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Presence of intervertebral discs alters observed stiffness and failure mechanisms in the vertebra

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ABSTRACT

Ex vivo mechanical testing is an essential tool for study of vertebral mechanics. However, the common method of testing vertebral bodies in the absence of adjacent intervertebral discs (IVDs) may limit the physiological relevance of the results. The goal of this study was to determine the influence of IVDs on vertebral mechanical properties and failure mechanisms. Rabbit thoracic vertebral bodies were tested with and without IVDs in a stepwise fashion that incorporated a micro-computed tomography scan at each loading step. The image sequences were analyzed using digital volume correlation to quantify deformations throughout the vertebral body. The observed deformation patterns differed substantially between the groups. Specimens tested with IVDs exhibited a slow increase in strain in the inferior and posterior regions, followed by a sudden increase in strain in the anterior cortex right at the yield point. In contrast, the highest strains in the isolated vertebral bodies were in the posterior regions throughout the test. Specimens tested with IVDs had lower stiffness (507.49 ± 184.73 N/mm vs. 845.61 ± 296.09 N/mm; $p=0.044$), higher ultimate displacement (2.00 ± 0.68 mm vs. 1.17 ± 0.54 mm; $p=0.043$), and higher maximum shear strains (e.g. top 25th percentile: 0.19 ± 0.11 vs. 0.06 ± 0.07 mm/mm; $p<0.0458$), and tended to have lower ultimate force (690.28 ± 160.25 N vs. 873.81 ± 131.48 N; $p=0.056$). Similar work to failure (648.15 ± 317.86 N-mm vs. 603.49 ± 437.95 N-mm; $p=0.844$) was observed between the two groups. These results indicate that testing vertebral bodies in the absence of IVDs can elicit artifactual failure mechanisms. These artifacts may be more prominent than the effects on vertebral strength and toughness.

1. Introduction

Mechanical testing of vertebrae *ex vivo* is a widely used and essential method in research on the effects of aging, genetics, disease, and drug treatments and other interventions on the mechanical behavior of the vertebra. For these mechanical tests, the adjacent intervertebral discs (IVDs) are often removed prior to testing in order to obtain better control over the loading conditions that are applied to the vertebra and to simplify calculations of vertebral stiffness. However, concerns have been raised that testing of isolated vertebrae produces highly idealized, non-physiological loading across the endplate (Yerby et al., 1998). Whether these loading conditions confound measurements of vertebral mechanical properties and failure mechanisms has not been established but is of central relevance to the design and interpretation of *ex vivo* mechanical tests.

The load distribution across the endplate depends on the anatomy and health of the adjacent IVDs. For healthy IVDs, the load distribution measured using stress profilometry is characterized by a high plateau of pressure in the nucleus pulposus, flanked by a steep drop in pressure over the approximate thickness of the annulus fibrosus (Adams et al., 2006; Pollintine et al., 2004). In degenerated IVDs, regions of high pressure occur instead in the annulus, and the percentage of the applied load distributed to the anterior half of the vertebra as compared to the neural arch increases dramatically from erect to flexed postures (Pollintine et al., 2004). In vertebrae with degenerated adjacent discs, trabecular bone is denser and stronger posteriorly than anteriorly (Hulme et al., 2007; Keller et al., 1989; Keller et al., 1993; Simpson et al., 2001), consistent with increased loading of the posterior region in erect postures. Moreover, the strength and fracture mode of vertebrae under axial compression can depend on IVD health (Hulme et al., 2007; Hansson and Roos, 1981). These studies highlight how variable the load distribution across the endplate can be and how these variations, by potentially altering the properties of the underlying bone tissue, might affect the mechanical response of the vertebral body.

This influence of loading conditions on vertebral mechanical behavior has been addressed using finite element (FE) models. By using different load distributions across the endplate to represent different severities of IVD degeneration, these studies have examined the effects of disc health on stress and strain distributions within the vertebra (Dai, 1998; Polikeit et al., 2004; Shirazi-Adl et al., 1984) and

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on the relative contributions of the cortical shell and trabecular centrum to vertebral strength and fracture risk (Buckley et al., 2006; Frei et al., 2002; Homminga et al., 2001; Silva et al., 1997). Interestingly, one of these studies predicted that the specific load distribution across the endplate had only a mild effect on estimates of vertebral strength, although the magnitude of the effect was largest for low-density vertebrae (Buckley et al., 2006). Other FE studies have examined the interplay between disc health and the spatial distribution of bone density in the vertebra, finding that as a result of bone adaptation in response to altered loading associated with IVD degeneration, bone density would be expected to decrease in the central transverse regions of the vertebra (Homminga et al., 2012). In contrast to the multitude of FE studies, the only experimental data available thus far comes from mechanical testing of slices of vertebrae with and without the disc tissues (Yerby et al., 1998) and from comparison of 2-D measurements of surface deformations of vertebrae to data on intra-discal pressure (Pollintine et al., 2010). True 3-D, experimental data are lacking.

The overall goal of this study was to determine the influence of the IVDs on vertebral mechanical properties and failure mechanisms. A method of digital volume correlation (DVC) (Hussein et al., 2012; Liu and Morgan, 2007) was used to track the deformation patterns that developed within the vertebral body prior to and at the onset of failure. The specific objectives were: (1) to quantify 3-D deformation and failure patterns in vertebral bodies tested with and without IVDs; and (2) to quantify the effect of the IVDs on vertebral mechanical properties.

2. Methods

2.1. Specimen preparation

Thoracic vertebrae (6 T9 and 6 T11) were dissected from fresh-frozen spines of female New Zealand White rabbits (18–28 weeks of age). All samples had partially open growth plates of similar thickness, as examined qualitatively using 3-D reconstructions of μCT images. Six vertebrae were harvested with the adjacent intervertebral discs intact ("V+D specimens") (Fig. 1A). The remaining six specimens consisted of only the vertebrae ("V specimens") (Fig. 1A). Posterior elements were removed, and the cranial and caudal ends of the specimens were potted in polymethyl methacrylate (PMMA). Care was taken during the potting process to align the superior–inferior axis of the vertebral bodies with the axial loading direction.

2.2. Mechanical testing and micro-computed tomography (μCT)

Each specimen was placed in a custom-designed, radiolucent, and loading device. The device was filled with saline to hydrate the specimen throughout the test. The specimen was potted in a rectangular-shaped dish, which fit snugly within an inset region within the loading device. Hence, the specimen as a whole was constrained from rotating inside the loading device (Fig. 1B), although the vertebral body in the V+D specimens was not itself constrained against rotation in any additional manner. After applying a pre-load of ~30 N for 20 min, the vertebral body was scanned via μCT (μCT 80, Scanco Medical, Brüttisellen, Switzerland; 36 μm/voxel) (pre-load scan). The specimen was then compressed axially in a stepwise fashion. For the V specimens, each loading step consisted of applying a compressive displacement of 0.25 mm applied at a rate of 0.15 mm/s. For the V+D specimens, the displacement increment was chosen such that the peak axial force that developed at the first loading step matched the average peak force observed at the first loading step in the V specimens. A μCT scan was performed at each step, following a 20-minute relaxation period. The duration of each μCT scan was approximately 90 min. The force was recorded right after load application, after the 20-minute relaxation period, and upon completion of the μCT scan to assess the amount of relaxation taking place throughout a load increment. The overall amount of force relaxation was approximately 70% of the initial peak force, and the majority (87%) of the relaxation occurred during the initial 20-minute period. The combination of stepwise loading and μCT imaging was continued until the ultimate force was reached (seven load increments on average). For both groups, the ultimate force was defined as the peak of the force–displacement curve constructed from the values of force recorded after the 20-minute relaxation periods.

2.3. 3-D measurement of deformation and failure

Digital volume correlation (DVC) was used to obtain direct, 3-D experimental measurements of the failure patterns in the vertebral body, as defined by the distributions of strains throughout the centrum (Hussein et al., 2012). DVC analyzes the images from the different loading steps in an automated fashion to track the movement and deformation of multiple, individual sub-regions throughout the entire centrum over the course of mechanical testing. In preparation for DVC, the images from the different loading steps were aligned to the pre-load scan using image registration (Scanco Medical, Brüttisellen, Switzerland). The sub-regions within the vertebral body were then defined by generating an irregular mesh, consisting of hexahedral sub-regions with side length ~1.44 mm, that conforms to the geometry of the vertebral body, using the IA-FEMesh software (The University of Iowa, Iowa City, IA) (Fig. 2C). The sub-region size was chosen based on results from a previous study (Liu and Morgan, 2007) which found that sub-regions with side length ~1.44 mm provide the best combination of spatial resolution, accuracy, and precision in the strain measurements in the rabbit vertebral body. The images from a given loading step were then analyzed together with the pre-load images using a custom, DVC algorithm (gradient-based, iterative, optimization technique) (Liu and Morgan, 2007) to determine the displacement and strain fields throughout the entire vertebral body (Fig. 2D) at each load increment. The measured strain fields correspond to strain experienced as the vertebra is loaded to failure. For this

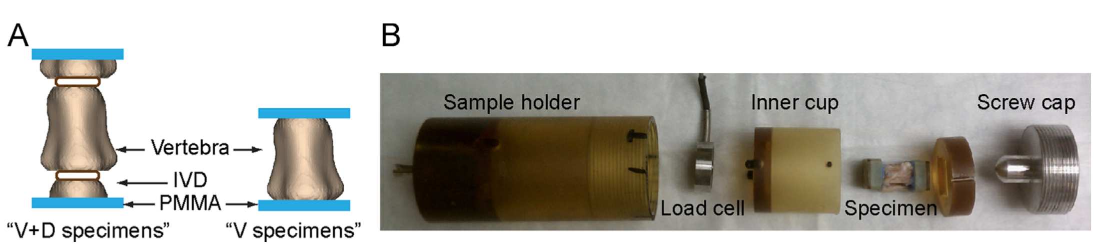

Fig. 1. (A) Schematic for "V+D specimens" and "V specimens"; and (B) components of the loading device.

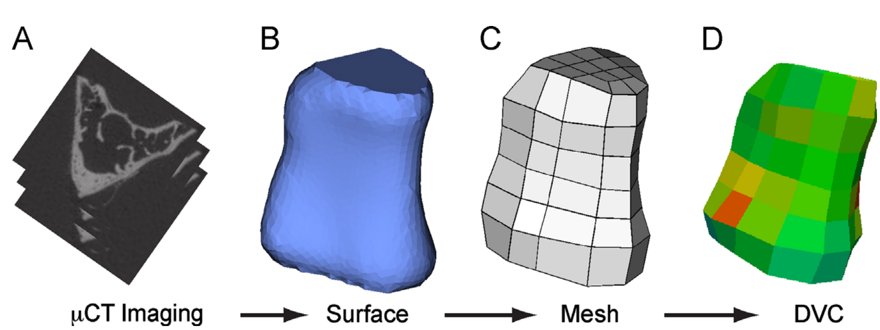

Fig. 2. Schematic for generating the 3D, irregularly shaped mesh to be used for DVC analyses of deformations in whole vertebral bodies.