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Verification of pure moment testing in a multi–degree of freedom spine testing apparatus

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Abstract

Background: Pure moment testing is a common method used in cadaveric spine testing. The fundamental basis for the widespread acceptance of applying a pure moment is uniform loading along the column of the spine. To our knowledge, this protocol has not been experimentally verified on a multi–degree of freedom testing apparatus. Given its ubiquitous use in spine biomechanics laboratories, confirmation of this comparative cadaveric test protocol is paramount.
Methods: Group A specimens (n = 13) were used to test the pure moment protocol, by use of 3 constructs that changed the number of involved vertebrae, orientation, and rigidity of the spine construct. Group B specimens (n = 6) were used to determine whether potting orientation, testing order, or degradation affected the range of motion (ROM) by use of 8 constructs. Each group was subjected to 3 cycles of flexion-extension, lateral bending, and axial torsion. The data from the third cycle were used to calculate the ROM for each method.
Results: Group A testing resulted in significant differences in ROM across the 3 constructs for lateral bending and axial torsion ($P < .02$) and trended toward a difference for flexion-extension ($P = .055$). Group B testing showed an increase in ROM across 8 constructs ($P < .04$) but no significant difference due to the orientation change.
Conclusion: The increased ROM across constructs observed in both groups indicates that the cause is likely the testing order or degradation of the specimens, with orientation having no observed effect. The data do not invalidate pure moment testing, and its use should persist.

Keywords: Pure moment testing; Lumbar spine; Spine biomechanics; Tissue degradation

In vitro biomechanical experimentation is commonly used to test spinal implants prior to clinical testing.[1] Wilke et al.[2] showed that the loads imposed during in vitro biomechanical testing are comparable to the loads observed in vivo, implying that it is an appropriate model for spinal testing. Pure moment testing is a commonly used method for in vitro biomechanical spine experimentation because the spine is not a uniform structure and it consists of multiple vertebrae connected by joints and ligaments, therefore requiring a specific method of loading.[3]

The pure moment conceptual framework is based on the presumption that uniform loading along the column of the spine in the absence of an applied shear force results from the application of a pure moment. This zero shear loading condition predicts a moment with constant magnitude at each functional spinal unit (FSU) along the spinal column, independent of fixation, number of involved FSUs, or orientation in the plane of loading.[4] These conditions make pure moment testing particularly attractive because it allows for a degree of standardization, which is necessary to compare data across experiments and laboratories.[3] This method is a useful simplifying tool and is the preferred method of testing because it allows for unconstrained motion of the spine.[3] Although a few aspects of the assumptions underlying this method of testing have been verified in the literature, such as shear forces,[4,5] other key elements require further scrutiny. This study will elucidate certain aspects of the pure moment protocol using a multi–degree of freedom testing apparatus to more thoroughly verify the assumption. In practice, deviations from the idealized framework of pure moment loading exist, with nominal shear loads being commonly observed and reported.[4,5] The orientation of the or-

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thogonal planes other than the plane of loading also has the potential to affect test outcomes.

The standard of biomechanical spine testing consists of using artificial models or fresh-frozen cadaveric spines and subjecting them to a load control protocol that uses the flexibility method. The flexibility method consists of applying known loads to a specimen and measuring its kinematic response. These loads are typically applied in flexion-extension, right and left lateral bending, and right and left axial torsion. In addition, the flexibility method using pure moment testing has been a template for a variety of studies, including cervical, thoracic, lumbar, and animal models.[6] However, cadaveric specimen testing can be affected by multiple factors, such as the orientation of the specimen with respect to the testing apparatus and tissue degradation due to mechanical testing or dehydration of the specimen.

The goal of this study was to verify the fidelity of a pure moment testing protocol on a custom-built multi–degree of freedom machine consisting of counteracting flexion-extension and lateral bending motors mounted on a biaxial material testing frame. The testing involved determining whether the moment applied at each FSU along the spine was constant and whether there were observable adjacent-level effects. Verifying this assumption is important because of the widespread applicability of the test protocol to research involving spine testing and authenticates the validity of previous and future studies. This was accomplished by use of the flexibility method on fresh-frozen cadaveric spine segments and comparing the measured range of motion (ROM) over different constructs. Under ideal conditions, there should not be a detectable significant difference in the ROM regardless of fixation, length of the specimen, or orientation in plane of loading.[3] Thus this experiment should uphold the assumption that pure moments are being applied and uniformly distributed down the spinal column. In addition, to investigate particular sources of error, specimens were tested for the effects of orientation during testing and degradation due to testing order.

In short, this study aims to verify the pure moment protocol by testing the following aspects of spinal testing. First, the main hypothesis is to determine whether the construct length (number of FSUs per segment) results in observable near or far adjacent-level effects. Second, the effects of potting technique on segmental loading and the resulting ROM were compared. Finally, the effects from the conditions on each specimen in this experiment were examined to establish the significance of mechanical testing and length of specimen exposure.

Methods

Rigid tracking body fixation (specimen preparation)

Two groups of specimens were used for this study: group A specimens were used to conduct the verification of the pure moment assumption, and group B specimens were used to determine the effect of potting orientation and tissue degradation. For each group, the fresh-frozen cadaveric spines were cleaned of muscle, loose connective tissue, and the anterior longitudinal ligament, with special care given to preserve the remaining intervertebral ligamentous structures. The ends of all specimens were dissected to fit within aluminum potting rings. Three wood screws were driven through the ends of the segments before potting to provide additional anchoring points. The specimens were potted with a thermosetting polymer, polyester resin, and hardener (Bondo; 3M, Atlanta, Georgia).

A rigid aluminum tracking body, containing 4 active light-emitting diodes, was permanently affixed to the anterior surface of each lumbar vertebral body with Bondo and aluminum sheet metal screws. Specimens were stored at −20°C, thawed at room temperature for at least 24 hours before each test, and stored at 4°C between tests. Each specimen was also intermittently sprayed with an isotonic solution to maintain its hydration level. Furthermore, specimens were wrapped in gauze soaked in an isotonic solution when not being tested.

Group A comprised 13 analyzed FSUs consisting of 4 L1-2 FSUs, 4 L2-3 FSUs, and 5 L4-5 FSUs. The 4 T12-L3 segments and 5 L4-S1 segments were dissected from 6 human lumbar spines (4 female and 2 male specimens; mean age, 58.3 years; age range, 24–71 years). The specimens were previously used in a pedicle screw–based fixation study with instrumentation at the L3-4 level. After full length testing, each spine was disarticulated into 2 segments at the L3-4 level and the ends of each segment (T12 and L3 for the upper segment and L4 and S1 for the lower segment) were repotted (Fig. 1A).

Group B consisted of 6 human lumbar specimens from T12 through sacrum (2 female and 4 male specimens; mean age, 60.8 years; age range, 52–68 years). The specimens used were from a previous pedicle screw–based posterior dynamic stabilization study. Some of the previously obtained data were analyzed for the untreated levels L1-L2 and L2-L3 for this analysis. After full length testing, each spine was disarticulated at L4-L5 and L4 was repotted to test the T12-L4 segment (Fig. 1B). The disarticulation was performed to address the effect of the angle of potting orientation. Because of the natural lordosis of the lumbar spine, the orientation of the specimen changes depending on the length of the specimen and which vertebrae are potted. Therefore, by changing the length of the specimens and the vertebra potted, the extent of lordosis of the specimen was reduced, causing a modification in the angle of the tracked vertebrae with respect to the loading apparatus.

In vitro testing (specimen configuration)

Each specimen was tested on a multi–degree of freedom custom-built machine composed of counteracting inferior and superior flexion-extension and lateral bending electric motors mounted on a biaxial (axial displacement and rota-

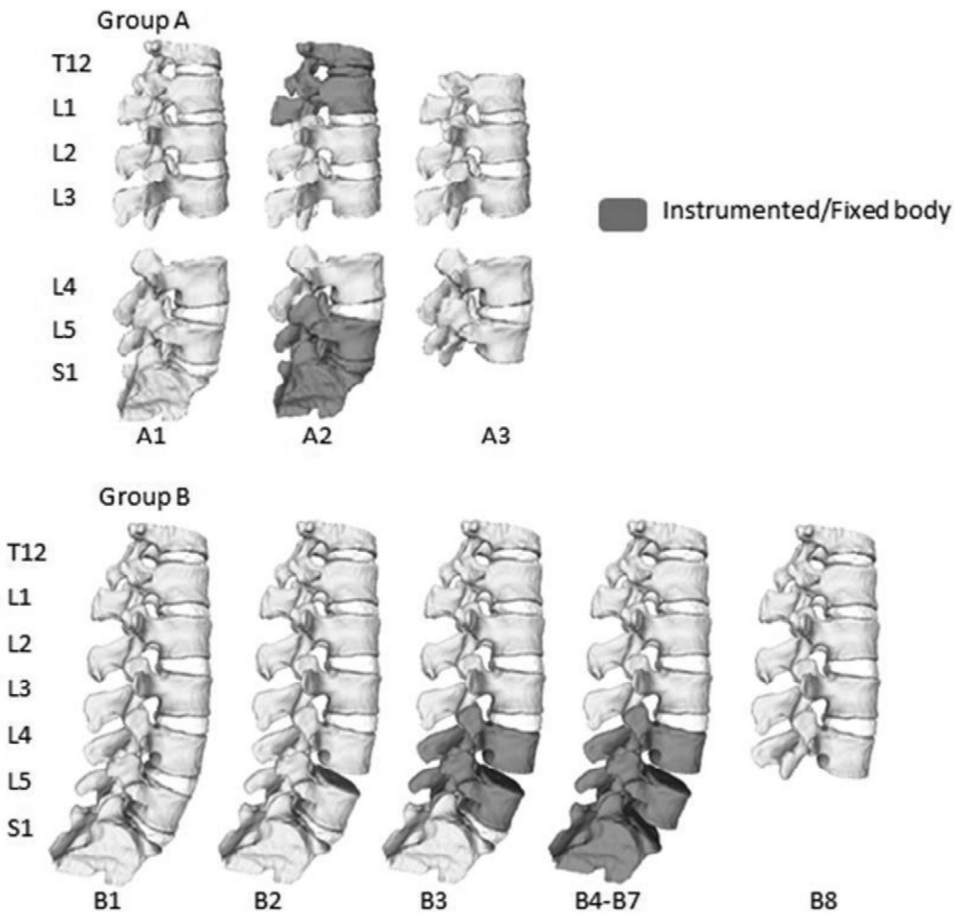

Fig. 1. Diagram depicting all constructs used for experiments, with shaded vertebra indicating an instrumented or fixed body. (A) In group A 3 constructs were used to test the pure moment assumption. (B) In group B 8 constructs were used to test for degradation due to testing order and change in orientation.

tion) pneumatically actuated test frame (Smart Test Series; Bose, Eden Prairie, Maryland). Pure moments were maintained by actively controlling counteracting motors to apply equal and opposing moments throughout testing as described by Kunz et al.[7] During each mode of loading, all off-axis actuators were fully unconstrained by commanding them to zero load or torque.

Specimens were subjected to a load control protocol in which 3 cycles of flexion-extension, lateral bending, and axial torsion moments with sinusoidal load profiles of frequency 0.005 Hz and amplitude $\pm$ 7.5 Nm were applied to the spine by the corresponding motors.

The kinematic response of each vertebral body was monitored with an Optotrak Certus motion capture system (Northern Digital Instruments, Waterloo, Ontario, Canada) with a manufacturer-stated accuracy of 0.1 mm to track the location of each pre-characterized tracking body with active light-emitting diode complex. Intervertebral ROM was calculated for each FSU tested. All values reported herein correspond to the intervertebral ROM for FSUs not destabilized or otherwise augmented with spinal implants or fixated with wood screws.

A total of 3 different constructs were used for group A specimens, depicted in Fig. 1A:

1. Construct A1—intact T12-L3 and L4-S1 segments
2. Construct A2—T12-L3 and L4-S1 segments with 2 3-inch wood screws driven into the specimen to fixate T12-L1 and L5-S1, respectively
3. Construct A3—intact L1-3 and L4-5 segments.

A total of 8 different tests were run on group B specimens in 6 different constructs, depicted in Fig. 1B:

1. Construct B1—intact T12-S1 segment
2. Construct B2—T12-S1 segment, destabilized at L4-5
3. Construct B3—T12-S1 segment, instrumented at a single level with motion preservation device at L4-5
4. Construct B4—T12-S1 segment, instrumented at 2 levels with motion preservation device at L4-5 and L5-S1
5. Construct B5—T12-S1 segment, instrumented with motion preservation device at L4-5 and rigid rods at L5-S1
6. Construct B6—retest of construct B4
7. Construct B7—retest of construct B4

8. Construct B8—T12-L4 segment with no instrumentation.

Analysis of kinematic data

The kinematic data acquired during each test were collected, and the third cycle of each of the tests was analyzed by use of custom written programs in a technical computing environment (MATLAB; The MathWorks, Natick, Massachusetts). The ROM for flexion-extension, lateral bending, and axial torsion was calculated as the range of Euler angles corresponding to each mode of loading.

Part A of this study consisted of the analysis of the data for the individual FSUs of L1-2, L2-3, and L4-5, none of which were fused during experimentation. For the analysis of group A data, the ROM of each analyzed FSU was determined, and statistical analysis was conducted to explore differences between testing conditions irrespective of intervertebral level. For group B data, only L1-2 and L2-3 were analyzed for change in ROM and compared with sequential constructs. Both analyzed FSUs from part B were not fused during experimentation.

Statistical methods

A 1-way repeated-measure analysis of variance test with Bonferroni correction was used to compare ROM for all constructs. A planned polynomial contrast was conducted for each specific mode of loading to detect any trends with respect to testing order for the same mode of loading. Group A consisted of a sample size of 13, and group B consisted of a sample size of 6. For all conditions, a significance level of $P = .05$ was used.

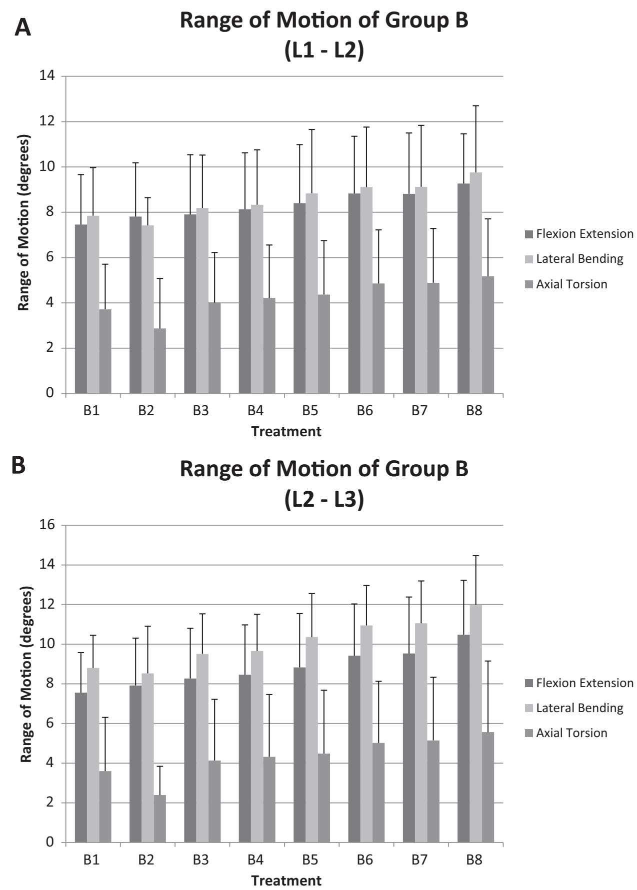

Fig. 3. ROM in degrees for group B in 3 modes of loading—flexion-extension, lateral bending, and axial torsion—at (A) L1-L2 and (B) L2-L3. Group B testing was performed to observe degradation of specimens over 8 treatments and change in orientation of specimens.

Results

The following results were obtained from the analysis of group A specimens, specifically the L1-2, L2-3, and L4-5 FSUs. The mean ROM of all FSUs for each tested condition is shown in Fig. 2. The flexion-extension, lateral bending, and torsion ROM of the specimens gradually increased between sequential construct tests (Fig. 2). Comparison between constructs A1 and A2 in flexion-extension did not show a significant difference in ROM but was trending toward one ($P = .055$). In lateral bending, a significant difference was found between constructs A1 and A3 and between constructs A2 and A3 ($P = .012$ and $P = .009$, respectively). Furthermore, a significant difference was found in torsion between constructs A1 and A3 and between constructs A2 and A3 ($P = .014$ and $P = .018$, respectively). By use of a planned polynomial contrast, a linear trend across testing order was observed to be significant in flexion-extension, lateral bending, and torsion ($P = .024$, $P = .004$, and $P = .005$, respectively).

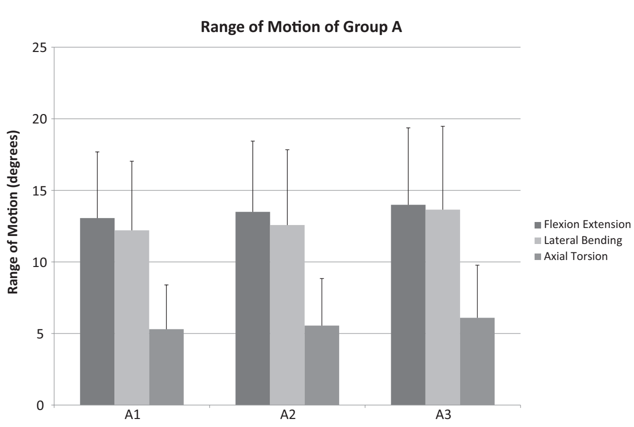

Fig. 2. ROM in degrees for group A, testing the pure moment assumption, in 3 modes of loading: flexion-extension, lateral bending, and axial torsion.

The following results were obtained from analysis of group B specimens, specifically the L1-2 and L2-3 FSUs. The ROM compared in each mode of loading is shown in Fig. 3. An increase in ROM over time for flexion-extension, lateral bending, and axial torsion was observed for the L1-2 and L2-3 FSUs. The significant differences comparing all 8 constructs for untreated levels are shown in Table 1. By use of a planned polynomial contrast, a linear trend was observed to be significant over the course of the 8 constructs

Table 1
Calculated P values from group B testing, indicating increase in ROM over 8 treatments

	B1		B2		B3		B4		B5		B6		B7		B8	
Treatment	L1-2	L2-3	L1-2	L2-3	L1-2	L2-3	L1-2	L2-3	L1-2	L2-3	L1-2	L2-3	L1-2	L2-3	L1-2	L2-3
Flexion-extension																
B1			>.999	>.999	>.999	>.999	.275	>.999	.144	.898	.006*	.211	.039*	.369	<.001*	.135
B2	>.999	>.999			>.999	>.999	>.999	.437	.178	.284	.035*	.037*	.217	.088	.005*	.056
B3	>.999	>.999	>.999	>.999			>.999	>.999	.025*	>.999	.017*	.044*	.037*	.066	.074	.047*
B4	.275	>.999	>.999	.437	>.999	>.999			>.999	>.999	.011*	.012*	.207	.068	.041*	.041*
B5	.144	.898	.178	.284	.025*	>.999	>.999	>.999			.791	.013*	>.999	.039*	.413	.03*
B6	.006*	.221	.035*	.037*	.017*	.044*	.011*	.012*	.791	.13			>.999	>.999	>.999	.113
B7	.039*	.369	.217	.088	.037*	.066	.207	.068	>.999	.039*	>.999	>.999			>.999	.189
B8	<.001*	.135	.005*	.056	.074	.047*	.041*	.041*	.413	.03*	>.999	.113	>.999	.189		
Lateral bending																
B1			>.999	>.999	>.999	>.999	>.999	>.999	.742	.727	.134	.329	.169	.289	.094	.162
B2	>.999	>.999			>.999	>.999	>.999	>.999	>.999	>.999	>.999	>.999	>.999	>.999	>.999	.608
B3	>.999	>.999	>.999	>.999			>.999	>.999	.667	.134	.049*	.006*	.114	.007*	.08	.014*
B4	>.999	>.999	>.999	>.999	>.999	>.999			.808	.322	.013*	.036*	.049*	.027*	.06	.033*
B5	.742	.727	>.999	>.999	.667	.134	.808	.322			.809	.72	.864	.086	.061	.013*
B6	.134	.329	>.999	>.999	.049*	.006*	.013*	.036*	.809	.72			>.999	>.999	.562	.215
B7	.169	.289	>.999	>.999	.114	.007*	.049*	.027*	.864	.086	>.999	>.999			.573	.095
B8	.094	.162	>.999	.608	.08	.014*	.06	.033*	.061	.013*	.562	.215	.573	.095		
Axial torsion																
B1			>.999	>.999	>.999	>.999	.57	.791	.348	.687	.059	.1111	.059	.126	.08	.391
B2	>.999	>.999			>.999	>.999	>.999	>.999	>.999	>.999	>.999	.006*	>.999	>.999	>.999	>.999
B3	>.999	>.999	>.999	>.999			.703	>.999	.424	.239	.115	.07	.102	.011*	.1	.227
B4	.57	.791	>.999	>.999	.703	>.999			.432	>.999	.206	.021*	.15	.06	.106	.311
B5	.348	.687	>.999	>.999	.424	.239	.432	>.999			.516		.386	.012*	.125	.255
B6	.059	.111	>.999	>.999	.115	.006*	.206	.07	.516	.021*		.535	>.999	.535	.5	>.999
B7	.059	.126	>.999	>.999	.102	.011*	.15	.06	.386	.012*	>.999				.964	>.999
B8	.08	.391	>.999	>.999	.1	.227	.106	.311	.125	.255	.5	>.999	.964	>.999		

* Significant difference ($P < .05$).

in L1-2, with $P < .001$ for flexion-extension, $P = .015$ for lateral bending, and $P = .014$ for axial torsion. The same trend of an increased ROM was observed in L2-3, with $P = .003$ for flexion-extension, $P = .004$ for lateral bending, and $P = .037$ for axial torsion.

Discussion

This study investigated whether a pure moment applied to a spinal construct during in vitro biomechanical testing resulted in uniform loading along the spine using a custom-built multi–degree of freedom machine. Verification of this method has been confirmed, and specific experimental variables including the nonuniform structure of the spine, the orientation of the spine outside the plane of loading, and the presence of nominal shear loads are important factors that may result in potential deviations from the ideal conditions inherent to the pure moment assumption. From analysis of group A data, changes in the rigidity of the specimens without changing specimen length (eg, construct A1 to A2) did not significantly affect ROM in all 3 modes of loading, indicating the absence of observable adjacent-level effects consistent with tenets of the pure moment protocol.

Significant differences between the differing length constructs (eg, constructs A1 and A3 and constructs A2 and A3) were not observed in flexion-extension but were observed in lateral bending and axial torsion. A significant difference was observed between both constructs A1 and A3 and constructs A2 and A3, with an increase in ROM for both comparisons in both modes of loading. In addition, for all 3 modes of loading, an increasing linear trend was observed to be significant for all constructs, with all P values less than .025. This statistically significant increase in ROM could be a result of the order in which each construct was tested and thus can be attributed to degradation of the specimen or other unquantifiable factors. Possible factors that could cause degradation include the extent of mechanical testing and dehydration of the specimen.

Furthermore, the increased ROM may be due to the change in the orientation of the spine after repotting, given that the change was biased toward a reduction in segmental lordosis. This could result in a reduction in the rotational coupling during these modes of loading, leading to an increase in ROM. However, the linear trend observed from group A only consisted of 3 data points, and further analysis of group B data was used to confirm the hypothesis that testing order contributed significantly to the increased ROM. In addition, because of the reuse of group A specimens, it was necessary to analyze group B data to appropriately determine the effects of extensive mechanical testing.

From analysis of group B data, the increasing trend was also observed to be significant in the ROM across 8 tests for both L1-2 and L2-3 FSUs. This provided more evidence in favor of the conclusion drawn from group A that the change of ROM was due to testing order of the constructs. In addition, significant differences were not found in sequential constructs (eg, constructs B1 and B2 and constructs B3 and B4), but differences were observed in constructs separated by multiple tests (eg, constructs B1 and B7 and constructs B2 and B6). These differences could be due to tissue degradation of the specimen, caused by the dehydration of the tissue or the amount of mechanical testing to which each specimen was subjected. From the data, there were no significant differences between constructs B7 and B8 attributable to potting orientation, supporting the conclusion that potting orientation was not a significant factor causing the increased ROM found from group A.

The main limitations of this study are related to the specific conditions of the specimen throughout the testing process. The amount of time each specimen was stored at room temperature and in the refrigerator (4°C) throughout the testing process was not quantified. Future experiments could monitor and standardize these conditions of each specimen throughout the testing process. The exact number of freeze-thaw cycles for each specimen is also unknown. This is because specimens were refrozen if consecutive constructs occurred over a weekend but were refrigerated if the consecutive constructs occurred on consecutive weekdays. However, multiple studies have shown that the number of freeze-thaw cycles and length of time frozen does not affect the mechanical properties of cadaveric muscles,[8] bones,[9,10] intervertebral discs,[11] and the entire lumbar spine.[12,13] The orientation of each vertebra relative to the loading mechanism was not monitored during experimentation. Future experiments could use this information to monitor the changes in vertebral orientation and correct accordingly. Finally, randomizing the testing order of different constructs should be performed in future studies when possible. This would ensure that the observed increases in ROM are not due to the amount of testing and would allow for effects due to the testing order to be observed.

After experimentation, it was concluded that there was a lack of evidence to show that the assumptions of pure moment testing are inappropriate for the presented testing protocol on a custom-built multi–degree of freedom testing machine. The observed increase in ROM of the specimen was likely due to degradation of the specimen from extensive mechanical testing and the specific conditions of each specimen. In addition, potting orientation was not likely a factor in the increased ROM but should be monitored in future experiments. Further experimentation could also investigate the specific causes of the increase in ROM over time. The subsequent testing would require a randomized testing order to ensure that degradation due to mechanical testing does not affect the results. The results of this study vindicate the assertion that the standard pure moment testing protocol is inappropriate for investigating adjacent-level effects resulting from the implantation of spinal stabilization devices. Future studies into such effects should include modifications to the protocol, such as the hybrid technique proposed by Panjabi et al.[14] In conclusion, the pure moment assumption is an important simplifying tool used in most spine biomechanical laboratories that was not invalidated, and it should continue to be used under appropriately controlled conditions.

Acknowledgments

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