Journal of Biomechanics 48 (2015) 3701–3708

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Exploring interactions between force, repetition and posture on intervertebral disc height loss and bulging in isolated porcine cervical functional spinal units from sub-acute-failure magnitudes of cyclic compressive loading

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ARTICLE INFO

Article history:
Accepted 18 August 2015

Keywords:
Intervertebral disc
Annulus fibrosus
Height loss
Bulging
Laser scanner

ABSTRACT

Most *in vitro* studies are limited in the ability to partition intervertebral disc (IVD) height loss from total specimen height loss since the net changes in the actuator position of the materials testing system simply reflect net changes to functional spinal units (FSUs) used for testing. Three levels of peak compressive force, three cycle rates and two dynamic postural conditions were examined using a full-factorial design. Cyclic compressive force was applied using a time-varying waveform with synchronous flexion/extension for 5000 cycles. Surface scans from the anterior aspect of the IVD were recorded in a neutral and flexed posture before and after the cyclic loading protocol using a 3D laser scanner to characterise changes in IVD height loss and bulging. A significant three-way interaction ($p=0.0092$) between the magnitude of peak compressive force, cycle rate and degree of postural deviation was observed in cycle-varying specimen height loss data. A significant main effect of peak compressive force ($p=0.0003$) was also observed in IVD height loss calculated from the surface profiles of the IVD. The relative contribution of IVD height loss (measured on the anterior surface) to total specimen height loss across experimental conditions varied considerably, ranging from 19% to 58%. Postural deviation was the only factor that significantly affected the magnitude of peak AF bulge ($p=0.0016$). This investigation provides evidence that total specimen height loss is not an accurate depiction of cycle-varying changes in the IVD across a range of *in vivo* scenarios that were replicated with *in vitro* testing.

1. Introduction

Specimen height loss is a dependent measure that is commonly examined in spine biomechanics *in vitro* studies. Previous research conducted by van der Veen et al. (2008) has demonstrated that deformation in the intervertebral disc (IVD), cartilaginous endplates and vertebrae all contribute to the total height loss of a functional spinal unit (FSU) mechanically loaded in compression. Unfortunately, most studies are limited in the ability to partition this height loss across each of these anatomical structures since the net differences in the actuator position of the materials testing system simply reflect overall changes to the entire osteoligamentous system of FSUs and mounting interfaces used for testing. Of clinical importance, exaggerated height loss has been linked to IVD bulging, which is a known mechanism for low back pain due to compression of the spinal cord in the spinal canal or impingement of the nerve roots in the neural foramina (Brinckmann et al., 1989; Cuchanski et al., 2011; Stokes, 1988; Wenger and Schlegel, 1997).

Therefore, the primary objective of this study was to explore interactions between: (i) the magnitude of the applied compressive force, (ii) cycle rate and (iii) degree of postural deviation on IVD height loss in isolated porcine cervical FSUs subjected to sub-maximal cyclic compressive loading. A secondary objective was to quantify the amount of pre/post-annulus fibrosus (AF) bulging across loading conditions to better understand the structural changes that occur in the IVD of intact FSUs under cyclic loading conditions.

2. Materials and methods

2.1. Specimen preparation

The cervical spines of 63 porcine specimens (mean age=6 months, weight=85 kg) were obtained following death and stored frozen at −20 °C. The cervical spine was separated into two FSUs for testing, which included two adjacent vertebral bodies and

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http://dx.doi.org/10.1016/j.jbiomech.2015.08.023
0021-9290/

the IVD at the level of c34 and c56; resulting in a total of 126 FSUs that were initially included in the study. Porcine cervical FSUs were used as surrogates for the human lumbar spine due to their anatomical and functional similarities (Oxland et al., 1991; Yingling et al., 1999), providing superior control over potential confounding factors that can impact the mechanical integrity of the tissues surrounding the intervertebral joint (e.g. age, diet, physical activity). The inclusion criteria for specimens included in this investigation required that FSUs met a non-degenerated disc quality (Grade 1) as outlined by Galante (1967). Before testing, frozen specimens were thawed at room temperature for a minimum of 12 h. Dissection of the cervical spine involved isolating the two FSUs of interest and carefully removing the surrounding musculature, leaving only the osteoligamentous structures intact, except for the portion of the anterior longitudinal ligament that attached directly to the anterior surface of the AF, which was removed to expose the IVD for surface profile scanning. Once the dissection protocol was complete, width and depth measurements of the two exposed endplates were recorded using digital calipers. These measurements were used to estimate an average intervertebral joint endplate surface area using the equation of an ellipse that were used as input for a regression equation, which was developed using porcine cervical FSUs, to approximate each specimen's ultimate compressive tolerance (UCT) without destructive testing (Parkinson et al., 2005). This allowed for normalisation of peak compressive loading across specimens. Next, the locations of the cartilaginous endplates on the anterior surface of the FSU were marked with three steel pins (0.5 mm diameter; 6 per specimen). One pin was placed at the centre of the IVD and two on the anterolateral sides (Fig. 1). This was done to facilitate pre/post-registration and alignment of the resultant 3D surface profiles obtained from the anterior surface of the IVD from each FSU. The superior and inferior vertebrae of the FSU were then fixed within custom-machined aluminium cups using a combination of wood screws (fixed 1 cm into the exposed endplates) and non-exothermic dental plaster (Denstone; Miles, Southbend, IN, USA). To prevent specimen dehydration throughout the dissection and potting procedures, all FSUs were kept hydrated with a saline mist (0.9% weight per volume [w/v] solution) that was applied approximately every 15 min.

2.2. Procedure

Potted specimens were mounted in a servo-hydraulic materials testing system (Model 8872; Instron, Canton, MA, USA), modified to apply flexion/extension motion to the FSU while under compressive load. Each specimen was free to translate in the anterior–posterior direction (via bearing tray), which enabled the centre of rotation to translate within the joint during the cyclic loading protocol. To minimise dehydration during the prolonged mechanical exposure, all specimens were wrapped in saline soak gauze (0.9% w/v solution) and plastic wrap. Each FSU initially received 15 min of static compressive force (300 N) to counter any post-

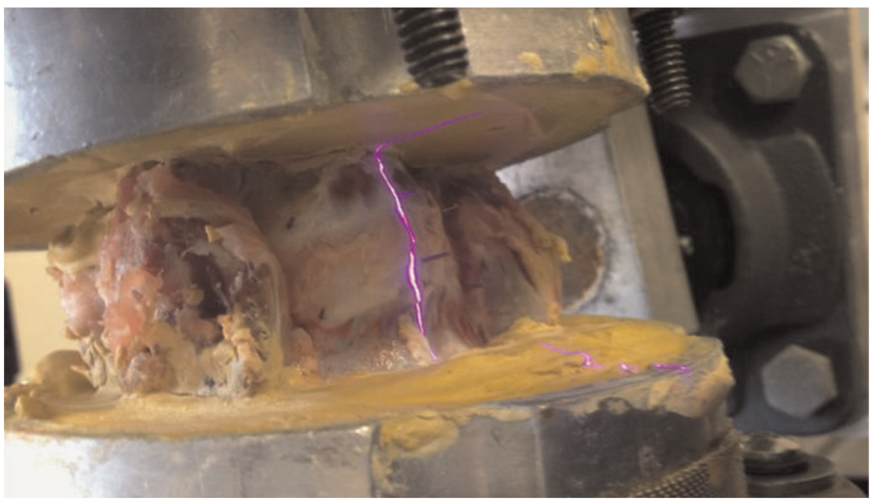

Fig. 1. Functional spinal unit potted in custom machined aluminium cups with non-exothermic dental plaster (yellow) and mounted in a modified materials testing system. Three stainless steel pins (0.5 mm diameter) were inserted into the superior and inferior cartilaginous endplates (6 total) to facilitate pre/post-registration of the 3D surface profile and identify the superior and inferior boundaries of the IVD. The visible (blue) laser on the anterior surface of the FSU was translated across the specimen to construct individual surface profiles. The test specimen is shown with the plastic backed saline infused gauze material removed that was in place during testing to prevent dehydration of test samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

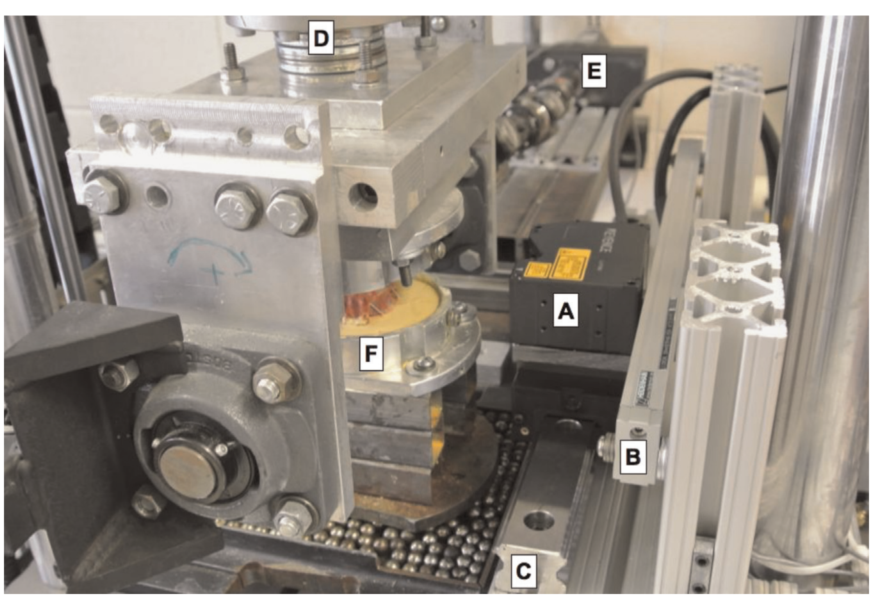

Fig. 2. Modified materials testing apparatus with 3D laser scanning system that was employed for testing. (A) 2D laser head, (B) linear encoder, (C) linear guide, (D) 20 kN load cell in-line with Instron actuator, (E) flexion/extension torque motor, and (F) functional spinal unit potted in custom machined aluminium cups with non-exothermic dental plaster.