaedic Surgeons, Park Ridge, IL, pp. 147–207.
[3] Kelsey, J. L., Mundt, D. F., and Golden, A. L., 1992, "Epidemiology of Low Back Pain," *The Lumbar Spine and Back Pain*, J. I. V. Malcolm, ed., Churchill Livingstone, New York, pp. 537–549.
[4] White, A. A., 1981, "Biomechanics of Lumbar Spine and Sacroiliac Articulation: Relevance to Idiopathic Low Back Pain," *Symposium on Idiopathic Low Back Pain*, A. A. White and S. L. Gordon, eds., Mosby, St. Louis, pp. 296–322.
[5] Buckwalter, J. A., 1995, "Aging and Degeneration of the Human Intervertebral Disc," Spine, **20**(11), pp. 1307–1314.
[6] Urban, J. P., Holm, S., and Maroudas, A., 1978, "Diffusion of Small Solutes Into the Intervertebral Disc: An In Vivo Study," Biorheology, **15**(3–4), pp. 203–221.
[7] Maroudas, A., 1975, "Biophysical Chemistry of Cartilaginous Tissues With Special Reference to Solute and Fluid Transport," Biorheology, **12**, pp. 233–248.
[8] Urban, J. P., Holm, S., Maroudas, A., and Nachemson, A., 1982, "Nutrition of the Intervertebral Disc: Effect of Fluid Flow on Solute Transport," Clin. Orthop. Relat. Res., **170**, pp. 296–302.
[9] Nachemson, A., Lewin, T., Maroudas, A., and Freeman, M. A., 1970, "In Vitro Diffusion of Dye Through the End-Plates and the Annulus Fibrosus of Human Lumbar Inter-Vertebral Discs," Acta Orthop. Scand., **41**(6), pp. 589–607.
[10] Holm, S., and Nachemson, A., 1982, "Nutritional Changes in the Canine Intervertebral Disc After Spinal Fusion," Clin. Orthop. Relat. Res., **169**, pp. 243–258.
[11] Horner, H. A., and Urban, J. P., 2001, "2001 Volvo Award Winner in Basic Science Studies: Effect of Nutrient Supply on the Viability of Cells From the Nucleus Pulposus of the Intervertebral Disc," Spine, **26**(23), pp. 2543–2549.
[12] Bibby, S. R., Fairbank, J. C., Urban, M. R., and Urban, J. P., 2002, "Cell Viability in Scoliotic Discs in Relation to Disc Deformity and Nutrient Levels," Spine, **27**(20), pp. 2220–2228.
[13] Urban, J. P., 2001, "The Role of the Physicochemical Environment in Determining Disc Cell Behaviour," Biochem. Soc. Trans., **30**(6), pp. 858–864.
[14] Adams, M. A., and Hutton, W. C., 1986, "The Effect of Posture on Diffusion Into Lumbar Intervertebral Discs," J. Anat., **147**, pp. 121–134.
[15] Oshima, H., Ishihara, H., Urban, J. P., and Tsuji, H., 1993, "The Use of Coccygeal Discs to Study Intervertebral Disc Metabolism," J. Orthop. Res., **11**(3), pp. 332–338.
[16] Beckstein, J. C., Sen, S., Schaer, T. P., Vresilovic, E. J., and Elliot, D. M., 2008, "Comparison of Animal Discs Used in Disc Research to Human Lumbar Disc," Spine, **33**(6), pp. E166–E173.
[17] Gu, W. Y., and Justiz, M. A., 2002, "Apparatus for Measuring the Swelling Dependent Electrical Conductivity of Charged Hydrated Soft Tissues," ASME J. Biomech. Eng., **124**, pp. 790–793.
[18] Bibby, S. R. S., Jones, D. A., Ripley, R. M., and Urban, J. P., 2005, "Metabolism of the Intervertebral Disc: Effects of Low Levels of Oxygen, Glucose, and pH on Rates of Energy Metabolism of Bovine Nucleus Pulposus Cells," Spine, **30**(5), pp. 487–496.
[19] Malda, J., Rouwkema, J., Martens, D. E., Le Comte, E. P., Kooy, F. K., Tramper, J., van Blitterswijk, C. A., and Riesle, J., 2004, "Oxygen Gradients in Tissue-Engineered PEGT/PBT Cartilaginous Constructs: Measurement and Modeling," Biotechnol. Bioeng., **86**(1), pp. 9–18.
[20] Jackson, A. R., Yuan, T. Y., Huang, C. Y., Travascio, F., and Gu, W. Y., 2008, "Effect of Compression and Anisotropy on the Diffusion of Glucose in Annulus Fibrosus," Spine, **33**(1), pp. 1–7.
[21] Haselgrove, J. C., Shapiro, I. M., and Silverton, S. F., 1993, "Computer Modeling of the Oxygen Supply and Demand of Cells of the Avian Growth Cartilage," Am. J. Physiol., **265**(2), pp. C497–C506.
[22] O'Hare, D., Winlove, C. P., and Parker, K. H., 1991, "Electrochemical Method for Direct Measurement of Oxygen Concentration and Diffusivity in the Inter-

vertebral Disc: Electrochemical Characterization and Tissue-Sensor Interactions," J. Biomed. Eng., **13**(4), pp. 304–312.
[23] Macpherson, J. V., O'Hare, D., Unwin, P. R., and Winlove, C. P., 1997, "Quantitative Spatially Resolved Measurements of Mass Transfer Through Laryngeal Cartilage," Biophys. J., **73**(5), pp. 2771–2781.
[24] Burstein, D., Gray, M. L., Hartman, A. L., Gipe, R., and Foy, B. D., 1993, "Diffusion of Small Solutes in Cartilage as Measured by Nuclear Magnetic Resonance (NMR) Spectroscopy and Imaging," J. Orthop. Res., **11**(4), pp. 465–478.
[25] Quinn, T. M., Kocian, P., and Meister, J. J., 2000, "Static Compression is Associated With Decreased Diffusivity of Dextrans in Cartilage Explants," Arch. Biochem. Biophys., **384**, pp. 327–334.
[26] Quinn, T. M., Morel, V., and Meister, J. J., 2001, "Static Compression of Articular Cartilage can Reduce Solute Diffusivity and Partitioning: Implications for the Chondrocyte Biological Response," J. Biomech., **34**(11), pp. 1463–1469.
[27] Ngwa, W., Geier, O., Stallmach, F., Naji, L., Schiller, J., and Arnold, K., 2002, "Cation Diffusion in Cartilage Measured by Pulsed Field Gradient NMR," Eur. Biophys. J., **31**(1), pp. 73–80.
[28] Chiu, E. J., Newitt, D. C., Segal, M. R., Hu, S. S., Lotz, J. C., and Majumdar, S., 2001, "Magnetic Resonance Imaging Measurement of Relaxation and Water Diffusion in the Human Lumbar Intervertebral Disc Under Compression In Vitro," Spine, **26**(19), pp. E437–E444.
[29] Drew, S. C., Silva, P., Crozier, S., and Pearcy, M. J., 2004, "A Diffusion and T2 Relaxation MRI Study of the Ovine Lumbar Intervertebral Disc Under Compression In Vitro," Phys. Med. Biol., **49**(16), pp. 3585–3592.
[30] Gu, W. Y., Yao, H., Vega, A. L., and Flagler, D., 2004, "Diffusivity of Ions in Agarose Gels and Intervertebral Disc: Effect of Porosity," Ann. Biomed. Eng., **32**, pp. 1710–1717.
[31] Himmelblau, D. M., 1964, "Diffusion of Dissolved Gases in Liquids," Chem. Rev. (Washington, D.C.), **64**, pp. 527–550.
[32] Gu, W. Y., Yao, H., Huang, C.-Y., and Cheung, H. S., 2003, "New Insight Into Deformation-Dependent Hydraulic Permeability of Gels and Cartilage, and Dynamic Behavior of Agarose Gels in Confined Compression," J. Biomech., **36**, pp. 593–598.
[33] Kraemer, J., Kolditz, D., and Gowin, R., 1985, "Water and Electrolyte Content of Human Intervertebral Discs Under Variable Load," Spine, **10**(1), pp. 69–71.
[34] Ohshima, H., Tsuji, H., Hiarano, N., Ishihara, H., Katoh, Y., and Yamada, H., 1989, "Water Diffusion Pathway, Swelling Pressure, and Biomechanical Properties of the Intervertebral Disc During Compression Load," Spine, **14**, pp. 1234–1244.
[35] Adams, M. A., and Hutton, W. C., 1983, "The Effect of Posture on the Fluid Content of Lumbar Intervertebral Discs," Spine, **8**(6), pp. 665–671.
[36] Iatridis, J. C., and ap Gwynn, I., 2004, "Mechanisms for Mechanical Damage in the Intervertebral Disc Annulus Fibrosus," J. Biomech., **37**, pp. 1165–1175.
[37] Maroudas, A., Stockwell, R. A., Nachemson, A., and Urban, J., 1975, "Factors Involved in the Nutrition of the Human Lumbar Intervertebral Disc: Cellularity and Diffusion of Glucose In Vitro," J. Anat., **120**(1), pp. 113–130.
[38] Travascio, F., and Gu, W., 2007, "Anisotropic Diffusive Transport in Annulus Fibrosus: Experimental Determination of the Diffusion Tensor by FRAP Technique," Ann. Biomed. Eng., **35**(10), pp. 1739–1748.
[39] Jackson, A. R., Yao, H., Brown, M. D., and Gu, W. Y., 2006, "Anisotropic Ion Diffusivity in Intervertebral Disc: An Electrical Conductivity Approach," Spine, **31**, pp. 2783–2789.
[40] Hsu, E. W., and Setton, L. A., 1999, "Diffusion Tensor Microscopy of the Intervertebral Disc Anulus Fibrosus," Magn. Reson. Med., **41**(5), pp. 992–999.