INTRODUCTION
Back pain is one of the major complaints of patients who visit doctors in the US. Degeneration of intervertebral disc (IVD) has been suggested to be one of the significant contributors to low back pain. Since IVD is the largest avascular tissue in the human body, poor nutrition supply (e.g., glucose and oxygen) may strongly affect the biosynthesis of extracellular matrix (ECM) due to its high energy demand [1]. Our previous theoretical study showed that dynamic loading may increase cellular glucose consumption within IVD by enhancing the transport of nutrients and metabolites [2]. It has also been demonstrated that dynamic loading promotes ATP production and release by IVD cells in three-dimensional agarose culture [3,4]. Our recent studies showed that static compression not only exhibits an intrinsic effect on ATP metabolism of IVD cells [4], but also alters ATP production of IVD by decreasing solute diffusivity in the disc [5]. However, the effect of dynamic compression on the ATP metabolism of IVD has not yet been studied. Therefore, the objective of this study was to investigate (1) the effect of dynamic compression on the ATP production of IVD and (2) differences between annulus fibrosus (AF) and nucleus pulposus (NP).

MATERIALS AND METHODS
Lumbar spines of 4–6 month-old pigs were obtained within 2 – 8 h of sacrifice. Functional spinal units (FSUs) were isolated from lumbar region of the spine by making parallel transverse cuts at the interface between vertebrae and endplates. The FSUs were placed in custom-designed compression chambers and cultured in high glucose Dulbecco’s Modified Eagle Medium (DMEM, Invitrogen Corp., Carlsbad, CA) containing 10% fetal bovine serum (FBS, Invitrogen Corp) and 1% antibiotic-antimycotic (Invitrogen Corp) in an incubator at 37°C overnight. The medium is continuously circulated with a flow rate of 1.1 ml/min; which is about twice the rate of blood flow to the disc in vivo. For the whole disc compression test, the disc height was measured at 3 locations on the disc surface. A custom-designed compression device was developed to apply compressive loading to the IVD. For the dynamic compression group, the discs were preloaded by 5% compressive strain and then subjected to 10% compressive strain at 1 Hz for 1 h in the incubator. The control group was left undisturbed in the incubator for the duration of the experiment. After the experiment, a transverse cut was made on each unit to expose the AF and NP. The tissue samples (~10 mm³) were harvested from the locations shown in Figure 1. The samples obtained from AF and NP regions were used to determine the ATP and DNA contents. ATP was released from tissue samples using perchloric acid treatment [1] and then determined using the Luciferin-luciferase method (Sigma, St. Louis, MO). The DNA content was measured based on the protocol described in a previous study [2]. The ATP contents were normalized by the DNA content. A student t-test was performed to examine differences in ATP content between the control and loaded groups for each anatomical region and between the AF and NP regions using Excel (Microsoft Inc, Redmond, WA).

RESULTS
Dynamic compression significantly increased the ATP content of NP tissues (p<0.001) (Fig. 2a). However, the ATP content of AF was significantly decreased by dynamic loading (Fig. 2a). The ATP content of NP tissue was significantly higher than that of AF tissue for both control and loading conditions (p<0.001) (Fig. 2b). Since dynamic loading promotes ATP production and release by IVD cells in agarose culture [3,4]. Our recent studies showed that static compression not only exhibits an intrinsic effect on ATP metabolism of IVD cells [4], but also alters ATP production of IVD by decreasing solute diffusivity in the disc [5]. However, the effect of dynamic compression on the ATP metabolism of IVD has not yet been studied. Therefore, the objective of this study was to investigate (1) the effect of dynamic compression on the ATP production of IVD and (2) differences between annulus fibrosus (AF) and nucleus pulposus (NP).

Figure 1 Harvest sites of AF and NP

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DISCUSSION
This study is the first to demonstrate the effect of dynamic compression on the ATP content in the IVD. It was found that dynamic compression promoted cellular ATP production in the NP region of the IVD. It confirms the findings of our previous studies [2,3], which showed that dynamic loading promoted ATP production of NP cells in agarose culture. Another factor for this effect may be an increase in transport of nutrients by dynamic loading [2]. However, a significant decrease in the ATP content of AF tissues under dynamic loading was contradictory to our recent finding that ATP production of AF cells in agarose culture was increased by dynamic loading. This discrepancy may be due to the differences in mechancial environment between the agarose culture and the complex ECM of the IVD. For instance, high hydrostatic pressure can be induced in the IVD under dynamic loading, but not in agarose culture. In addition, significantly higher ATP content found in the loaded NP tissues is in concurrence with our previous studies [2,3], indicating that NP cells exhibit high metabolic activities than AF cells. Since dynamic loading can promote ATP release from IVD cells [2,3], it is expected that ATP accumulation will occur in the IVD under dynamic loading. Since extracellular ATP can cause tissue calcification [6] and mediate a wide variety of biological responses via purinergic receptors [7], extracellular ATP may play an important role in NP metabolism and endplate calcification, which is closely related to disc degeneration. Further works are required to investigate the effect of dynamic loading on ATP release and metabolic pathway of IVD.

SIGNIFICANCE
Due to the avascular nature of IVD, malnutrition has been suggested as a major cause of disc degeneration. Since ECM biosynthesis is an energy demanding process, this study provided a better understating in ATP metabolism of IVD under mechanical loading.

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REFERENCES

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