INTRODUCTION

The intervertebral disc (IVD) is the largest avascular structure in the human body, and is comprised of three major components: the annulus fibrosus (AF), nucleus pulposus (NP), and cartilaginous end-plate (CEP). Compromised disc nutrition is believed to be the key factor in the development of disc degeneration which has hampered previous tissue engineering or repair efforts. The balance between the rate of nutrient transport through the matrix and the rate of consumption by disc cells determines the local nutrient concentration gradient within the IVD. Previous studies have shown a dependence of oxygen consumption rates (OCR) for animal IVD cells on oxygen tension, pH levels, and glucose concentrations [1-3]. However, the OCR of human IVD cells has not been investigated in this same manner. The objective of this study is to determine the OCR of human IVD cells at various glucose concentrations and to examine differences in OCR among the AF, NP and CEP cells.

METHODS

Human lumbar IVD tissues were obtained as to-be-discarded surgical waste from patients undergoing surgery for degenerative disc disease (total n=5 patients, aged 43–62 years old, degeneration Grade 3 or 4). Disc tissues were well rinsed and grossly separated into AF, NP, and CEP according to anatomic appearance. Cells were released from these three regions by enzyme digestion and cultured in high glucose (25 mM) DMEM with 10% FBS. Cells for all experiments were obtained after two passages. Before OCR measurements, cells from each region were separated into 3 groups that were incubated in DMEM with glucose concentrations of 1, 5, and 25 mM for another 24 hours (media contained no FBS and was buffered to a pH of 7.4). Cell suspensions of a million cells per mL were placed into the metabolism chambers (Instech Laboratories, Plymouth Meeting, PA). The chamber was sealed and real time dissolved oxygen concentrations in the medium were recorded by a fiber optic oxygen sensor (Ocean Optics, Dunedin, FL) Experiments were stopped when oxygen concentrations fell below 0.1%. By curve-fitting the recorded oxygen concentration data [2], the Michaelis-Menten model of enzyme kinetics (Fig. 2). Although there were no significant differences in Km, significant effects due to the glucose level were found for Vmax in all three regions. The cells cultured in the highest glucose medium (25 mM) exhibited a smaller Vmax than those cultured in the lowest glucose medium (1 mM). The averaged OCR of NP cells was significantly higher than those of AF and CEP cells (Fig. 3).

DISCUSSION

Generally, cells exhibit a higher rate of glycolysis in high glucose culture. Inhibition of cellular respiration due to the high rate of glycolysis, described as the Crabtree effect, has been demonstrated in porcine AF cells, but not in porcine NP cells [2]. Low glucose did not stimulate oxygen consumption for either bovine AF or NP cells [1,3]. This study found that the Crabtree effect existed in human IVD cells from all three regions.

This study also found that oxygen consumption rates of NP cells were significantly greater than those of AF and CEP cells in human IVD. This is consistent with previous studies for porcine and bovine IVDs. However, the OCR of human IVD cells measured in this study are about 5-6 times higher than porcine and bovine IVD cells (Fig. 3). These differences between human and animal IVD cells might be attributed to intrinsic differences between species, as well as degenerated samples and monolayer culture techniques adopted in this study.

In summary, this study outlined oxygen consumption properties at different glucose conditions in three regions of human IVD. It will provide more evidence to further analyze the nutrient metabolism mechanism in the human IVD. The results of this study also provide valuable data for theoretically predicting the oxygen distribution and transport inside human IVD.

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REFERENCE