The Influence of Harvesting Methods For The Development Of An *In-vitro* Intervertebral Disc Model

B. Jim[1], O. Arlet[1], L. Beckman[1], T. Steffen[1]

[1]*Orthopaedic Research Lab, McGill University, Montreal, Canada*

INTRODUCTION: There have been a large variety of animal models used to study the intervertebral disc. However, there has yet to be a live ex-vivo model that provides of a platform for long-term, dynamic loading, disc tissue studying and culturing. In this study the impact of harvesting method on the mechanical properties and nutrition diffusion of bovine coccygeal intervertebral discs under cyclic dynamic load was monitored. The objective was to determine the most suitable harvest method for the continued development of an in-vitro intervertebral disc model.

METHODS: Fresh bovine coccygeal intervertebral discs (n=21) were harvested with 3 different techniques: without endplates (NEP) (widely accepted method for IVD harvest[1]), with the cartilaginous endplates (CEP) (our newly developed method), with bony endplates (BEP) simultaneously from the same tail. The tissues were loaded in axial compression over 6 days under repeated dynamic 0.1-1.0 MPa for 12 hrs at 0.3 Hz and static load at 0.1MPa for 12 hrs (minimum physiological load to counter swelling[2]). The loading, in an environment mimicking physiological conditions, was facilitated by our novel bioreactor developed to incorporate computer controlled dynamic axial loading while collecting mechanical load-displacement data. To determine nutrient diffusion, the disc tissue was labeled with $^{35}SO_4$ on day 5 for 1 complete load cycle, 24 hrs. The stress-relaxation mechanical behavior of the tissue was fit using a Kelvin model[3] to determine time constants and permanent strain. Disc tissue was dissected into 6mm (superior-inferior) annular and nuclear plugs and further sliced horizontally into 1mm layers. Nutrient diffusion through the tissue was mapped by counting ^{35}S diffused into the 1mm segments.

RESULTS: The creep and recovery time constant, ranked from greatest to lowest, were CEP>BEP>NEP, indicating that the NEP method lost height and recovered height quickest. Permanent strain reached a steady state for both BEP and CEP methods but showed a tendency to further strain for NEP (Fig. 1) in the creep. Nutrition diffusion, ranked from greatest to lowest, in the nucleus, were NEP>CEP>BEP where BEP S35 count = 50% <CEP S35 counts. (Fig. 2)

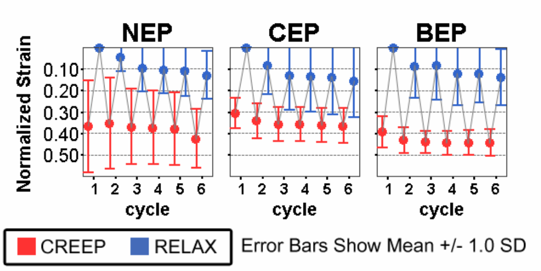

Fig. 1: Normalized maximum strain over the 6 loading cycles. Maximum strain during creep cycle is shown in red and relaxation shown in blue for each harvesting method.

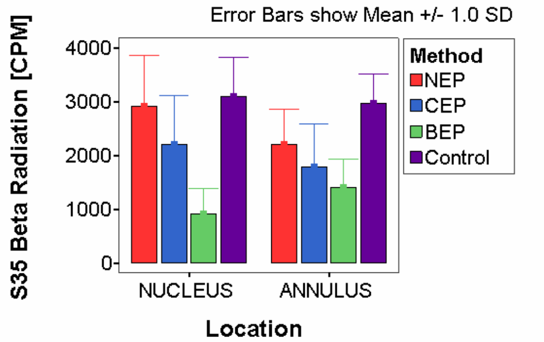

Fig. 2: CPM count for each harvest method and control. Mean nucleus counts shown first followed by annular counts.

DISCUSSION & CONCLUSIONS: The data demonstrated that the harvest method is critical in both mechanical stability and nutrient supply for long term tissue culturing. Although in nutrient diffusion the NEP method resulted with highest diffusion in both the nucleus and the annulus the CEP harvest method proved to be the best overall candidate for future in-vitro IVD model development.

REFERENCES: [1]C.R. Lee, L. Poveda, J.C. Iatridis, and M. Alini (2004) *Trans ORS* **28**:838 [2]J.P. Urban and J.F. McMullin (1998) *Spine* **13**(2):179-87 [3] Y.C. Fung, (1965). *Foundations of Solid Mechanics*. Prentice-Hall