INTRODUCTION
Abnormal mechanical stress is considered as a contributing factor to the degeneration of intervertebral disc (IVD). Several studies reported that non-physiological stresses can cause or accelerate biological symptoms leading to disc degeneration. Previous studies have been limited to studying the effects of compressive forces or shear force alone due to practical limitations associated with study designs and in vivo animal models. An in vitro system that can: 1) overcome in vivo limitations and 2) simulate near physiological loading regime in a biologically conducive environment is essential to further our understanding of IVD mechanobiology and degeneration.

We previously showed that IVD motion segments could be kept viable for at least 14 days in culture [1]. We developed a biomechanical culture system (BDCS) that can apply combined loading on IVD motion segments in a controlled environment during culture. The objective this study was to test the feasibility of the BDCS using rat IVD motion segments. For evaluation purposes, we evaluated tissue cell viability and morphological changes in addition to tissue mechanical properties, to ascertain effects of chronic load on IVD mechanical integrity.

METHODS:
Harvest of IVD motion segments: Six Sprague Dawley rats (~300g) were used for this study. Lumbar motion segments L5-6 were harvested from rats using methodology previously described [1].

Culture and loading protocol: Motion segments were anchored in the custom built in vitro BDCS inside a standard incubator and bathed in complete medium [1]. The compression group (n=3) underwent static compressive stress of 0.25MPa and 0MPa for 12 hours each simulating a diurnal cycle. The combined load group (n=3) underwent 1.5N and 0N of shear force for 12 hours each along with 0.25MPa and 0MPa of compressive stress with similar duration as the compression group. Loading and unloading was continued for 7 days.

Mechanical property measurement: Mechanical properties were measured at Day 0 and Day 7 of the loading protocol. Kruskal-Wallis One-Way ANOVA was used for statistical analyses for difference between loading types.

Quasi-static compression: The specimen was compressed by 10% of its original disc height (DH). 10% compression was achieved in 2 seconds.

Cyclic compression: The specimen was compressed to 8% of its DH in 2 seconds and cyclically compressed to 12% of its DH with amplitude of 4% DH at a frequency of 0.1 Hz.

Cell viability and tissue morphology: After 7 days of loading, the specimens were treated with Nitroblue Tetrazolium (NBT) to determine cell viability. Mid-sagittal sections (5 μm thick) from each paraffin-embedded disc were mounted with Vectashield mounting medium containing 4’, 6- Diamidino-2-phenylindole (DAPI) (Vector Laboratories) to label nuclear material. In addition, Hematoxylin and Eosin (H&E) and Safranin-O stained sections were used for tissue morphology. Tissue analyses, cell viability counts and statistical analyses were performed as previously discussed [1]

RESULTS:
Histologically, other than the presence of a collapsed nucleus pulposus(NP), there were no evident signs of cell migration or degenerative changes in either of the loaded groups [Fig1]. Safranin-O sections for all groups showed proteoglycan (PG) loss, except that the loaded groups showed a bright band of staining at the inner anulus region at the transition zone that was not evident in control [Fig1]. In the NP region, the compression group [Fig1B] showed clustering of NP cells in a striated pattern. In the combine load group [Fig1C], the NP cells formed small round clusters but did not form striated patterns as seen in the compression group. Clustering of NP cells and striation patterns in the NP were not evident in control group.

Mean Cell viability percentages of the compressive load group were not significantly different from combined load group in all three regions (EP-Endplate, NP-Nucleus Pulposus and AF-Anulus Fibrosus) that were considered for cell viability counts [Table1]. Cell density observed in both loaded groups were comparable to non-loaded controls.

All loaded specimens irrespective of the loading type showed a loss of static and dynamic compressive modulus after 7 days of loading [Table 2]. Both loaded groups exhibited decreases in peak stress values after 7 days of loading. However, there was no significant difference between the loading groups in terms of static/dynamic modulus or peak stress.

DISCUSSION
No significant difference in cell viability between the load and non-loaded groups suggests no adverse effect of chronic load application on the cell viability, while morphological changes and PG variation in the NP indicate possible differential response to load types. These clearly showed that it is feasible to study IVD mechanobiology under in vitro conditions using our BDCS. Results of this study also showed that the BDCS allows the measurement of the changes in biomechanical properties of the IVD over time. However, PG loss in loaded as well as control IVDs suggests that culture conditions needs to be refined further to minimize PG loss for better studies of IVD mechanobiology.

Responses of cell to different loadings are expected to occur more slowly in in-vitro than in-vivo, and immunohistochemical analyses are recommended to detect early cell responses. In conclusion, in vitro mechanobiology of IVD motion segment is feasible and our model is a promising candidate for studying the pathomechanics of IVD degeneration and the effects of mechanical stimulation on the biology of IVD cells.

REFERENCE: