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The effect of six degree of freedom loading sequence on the in-vitro compressive properties of human lumbar spine segments

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ABSTRACT

The complex, direction-dependent, poro-viscoelastic properties of the intervertebral disc (disc) suggest that investigations of the six degree of freedom (6DOF) behaviour may be susceptible to inter-test variation in mechanical response if the disc does not return to initial conditions between loading directions. No studies have quantified the effects of sequential multi-directional loading on the consistency of the compressive response of the disc throughout a 6DOF testing protocol. Therefore, the objective of this study was to determine the effect of 6DOF loading on the compressive properties (stiffness and phase angle) of human discs, as evaluated by a reference compression test performed after each single DOF test. Fourteen intact human functional spinal units (FSU) were tested in each of $\pm$6DOFs (shear directions followed by bending and compression) across four orders of magnitude loading frequencies (0.001–1 Hz), followed by reference compression tests while subjected to physiological preload, hydration, and body temperature conditions in a hexapod robot. Repeated measures ANOVA revealed significant within-subjects effects between the reference compression tests for modulus ($p < 0.001$), stiffness ($p < 0.001$), and phase angle ($p = 0.008$). Significant post-hoc pairwise comparisons were initially seen between the control and other reference compression tests for stiffness and modulus after the shear DOFs, however, no significant differences were present after the final reference compression test compared to control. More pronounced effects were seen for stiffness in comparison to modulus and phase angle. These effects may be due to three potentials factors, which include the sequence of testing, the cohort of degenerative specimens, and/or cumulative creep due to the constant application of a follower load. While the sequence of test directions was chosen to minimise the biphasic effect, there may be other sequences, which could result in minimal changes in compressive properties.

1. Introduction

The human spine is a complex structure that allows for multi-directional, six degree of freedom (6DOF) movements under dynamic loads during daily activities. Understanding how the spine responds to those movements and loads is critical for the development of new spinal implants and surgical treatments for intervertebral disc (disc) injuries. To experimentally measure this response in-vitro, excised human spinal segments are mechanically tested to obtain viscoelastic and poroelastic properties of the disc. These properties have been extensively studied under uniaxial compression (Beckstein et al., 2008; Koeller et al., 1984; Virgin, 1951). However, loading of the spine during daily life is not limited to compression, therefore it is important to measure its behaviour in all 6DOF motions (anteroposterior/lateral shear, axial rotation, lateral bending, flexion and extension, and compression).

Panjabi et al. (1976) first proposed applying six forces and six moments on thoracic spinal segments to obtain three-dimensional loading curves of the disc. Later, Patwardhan et al. (1999) demonstrated the need for a compressive axial follower load on lumbar spinal columns (L1-S1) during 6DOF testing, in order to better mimic physiological conditions. Various 6DOF loading devices and corresponding testing protocols have been developed to apply those forces and moments. For example, a system of pulleys and weights (Panjabi et al., 1981), cables and linear actuators (Lysack et al., 2000; Patwardhan et al., 1999), or stepper motors and linear bearings (Goertzen et al., 2004; Wilke et al., 1994) have been used. More recent technologies have allowed the use of a robotic arm (Thompson et al., 2003) or a Stewart platform

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(Ding et al., 2014; Stokes et al., 2002) for application of more accurate 6DOF loading on biological specimens and measurement of mechanical properties.

For enabling reproducible in-vitro testing and comparison of results amongst different laboratories, a standard for 6DOF testing protocols was established (Panjabi, 1988; Wilke et al., 1998). These include a maximum test duration of 20 h, and a loading rate of 0.5–5°/s. Longer testing durations without protease inhibitors, antibacterial and antifungal agents can change disc properties and damage disc tissue (Wilke et al., 1998). Slower loading rates induce a creep effect and faster rates increase the effect of mechanical system inertia (Wilke et al., 1998). However, the sequential order in which 6DOF loading directions should be applied was not defined in that protocol.

Due to the biphasic behaviour of the disc, the sequence of 6DOF testing directions should be considered to minimise the influence of load history on subsequent tests (Costi et al., 2008). The change in volume during bending and compression promotes fluid exudation, and an accompanying reduction in disc height. It is likely that the subsequent behaviour of the disc will be influenced by its load history in these directions. In torsion and shear there are minimal changes in disc volume and fluid flow, resulting in primarily an intrinsic (solid phase) viscoelastic response (Costi et al., 2008). There is limited knowledge on how the biphasic phenomena affects degenerated discs because the degradation of the tissue structure, and loss of water content would be expected to diminish the biphasic response in favour of an increase in the viscoelastic response (Adams, 2004; Galbusera et al., 2014). These direction-dependent differences may contribute to inter-test variation in mechanical response during 6DOF loading regimes. Therefore, it is reasonable to propose that studies investigating 6DOF mechanical properties of the disc should be designed to ensure that it returns to initial conditions prior to commencing testing in the next loading direction.

Many studies have tested loading directions sequentially, without indicating if a recovery period in between tests was applied (Panjabi et al., 2001; Wilke et al., 1994; Yamamoto et al., 1989; Zirbel et al., 2013). In contrast, Lysack et al. (2000) applied five minutes of creep recovery between each DOF, however no disc properties were measured. In pilot studies, Costi et al., 2008 determined that subsequent to an overnight (12+ h) equilibrium compressive preload at 0.4 MPa, a 5 min compressive creep recovery period at 0.4 MPa after each shear and rotation DOF returned the disc to equilibrium hydration levels (unpublished data). A recovery period of 30 min was required for compression and ten minutes for bending DOFs (Costi et al., 2008). Recovery periods between 6DOF tests may be important for minimising the variation in measured disc mechanical properties. To the authors' knowledge, no studies have quantified the effects of sequential multi-directional loading on the consistency of the compressive response of the disc throughout a 6DOF testing protocol.

The primary aim of this study was to determine whether the compressive viscoelastic properties of human spine segments are altered during 6DOF loading, as evaluated by a reference compression test performed after each direction. The second aim was to determine whether the compressive properties differed during 6DOF loading with disc degenerative grade. The third aim was to compare compressive properties between intact spine segments (with facet joints) and isolated discs (without facet joints). We hypothesised that there will be no differences in stiffness, modulus and phase angle (a measure of viscous damping) between the initial and subsequent reference compression tests for each aim.

2. Methods

2.1. Specimen preparation

Fourteen vertebra-disc-vertebra functional spinal units (FSUs), including the posterior elements, were dissected from human lumbar spines Levels: $5 \times$ L1-2, $3 \times$ L2-3, $4 \times$ L3-4, $2 \times$ L4-5, mean (SD) age: 76.2 (11) years. Lumbar spines were stored at -30 °C and then thawed at room temperature for at least three hours, after which careful dissection of soft tissue surrounding the vertebrae and disc was performed, preserving both the anterior and posterior longitudinal ligaments, and the facet joint capsules. The superior and inferior vertebral surfaces were cut parallel to the mid-transverse plane of the disc, re-frozen until the day prior to testing, and thawed overnight in the refrigerator followed by three hours at room temperature. Axial and lateral radiographs were taken of all specimens to determine average disc height (using posterior, middle, and anterior measurements), disc area, and the instantaneous axis of rotation of the disc relative to the cups, which was used as the centre of rotation for the rotational tests (Pearcy et al., 1984). The vertebral bodies were then embedded in fixation tooling using Wood's Metal (Amin et al., 2015). The tooling consisted of a 316 grade stainless steel cup for each vertebra that standardised the volume occupied by the specimen and Wood's Metal. The inferior cup had a hollow-wall where water at 37 °C was circulated. Phosphate buffered saline (PBS) and protease inhibitors were placed inside the inferior cup with the FSU, to create a bath environment (refer to Mechanical Testing Protocol for details). A potting alignment device was used to ensure that the inferior and superior cups were parallel with the mid-transverse plane of the disc and to align the specimen with the hexapod robot's coordinate system. Tapers and slots were machined into each cup to restrict movement between the cup and the potting material. The potted FSU was then coupled to a custom 6DOF hexapod robot (Fig. 1) (Ding et al., 2014), running an adaptive velocity-based 6DOF load control scheme (Lawless et al., 2014).

2.2. Mechanical testing protocol

Since viscoelastic tissue properties are temperature and hydration dependent (Costi et al., 2002; Pflaster et al., 1997; Race et al., 2000), the specimens were immersed in a 0.15 M phosphate buffered saline bath at 37 °C throughout testing (Costi et al., 2008). To reduce putrefaction and tissue autolysis, protease inhibitors, antibacterial and antifungal agents were added to the bath (Costi et al., 2008). Each FSU was subjected to a 12 h axial compressive preload equivalent to a nucleus pressure of 0.1 MPa that represented the unloaded lumbar disc during sleeping (Wilke et al., 1999) to reach hydration equilibrium. The relationship between the applied external FSU compressive stress and nucleus pressure is linear, with nucleus pressure being greater by a factor of approximately 1.5 (Nachemson et al., 1964). This factor was used to calculate the required external compressive force based on the unloaded disc area, to generate the equivalent nucleus pressure. The unloaded disc area was estimated based on the formula $0.84 \times \mathrm{AP} \times \mathrm{LAT}$ (Nachemson et al., 1964), where AP and LAT were the largest anteroposterior and lateral dimensions of the inferior and superior vertebrae, averaged over three measurements using x-rays before potting the FSU.

A compressive axial follower preload (Patwardhan et al., 1999), equivalent to a nucleus pressure of 0.5 MPa was applied to all tests under 6DOF load control, where all off-axis forces and moments were minimised to zero. After hydration equilibration, an initial reference compression test (control) was conducted using a haversine waveform at 0.1 Hz for eight cycles with an amplitude of 0.6 MPa. This loading regime subjected the FSU to a 1.1 MPa equivalent nucleus pressure to approximate the in-vivo intradiscal pressure recorded during standing (Wilke et al., 1999). The FSU was then subjected to a suite of 6DOF tests, with a reference compression test performed after each direction (Fig. 2).

The FSU underwent dynamic haversine displacements/rotations in each DOF with a control protocol that drove the primary axis in position control while minimising coupling forces/moments in the other 5DOF via real-time load control (Lawless et al., 2014). The sequence of loading directions was chosen to theoretically minimise the biphasic effect (Costi et al., 2008): shear and axial rotation tests were conducted first, followed by bending and axial compression. Displacement amplitudes were: ± 0.6 mm for all shear tests (anterior, posterior left/right lateral shear); $\pm 2°$ for left/right axial rotation; $\pm 3°$ for left/right lateral bending; 5° for flexion, and 2° for extension (Costi et al., 2008; Lu et al., 2005; Pearcy and Tibrewal, 1984; Stokes and Frymoyer, 1987). In axial compression, a 1.1 MPa equivalent nucleus pressure was applied as described previously.

For each DOF, five cycles at 1 Hz, 0.1 Hz, and 0.01 Hz were applied, followed by a compressive creep recovery at 0.1 MPa equivalent nucleus pressure for either five minutes (shears/rotations), 10 min (bending), or 30 min (compression). After creep recovery, two cycles at 0.001 Hz were applied followed by another recovery period. After the dynamic tests, the disc underwent stress relaxation for five minutes (0.6 mm for shears and compression; 2° for flexion, extension, and axial rotation; 3° for lateral bending), and creep for five minutes (300 N for shears; 10 Nm for flexion, extension, and axial rotation; 20 Nm for lateral bending; 1.1 MPa for compression) with a creep recovery period in between. A final recovery period was conducted after the creep tests.

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