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Needle puncture in rabbit functional spinal units alters rotational biomechanics

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Abstract

Study Design—An in vitro biomechanical study for rabbit lumbar functional spinal units (FSUs) using a robot-based spine testing system.

Objective—To elucidate the effect of annular puncture with a 16G needle on mechanical properties in flexion/extension, axial rotation, and lateral bending.

Summary of Background Data—Needle puncture of the intervertebral disc has been shown to alter mechanical properties of the disc in compression, torsion, and bending. The effect of needle puncture in FSUs, where intact spinal ligaments and facet joints may mitigate or amplify these changes in the disc, on spinal motion segment stability subject to physiological rotations remains unknown.

Methods—Rabbit FSUs were tested using a robot testing system whose force/moment and position precision were assessed to demonstrate system capability. Flexibility testing methods were developed by load-to-failure testing in flexion/extension, axial rotation, and lateral bending. Subsequent testing methods were used to examine a 16G needle disc puncture and No. 11 blade disc stab (positive control for mechanical disruption). Flexibility testing was used to assess segmental range-of-motion (°), neutral zone stiffness (Nm/°) and width (° and Nm), and elastic zone stiffness before and after annular injury.

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Conflicts of Interest: Regarding conflicts of interest, there are none declared.

Results—The robot-based system was capable of performing flexibility testing on FSUs—mean precision of force/moment measurements and robot system movements were less than 3% and 1%, respectively, of moment-rotation target values. Flexibility moment targets were 0.3 Nm for flexion and axial rotation and 0.15 Nm for extension and lateral bending. Needle puncture caused significant ($p<0.05$) changes only in flexion/extension range-of-motion and neutral zone stiffness and width (Nm) compared to pre-intervention. No.11 blade-stab significantly increased range-of-motion in all motions, decreased neutral zone stiffness and width (Nm) in flexion/extension, and increased elastic zone stiffness in flexion and lateral bending.

Conclusions—These findings suggest that disc puncture and stab can destabilize FSUs in primary rotations.

INTRODUCTION

Intervertebral disc degeneration (IDD) is a leading cause of back pain, a prevalent world-wide health problem¹. The causes of disc degeneration are multifactorial, including associations with mechanical damage. Animal models of disc degeneration show associations between altered mechanical loading, including segmental instability and disc injury, and in the induction of degenerative changes in the disc². Rabbit models of IDD induced by needle puncture are widely used to study the course of degeneration^{3–7}; however, the initial resultant changes in spinal mechanics remain unknown.

Recent studies show that annular puncture causes immediate changes to disc mechanics^{8–11}. Examining disc mechanics in a rat tail, Michalek et al. observed that needle puncture reduced dynamic compressive stiffness, but decreases were not sensitive to needle diameter⁸. In contrast, torsional stiffness changed as a function of needle diameter⁸. A recent study using bovine caudal discs in rotational motions found that torsional stiffness and disc height were sensitive to annular puncture⁹. Based on additional testing and a literature review, Elliott et al. proposed that the needle diameter-to-disc height ratio was critical to biomechanical and subsequent biochemical changes in the disc¹⁰. They categorized needle-to-disc height ratios <0.25 as benign, >0.4 as inducing degenerative changes, and intermediate ratios as variable. While these studies have elucidated mechanical responses to needle puncture in discs, biomechanical effects have not been studied in functional spinal units (FSUs) where spinal ligaments and facet joints, which may mitigate or amplify these mechanical changes, are retained to preserve in-situ loading.

Additionally, the effect of needle stab on moment-rotation kinetics from segmental flexibility testing has not been investigated. Flexibility testing of spinal segments enables characterization of stability, which involves motion restriction by the facets and posterior ligamentous complex. Flexibility testing defines segmental range-of-motion (ROM), elastic zone (EZ) stiffness, and most sensitively, neutral zone (NZ) stiffness and width. In-vitro, the NZ, or the low-stiffness region of the moment-rotation curve, is governed largely by nucleus pulposus composition and interactions with surrounding structures^{11–15}, whereas the high-stiffness EZ is regulated by annular fibers¹⁵, spinal ligaments^{16, 17} and bony contact¹⁸. Grauer et al. adapted human lumbar spine flexibility testing methods to rabbit lumbar segments (L4–L7) and demonstrated the ability to detect differences in lumbar fusions¹⁹.

This type of testing has not been used, however, to evaluate FSUs in disc puncture models. Thus, testing methods and parameters had to be established for rabbit FSU flexibility testing.

Our research group has established a reliable model of disc degeneration based on acute injury and putative mechanical changes in the disc following annular puncture with a 16 gauge (16G) needle^{4, 20, 21}. Changes in axial mechanics in degeneration and therapeutically treated discs have been demonstrated 12 weeks after puncture⁵, yet nothing is known about initial mechanical changes in this degeneration model. Further, rotational rabbit FSU mechanics have not been thoroughly investigated. Thus, we established rabbit FSU flexibility testing methods to test the immediate in-vitro mechanical effect of 16G needle puncture on rotational biomechanics. We hypothesized that a 16G needle puncture would destabilize FSUs evidenced by (1) increased ROM in all segmental rotations, (2) reduced NZ stiffness in flexion/extension (F/E), and decreased EZ stiffness in axial rotation (AR) compared to pre-puncture controls.

MATERIALS AND METHODS

All study procedures were performed under the oversight of the Institutional Animal Use and Care Committee (University of Pittsburgh, Pittsburgh, PA).

Specimens Preparation

Lumbar spines were freshly harvested from female, 6–9 month old New Zealand White rabbits (2.0–2.5 kg). L3–4 FSUs were isolated by removing surrounding muscle, adipose tissue, and transverse processes. FSUs were immediately fixed with epoxy resin (Bondo, Atlanta, GA) and stored in 0.9% NaCl at 4°C overnight. On the following day, specimens were placed in custom fixtures and mounted within a robot-based spine testing system for rotational testing (Fig. 1). Specimens were wrapped in saline-soaked gauze and sprayed with saline throughout testing to prevent desiccation.

Device Description & Characterization

The robot-based spine testing system consisted of a serial-linkage robot (Staubli RX90, Staubli Inc., Duncan, SC), an on-board universal force sensor (UFS Model 90M38A-150, JR3 Inc., Woodland, CA) and custom fixtures (Fig. 1). The robot was controlled via a program written in MATLAB (Mathworks Inc., Natick, MA) and operated under adaptive displacement control in a quasi-static manner as described previously²². A flexibility test was simulated by producing a pure moment about an updating, preferred center of rotation²³ where off-axis forces were minimized at each step (0.5–1.0° step size) in the quasi-static movement (0.05–0.1°/s). Coupled rotation can occur, particularly in AR and LB, as the off-axis force and moment minimization allows primary moment to build up about an updating center-of-rotation.

The system load and position precision were assessed for rabbit FSU testing; acceptable precision targets were set to be less than 5% of applied moments and rotations. Load repeatability of six degree-of-freedom (DOF) forces/moments (0–90N /0–11 Nm detection range with 0.27N/0.0023 Nm resolution) was calculated during unloaded (robot only) and loaded (FSU attached) F/E. Position precision was quantified using a five-camera motion

tracking system (VICON 460, Vicon, Centennial, CO) in F/E and AR. Euler angles and displacements were analyzed by taking the root-mean square error over ten cycles for each step in the motion path.

Destructive Testing: Load-to-Failure

Destructive testing was performed to determine appropriate moment targets for flexibility tests in each rotational motion. FSUs were subjected to 10N of constant compression, scaling to body weight similar to other quadruped studies^{24, 25}, randomly assigned to flexion, extension, AR or lateral bending (LB) (n=3–5), and rotated in the testing system until failure marked by a pronounced decrease in primary moment (Fig. 2).

Non-destructive Testing: Flexibility Testing

From the failure moments of destructive tests, moment targets for non-destructive testing were selected based on the following criteria: moment targets (i) exceed the NZ, (ii) fall below 50% of the mean failure moment and (iii) promote consistency of targets across motions.

Using the moment targets listed in the Results, FSUs were tested non-destructively in flexion, extension, AR, and LB. FSUs were rotated to the moment target with 10N of constant compression three times in each motion; the third cycle was used for analysis. They were then subjected to an intervention (i.e. needle puncture or blade stab) or to no intervention (i.e. control) and were re-tested to permit pre-and post-intervention comparisons for each FSU. To reduce the effects of tissue strain history, FSUs were allowed to recover for 15 minutes at room temperature immersed in saline prior to re-testing. Intact control FSUs (n=5) were tested identically as before, and experimental FSUs (n=8) were subjected to a 16G needle puncture or to a positive control for mechanical disruption, a lateral, mid-height, full disc-width blade (No.11) stab. Mechanical outcomes were determined both pre- and post-intervention for each specimen.

No.11 Blade Stab

To serve as a positive control for severe damage-induced changes in biomechanics, a No. 11 blade was inserted laterally on the left side to the full disc width in rabbit FSUs (n=8) similar to the injury model used by Lipson and Muir²⁶. FSUs were tested intact, stabbed, and then re-tested.

16G Needle Puncture

A 16G needle was inserted on the left side to a 5 mm depth using the anterolateral approach (n=8) performed surgically in the disc degeneration model⁴. Similar to the blade-stabbed FSUs, punctured specimens were tested intact, punctured, and re-tested.

Mechanical Outcomes

Overall ROM for each motion were calculated by summing the rotation angle at the moment targets in flexion and extension, right and left AR, and right and left LB, respectively. Changes in ROM were calculated as a percent change between pre-and post-intervention ROM. EZ stiffness (Nm/°) was calculated within the linear portion of the moment-rotation

curve for each motion (0.2–0.3 Nm in flexion and AR, 0.08–0.15 Nm in extension and LB). Because only F/E possessed consistent sigmoidal moment-rotation curves, NZ analyses were performed for F/E (described below) but not AR or LB. NZ parameters were determined by fitting a double sigmoidal function to moment-rotation data to define the NZ as the high compliance region demarcated by extrema of the second derivative (Fig. 3) as described by Smit et al.²⁷.

$$D = \frac{1}{1 + e^{-(a1 + b1 * L)}} * c1 + \frac{1}{1 + e^{-(a2 + b2 * L)}} * c2 + d$$

NZ width was defined in two ways—classically, by rotation angle between the inflection points, and alternatively, as the difference in moment between inflection points. NZ stiffness was designated as the inverse of the slope of a linear fit (°/Nm) in the NZ. Change in EZ and NZ parameters was calculated as a percent change with intervention as it was for ROM.

Statistical Analysis

Paired t-tests were used to compare within groups (pre-and post-intervention); $p < 0.05$ was set to determine statistical significance. An independent analysis of variance (ANOVA) with Bonferroni correction ($p < 0.0125$) was performed in MATLAB (The Mathworks, Inc.) to compare between groups.

RESULTS

System Characterization

Precision of force/moment detection and robotic system positioning are depicted in Fig. 4. In F/E, mean force (mean of F_x , F_y , & F_z) and moment (mean of M_x , M_y , & M_z) precision were 0.25 ± 0.05 N and 0.009 ± 0.002 Nm, respectively. Mean kinematic translational precision (mean D_x , D_y , & D_z) for F/E and AR were 0.194 ± 0.063 and 0.081 ± 0.022 mm, respectively. Similarly, mean kinematic rotational precision (mean R_x , R_y , & R_z) were 0.113 ± 0.038 and $0.038 \pm 0.006^\circ$ for F/E and AR, respectively. Moment precision error was $< 3\%$ of moment targets, and rotational precision was $< 1\%$ of rotation angles.

Load-to-Failure

Failure loads varied based on motion (Fig 2): 0.86 (0.64–1.12] Nm in flexion, 0.55 (0.21–0.83) Nm in extension, 0.64 (0.49–0.74) Nm in AR, and 0.46 (0.27–0.74) Nm in LB. AR and extension failed at the endplate, whereas LB and flexion failed in the posterior ligaments. Therefore, moment targets for non-destructive loading were selected: 0.3 Nm for flexion and LB and 0.15 Nm for extension and AR.

Disc Disruption: No. 11 blade and 16G needle

Mean values before and after intervention for ROM and EZ for each motion and NZ properties for F/E are depicted in Table 1. Changes in NZ properties, EZ properties, and ROM before and after disc disruption are shown in Figs. 5, 6, and 7, respectively. The intact group confirmed no effect of re-testing FSUs; there were no significant differences within

samples before and after the 15 minute recovery period in saline. The No. 11 blade stab increased ROM in all motions ($p<.001$, $p=0.001$, and $p=.006$ in F/E, AR, and LB, respectively), increased NZ width (Nm) ($p=0.03$), decreased NZ stiffness ($p=0.004$), and increased EZ stiffness in flexion ($p=0.046$) and LB ($p=0.039$). A trend toward decreased EZ stiffness in AR across groups was observed. The 16G needle puncture caused significant changes in biomechanical properties, but changes occurred only in the NZ ($p=0.007$ and 0.003 for NZ width and stiffness, respectively) and ROM in F/E ($p=0.005$). Needle puncture, unlike blade stab, did not significantly influence ROM in AR or LB or EZ stiffness in any motion. Comparisons between groups showed significant differences between stabbed and intact FSUs' ROM for each motion ($p=0.011$, $p=0.004$, and $p=0.003$ in F/E, AR, and LB, respectively) and NZ stiffness in F/E ($p=.009$; Fig. 5). The punctured FSU group significantly differed from intact FSUs in NZ stiffness in F/E ($p=0.023$; Fig 5); differences in all other parameters and motions were not significant.

DISCUSSION

This study is the first to quantify the biomechanical effects of annular puncture in physiologic rotations using flexibility testing of lumbar functional spinal units. The purpose of this investigation was to quantify the immediate biomechanical effects of the annular puncture used to initiate degeneration in a rabbit intervertebral disc degeneration model⁴. The key findings of this study were that needle puncture reduced NZ stiffness and increased ROM in F/E. Reduced stiffness and hypermobility indicate reduced stability of the FSU following a 16G needle puncture of the disc. Segmental instability can induce disc degeneration^{28, 29} and likely contributes to the progression of disc degeneration in the rabbit IDD model.

While numerous recent studies have demonstrated immediate changes in intervertebral disc mechanics following needle puncture^{8, 10, 30–34}, the effect of puncture in the context of the FSU has not been studied. The current study extends previous findings to the segmental level where preserved facets and ligaments could amplify or mitigate the change in stiffness seen in disc-only testing. FSU rotational testing showed significant decreases in NZ stiffness in F/E, which depend largely on NP pressurization¹¹, for both the 16G puncture and No. 11 blade stab. Interestingly, the magnitude of reduction in NZ stiffness with puncture (22–23%) is similar to the ~20% reduction in compressive properties observed by Michalek et al.⁸. Changes in EZ stiffness and overall ROM, which have greater dependence on the AF and surrounding structures of the FSU, were evident in stabbed FSUs. A trend of decreasing torsional EZ stiffness between needle puncture and disc stab is in line with results from Michalek et al. who found torsional stiffness to be the most sensitive metric to annular injury⁹ and likely reflects the greater extent of fiber damage in a lateral stab injury compared to a needle puncture. These data are consistent with observations that properties dependent on NP pressurization are not related to defect size, but properties dependent on AF vary with injury magnitude^{30, 32}.

This study shows that a 16G needle puncture inserted in rabbit lumbar discs, similar to multiple in-vivo IDD models^{3, 4, 7, 35–37}, compromises segmental stability in addition to directly injuring the disc. Previous studies show mixed results on the ability of segmental

instability alone to induce degeneration^{28, 29, 38}. Examining disc needle puncture, Elliott et al. found a dependence of biological and mechanical changes on needle diameter relative to disc height, and a ratio >0.4 , as in this study, consistently results in degenerative changes¹⁰. Thus, while direct injury to the disc likely drives degenerative changes in rabbit lumbar degeneration models, induced instability may reinforce and add to degenerative changes.

Methods were developed to adapt a serial-linkage robot-based spine testing system used previously for human FSU testing²³ to rabbit FSUs. Numerous spine testing systems capable of pure moment flexibility testing could be used or adapted for similar testing. System precision is deemed adequate for rabbit FSU testing based on comparing load targets to force/moment measurement precision (an order-of-magnitude greater) and maximum rotation angles to rotational precision (three orders-of-magnitude greater). Force and position variability were comparable to a similar system used for rabbit testing³⁹. Load-to-failure testing determined load targets for flexibility testing, and results agreed closely for flexion and AR (0.3 Nm) with Grauer et al., who used body-weight matched load targets (0.27 Nm) derived from human lumbar testing for all rotations of rabbit lumbar segment testing⁴⁰. While our data demonstrate FSUs require smaller targets in extension and LB, differences in control of FSUs and age of specimens may account for differences in moment targets. Future therapeutic and multiple DOF mechanobiological testing will benefit from development of these testing methods.

The rabbit lumbar FSU was chosen because rabbit IDD models^{3, 4, 7, 35–37} are commonly used to study regenerative therapies^{6, 41–45} and cellular mechanisms of disc degeneration^{37, 46, 47}. The use of a rabbit model to study IDD, however, is limited by its smaller size, greater annular water content⁴⁸, and greater segmental laxity relative to humans⁴⁰. Nevertheless, Grauer et al. identified similar patterns to humans in lumbar ROM⁴⁰, and Beckstein et al. illustrated comparable normalized axial mechanics⁴⁸. While this study is consistent with the classification of Elliott et al., it cannot address the effect of a needle diameter-to-disc height threshold because smaller needle diameters were not used. Further, disc height changes were not measured, though they have been shown to be sensitive to annular disruption⁹. The 6–9 months old rabbits in this study, while similar to those used by Beckstein et al.⁴⁸, were younger than those used in flexibility studies (>10 months old)^{19, 40}. Finally, lack of muscle simulation, while less problematic in FSUs than multilevel testing, limits direct translation of these results.

Future studies need to include mechanical analysis of rabbits punctured in-vivo to characterize the effect of remodeling. Moreover, to better address the consequences of needle puncture, novel organ culture systems need to be adapted to flexibility testing to permit simultaneous assessment of cell viability, metabolism and matrix remodeling. Nevertheless, the implications of these results extend beyond animal models as annular injuries are common and thought to induce or accelerate disc degeneration. Reduction in segmental stability, which can result from annular injury, likely augments degenerative changes. Promoting stability of injured motion segments may be beneficial in slowing the progression of degeneration.

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Figure 1.

Lumbar spines were sectioned into (A) functional spinal units (FSU: L3–4) and each FSU was (B) fixed with epoxy resin and (C) mounted to a robot-based spine testing system.

Figure 2.

Representative load-to-failure moment-rotation curves in primary spinal rotations. Examples of typical epiphysis (left) and posterior ligament (right) failure are inserted.

Figure 3.

Representative moment-rotation curves for flexion-extension with fitted double sigmoid function (solid line) are plotted. The methods for neutral zone (NZ) determination are illustrated; first and second derivatives are shown in dashed and dotted lines, respectively. NZ width (NZ_w), NZ compliance (NZ_c), and elastic zone (EZ) compliance (EZ_c) are depicted.

Figure 4.

Repeatability (mean root mean square $\pm$ SD) of (a) robotic kinematics—i.e. robot relative to the base mounting fixture (D-displacement, R-rotation)—and (b) force/moment repeatability of the universal force sensor (F-force, M-moment) with and without an attached specimen in bending (flexion/extension) and torsion (axial rotation) across $n=3$ trials.

Figure 5.

Change in flexion/extension neutral zone (NZ) width—measured by angle (°) and moment (Nm)—and NZ stiffness (Nm/°) 15 minutes after intervention for intact (no intervention), 16G needle puncture, and No.11 blade stab. Significant differences ($p < 0.05$) between pre- and post-intervention are marked with (*) and significant differences between groups ($p < 0.0125$) are marked with bars. Error bars represent standard deviation of normalized change.

Figure 6.

Change in elastic zone (EZ) stiffness (Nm/°) 15 minutes after intervention: intact (no intervention), 16G needle puncture, and No.11 blade stab. Significant differences ($p<0.05$) between pre- and post-intervention are marked with (*); differences between groups were not significant ($p<0.0125$). Error bars represent standard deviation of normalized change.

Figure 7.

Change in total range of motion (ROM, °) 15 minutes after intervention: intact (no intervention), 16G needle puncture, and No.11 blade stab. Significant differences ($p < 0.05$) between pre- and post-intervention are marked with (*) and significant differences between groups are marked with bars ($p < 0.0125$). Error bars represent standard deviation of normalized change.

Table 1

Moment-rotation properties (neutral zone (NZ) width, NZ stiffness, elastic zone (EZ) stiffness, and range-of-motion (ROM) in flexion/extension, axial rotation and lateral bending

State	Time	Flexion/Extension				Axial Rotation				Lateral Bending	
		NZ Width (°)	NZ Width (Nm)	NZ Stiffness (Nm/°)	ROM (°)	EZ Stiffness (Nm/°)	ROM (°)	EZ Stiffness (Nm/°)	ROM (°)	EZ Stiffness (Nm/°)	ROM (°)
No Intervention	<i>Pre</i>	15.72 (1.53)	0.086 (0.026)	0.005 (0.001)	30.29 (0.09)	0.051 / 0.068 (0.028 / 0.022)	7.79 (0.78)	0.087 (0.038)	15.13 (2.22)	0.060 (0.025)	
	<i>Post</i>	16.05 (0.58)	0.086 (0.023)	0.005 (0.001)	31.05 (0.04)	0.049 / 0.076 (0.021 / 0.049)	7.89 (0.81)	0.098 (0.031)	14.29 (3.15)	0.063 (0.035)	
16G Needle Puncture	<i>Pre</i>	15.24 (1.09)	0.088 (0.021)	0.006 (0.001)	31.70 (0.10)	0.047 / 0.044 (0.038 / 0.029)	10.15 (2.40)	0.074 (0.030)	23.36 (7.94)	0.046 (0.033)	
	<i>Post</i>	15.35 (1.30)	0.068 (0.018)	0.004 (0.001)	33.17 (0.03)	0.052 / 0.057 (0.032 / 0.031)	10.62 (2.73)	0.079 (0.036)	22.99 (8.16)	0.054 (0.031)	
No.11 Blade Stab	<i>Pre</i>	17.11 (3.13)	0.107 (0.035)	0.005 (0.001)	37.19 (0.07)	0.026 / 0.027 (0.009 / 0.009)	11.28 (2.35)	0.061 (0.023)	24.63 (6.93)	0.027 (0.009)	
	<i>Post</i>	18.05 (1.69)	0.070 (0.013)	0.004 (0.001)	39.95 (0.03)	0.035 / 0.042 (0.008 / 0.032)	12.81 (1.99)	0.061 (0.024)	26.81 (6.94)	0.039 (0.020)	