Vertebral endplate porosity increases with age and disc degeneration
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ABSTRACT INTRODUCTION
Intervertebral disc degeneration occurs as a cascade of biologic and structural events that may interrelate with the adjacent vertebra. Since the intervertebral disc is avascular, it is dependent on capillaries within the adjacent vertebrae for nutrition. Consequently, it has been hypothesized that endplate calcification may be a causative factor for increased disc degeneration. The endplate is composed of a thin cartilage layer supported by a subchondral bone substrate. Together they play a dual, conflicting role of being strong (so as to support intervertebral disc pressure) and porous (to facilitate transport to and from the disc nucleus). In this study we investigated the relationship between subchondral bone morphology and disc degeneration characterized by age, cell density and GAG content of disc nucleus.

METHODS
Thirteen cadaveric human lumbar spines (L1-S1) were obtained from donor banks with ages ranging from 35-85 y.o. The vertebral bodies were sectioned in the horizontal mid-plane using a precision bone saw to obtain specimens consisting of half-vertebra/disc/half-vertebra. While frozen, 100 core specimens were harvested axially thru the vertebral/disc center using a 8.25 mm (inner diameter) diamond coring tool (Starlitech Industries, Rosemont, PA) and a drill press (Figure 1). The nucleus was next carefully separated from the cartilage endplates stored at -20°C for subsequent cell density assessment.

Next, the vertebra/endplate portions of the cores were imaged at 8 μm resolution using micro-CT (μCT 40, Scanco Medical, Brütisellen, Switzerland). Custom image analysis software was developed to define the bony endplate margin and calculate depth-dependent bone porosity using MATLAB software. Over 900 slices were created per sample, the grayscale images were binarized, then a custom algorithm was used to automatically identify and select the subchondral bone surface just below the endplate cartilage.

An ROI mask was chosen that extended to a 2 mm depth from the subchondral bone surface (Figure 2). Four parameters were measured: Pore Fraction PF (1-BV/TV), trabecular thickness (Tb.Th), Pore diameter (Po.D) and Pore number (Po.N). These parameters were compared to age, cell density and GAG content. Nucleus cell density was estimated using PicoGreen quantification of DNA. In addition, nucleus glycosaminoglycan (GAG) content was quantified using the dimethylmethene blue (DMMB) method using supernatant from nucleus digestion.

Correlation procedures were used to estimate the effects of specimen variables on the dependent parameters of interest (JMP version 7.1).

RESULTS
The mean and standard deviation for PF was 0.69±0.09%, the mean for Tb.Th was 0.17±0.04 mm, Po.D was 0.53±0.12 mm and Po.N was 2.88±0.52/mm. The PF and Po.D increased with age (R²=0.39; p<0.0001 and R²=0.36; p<0.0001 respectively), and decreased with GAG content (R²=0.23; p<0.0001 and R²=0.20; p<0.0001 respectively). Tb.Th decreased with age (R²=0.20; p<0.0001) and increased with GAG content (R²=0.14; p<0.0013). Po.N decreased with age (R²=0.07; p<0.0001). Also, Po.N increased with GAG content (R²=0.05 and p=0.07). PF, Tb.Th, Po.D and Po.N did not demonstrate any statistically significant relationship to cell density (p>0.05).

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REFERENCES

DISCUSSION
We observed the vertebral endplate becomes more porous and trabeculae become thinner with age, indicated by an increase in pore size and a decrease in pore number. Calcification in the endplate surface was not observed to increase with age as it had been previously reported. Our data are consistent with the well-known progression of vertebral osteoporosis with age and do not suggest there is an additional pathomechanism in effect at the endplate surface that adversely affects disc cell function. Decreased endplate bone density did correlate with decreased nuclear GAG content and indicates endplate weakening progresses with decreases in endplate swelling pressure in accordance with Wolff’s Law.

Other endplate features that may adversely affect disc cell function, like capillary density, are currently under investigation.

REFERENCES