Effect of Cigarette Smoke Exposure on Intervertebral Disc Remodeling Using a Sprague Dawley Rat Model

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INTRODUCTION: Back pain is a major socio-economic burden and is correlated with many different risk factors including cigarette smoking. Around 70% of the general population experiences back pain at some point in their lifetime with the annual prevalence being between 15% and 45% percent. Epidemiological evidence further suggests that back pain and intervertebral disc (IVD) degeneration occurs more frequently in smokers than non-smokers. In an identical twins study, a 20% greater MRI IVD degeneration score was found in the smoking group. Previous research indicates that tobacco smoke exposure can affect the IVD either through a direct pathway – alterations in cell function and local cytokine levels, or an indirect pathway – altered nutrient supply resulting from vasoconstriction and decreased hemoglobin for oxygen transport. The intrinsic mechanism which induces IVD degeneration remains unknown. Our previous research using a Sprague Dawley (SD) rat animal model aimed to fill this knowledge gap by showing that two months of cigarette smoke exposure could accelerate IVD remodeling as evidenced by endplate calcification, decreased cell viability, decreased solute transport through the disc, and increased equilibrium modulus. The objective of this study is to examine the impact cigarette smoke exposure has on IVD morphology and which components of the IVD [i.e., nucleus pulposus (NP), annulus fibrosis (AF), cartilage endplate (CEP)] play a dominant role in remodeling using the SD rat model. Micro-CT (μ-CT) and multiphoton excitation (MPE) imaging, histology and immunofluorescent staining, and a principal component analysis (PCA) will be performed to determine the dominant predictors for disc degeneration.

METHODS: Twenty-four six-month old male SD rats, obtained with institutional approval, were randomly assigned into non-smoke (n=12) and smoke (n=12) groups. The rats in the smoke group underwent daily smoke exposure for two months. During exposure, the animals were housed in a programable custom-built smoke exposure apparatus (Teague Enterprises) for two hours on weekdays and one hour on weekends. Serum cotinine levels (368 ± 82 ng/ml) were measured to ensure dosage was comparable to that of the average addicted human smoker and total particulate matter (200.6±73 mg/m^3) was measured to ensure dosage was comparable between specimens. The effect of smoke exposure and smoke cessation were examined by euthanizing the rats at two different time points, after smoke exposure (2 months) and after a period of smoke cessation (2 months smoke exposure + 5 months cessation). The lumbar portion of the spines were removed, and micro-CT imaging was performed on lower motion segments (L5-L6). The data acquired was processed using Amira, Geomagic Wrap, and MATLAB and the properties of the bony endplate (average object thickness, average space thickness, linear object density, cortical fraction, total volume, and flatness) were obtained. Spines were then fixed in formaldehyde and divided into motion segments. A custom build multiphoton microscope was used to acquire SHG and fluorescent images of upper motion segments (L1-L2) in each group. The images were further processed and stitched using Fiji ImageJ. Fixed discs (L5-L6) were sectioned from their midplane and stained with Hematoxylin and Eosin (H&E) and Safranin-O/Fast Green (Saf-O/FG). The stained slides were imaged with a brightfield microscope and a histopathology scoring system for intervertebral disc degeneration: an initiative of the ORS spine section was used to evaluate degeneration in the discs. Additional slides were stained with TUNEL and counterstained with DAPI to examine apoptotic rates of different regions within the disc. A PCA was preformed using the previous solute diffusion data and the new histologic and μ-CT morphologic data to determine which area of the disc was most sensitive to smoke exposure. Two-way ANOVA tests were performed to determine statistical significance.

RESULTS: Micro-CT analysis showed an increase in the trabecular object thickness for the superior endplate and a decrease in trabecular space thickness in the superior and inferior endplate for the 7-month smoke groups relative to the controls and 2-month smoke groups (Fig.1a-d). No change was seen in the inferior trabecular average object thickness. Cortical volume fraction increased while total volume decreased in the superior and inferior endplate regions of the 7-month smoke groups relative to the controls and 2-month smoke groups. Micro-CT data also showed an increase in flatness of the central region of the inferior CEP. MPE imaging showed a decrease in AF fiber organization and an increase calcification in the CEP of the smoke groups (Fig. 2a-d). Histological grading showed increases in degeneration in the 7-month groups relative to the 2-month groups and in the smoke group compared to the control group. Immunofluorescent staining showed an increase in the TUNEL ratio from the 2-month control group to the 2-month smoke group and a decrease from the 7-month control group to the 7-month smoke group for all regions of the disc except the superior CEP (Fig. 3). Additionally, it showed a decrease in cell density for the 7-month smoke group of the inferior CEP region relative to the controls and 2-month smoke groups. No significant change was seen in the cell density of the superior CEP. PCA analysis showed that the endplate morphological remodeling was the most dominant predictor of disc degeneration.

DISCUSSION: This study demonstrated that two months of cigarette smoke exposure altered the morphology of the IVD as evidenced by the changes in bony endplate structure, cartilage endplate cellularity, AF fiber organization, and NP structure and cellularity present after smoke exposure and smoke cessation. Consistent with changes in solute transport and mechanical properties seen in our previous studies, the most severe effect of smoke exposure was found in the 7-month smoke group (2 months smoke exposure + 5 months smoke cessation). This indicated there is limited benefit of smoke cessation on disc degeneration and that smoke cessation alone cannot fix the damage caused by prior smoke exposure. PCA testing revealed the endplate region to have the most sensitive variables when examining the difference between groups. The endplate remodeling could be explained by the increased apoptotic rates seen in the 2-month smoke group and decreased cell density in the 7-month smoke group.

SIGNIFICANCE/CLINICAL RELEVANCE: This study filled knowledge gaps by 1) demonstrating the effect of cigarette smoke exposure on IVD remodeling in a SD rat model; and 2) establishing the endplate region as the most susceptible region in smoke exposure induced pathophysiology.

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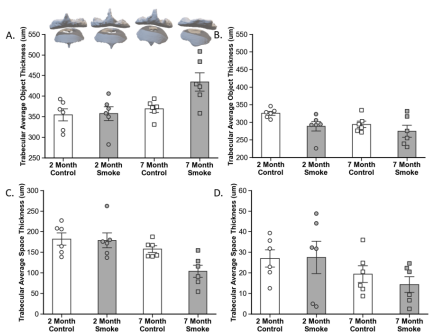

Fig. 1. Micro-CT analysis of trabecular average object thickness for **(A)** superior and **(B)** inferior endplates and of trabecular average space thickness for **(C)** superior and **(D)** inferior endplates.

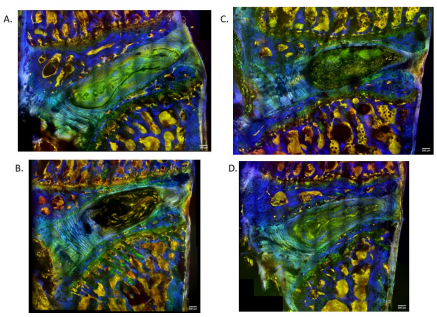

Fig. 2. Representative fluorescent imaging of 2-month **(A)** control and **(B)** smoke exposed and 7-month **(C)** control and **(D)** smoke exposed IVDs.

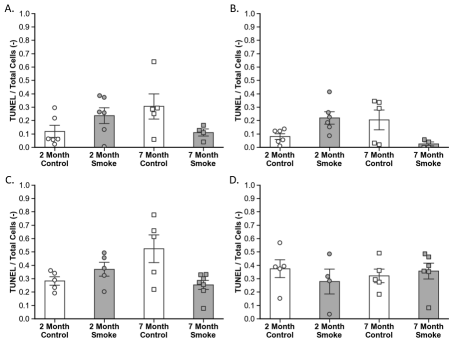

Fig. 3. TUNEL positive ratio for the **(A)** posterior AF, **(B)** NP, **(C)** Inferior CEP, and **(D)** Superior CEP regions of the disc.