

# Limitations of current in vitro test protocols for investigation of instrumented adjacent segment biomechanics: critical analysis of the literature

David Volkheimer<sup>1</sup> · Masoud Malakoutian<sup>2</sup> · Thomas R. Oxland<sup>2,3</sup> · Hans-Joachim Wilke<sup>1</sup>

Received: 24 September 2014/Revised: 17 May 2015/Accepted: 18 May 2015/Published online: 3 June 2015  
© Springer-Verlag Berlin Heidelberg 2015

## Abstract

**Purpose** Accelerated degenerative changes at intervertebral levels adjacent to a spinal fusion, the so-called adjacent segment degeneration (ASD), have been reported in many clinical studies. Even though the pathogenesis of ASD is still widely unknown, biomechanical in vitro approaches have often been used to investigate the impact of spinal instrumentation on the adjacent segments. The goal of this review is (1) to summarize the results of these studies with respect to the applied protocol and loads and (2) to discuss if the assumptions made for the different protocols match the patients' postoperative situation.

**Methods** A systematic MEDLINE search was performed using the keywords “adjacent”, “in vitro” and “spine” in combination. This revealed a total of 247 articles of which 33 met the inclusion criteria. In addition, a mechanical model was developed to evaluate the effects of the current in vitro biomechanical test protocols on the changes in the adjacent segments resulting from different stiffnesses of the “treated” segment.

**Results** The surgical treatments reported in biomechanical in vitro studies investigating ASD can be categorized into fusion procedures, total disc replacement (TDR), and dynamic implants. Three different test protocols (i.e. flexibility, stiffness, hybrid) with different loading scenarios (e.g. pure moment or eccentric load) are used in current biomechanical in vitro studies investigating ASD. According to the findings with the mechanical model, we found that the results for fusion procedures highly depend on the test protocol and method of load application, whereas for TDR and dynamic implants, most studies did not find significant changes in the adjacent segments, independent of which test protocol was used.

**Conclusions** The three test protocols mainly differ in the assumption on the postoperative motion behavior of the patients, which is the main reason for the conflicting findings. However, the protocols have never been validated using in vivo kinematic data. In a parallel review on in vivo kinematics by Malakoutian et al., it was found that the assumption that the patients move exactly the same after fusion implemented with the stiffness- and hybrid protocol does not match the patients' behavior. They showed that the motion of the whole lumbar spine rather tends to decrease in most studies, which could be predicted by the flexibility protocol. However, when the flexibility protocol is used with the “gold standard” pure moment, the difference in the kinematic changes between the cranial and caudal adjacent segment cannot be reproduced, putting the validity of current in vitro protocols into question.

---

**Electronic supplementary material** The online version of this article (doi:10.1007/s00586-015-4040-9) contains supplementary material, which is available to authorized users.

---

✉ Hans-Joachim Wilke  
hans-joachim.wilke@uni-ulm.de

<sup>1</sup> Institute of Orthopaedic Research and Biomechanics, Centre of Musculoskeletal Research, Ulm University, Helmholtzstrasse 14, 89081 Ulm, Germany

<sup>2</sup> Department of Orthopaedics, University of British Columbia, Vancouver, Canada

<sup>3</sup> Department of Mechanical Engineering, University of British Columbia, Vancouver, Canada

**Keywords** In vitro experiments · Kinematics · Range of motion · Adjacent segment degeneration · Biomechanics

## Introduction

Accelerated degenerative changes at intervertebral levels adjacent to a spinal fusion, the so-called adjacent segment degeneration (ASD), have been reported in clinical studies with a steadily increasing rate [1, 2]. Radiographic changes in the lumbosacral spine at segments cranial and caudal to a previous fusion span a broad range from 11 to 100 %, whereas clinical complaints appeared in only 0–28 % [1].

Controversy exists about the actual rate of ASD and the assumption of a fusion-related disease [1–3]. Although several risk factors have been identified, the pathogenesis of ASD is still widely unknown. However, it is often anticipated that the development of ASD is related to an adaptive hypermobility in segments adjacent to an instrumented fusion.

To scrutinize this hypothesis, *in vivo* motion of the patients' spine has been primarily evaluated using static radiography and videofluoroscopy. For fusion procedures, it was shown in a parallel review article by Malakoutian et al. that the cranial adjacent segment can be susceptible to the development of hypermobility in some cases, whereas at the inferior segment, either no changes were found or the motion decreased in the sagittal plane [4]. For total disc replacement (TDR) and other flexible implants, compensatory alterations in adjacent segments were seen rarely, apparently corroborating the protective potential of these devices.

To investigate implant-related biomechanical changes in the adjacent segments, *in vitro* studies have primarily focused on motion parameters such as range of motion (ROM) and neutral zone (NZ). Additionally, the load sharing between the anterior and posterior column of the spine, characterized by absolute facet loads as well as intradiscal pressure (IDP) and lamina strains, has been investigated after varying surgical treatments. However, conflicting results were found for the different parameters.

*In vivo* animal studies offer the opportunity to perform arthrodeses in spines unaffected from a pre-existing degeneration, thereby allowing one to discriminate between changes due to fusion or naturally progressing degeneration. Canine studies revealed that the susceptibility of neighboring intervertebral discs to adverse cellular and metabolic stimuli strongly depends on the genetics of the respective breed [5, 6]. In a rabbit model, changes in the structure of the annulus fibrosus at adjacent segments were noted already 3 months after surgical immobilization [8], whilst in a goat model, no signs of degeneration were found after 6 months [7]. These conflicting results highlight the limited transferability of these data to human, due to deviations in anatomy [9], biology [10] and loading condition [11] in animal models.

The goal of this review is to discuss the limitations of the current *in vitro* biomechanical test protocols developed for investigation of the adjacent segment degeneration due to spinal instrumentation. To support the reader with a basic knowledge of the theoretical background, an overview of experimental designs is given. The characteristics of different experimental protocols are demonstrated by means of a mechanical model. Thereafter, literature of *in vitro* biomechanical studies on adjacent segment effects is summarized. The studies are critically analyzed with respect to the experimental set-ups, applied loads, and underlying biomechanical principles. Finally, the validity of the assumptions made for the different protocols is discussed.

## Materials and methods

A MEDLINE search was performed using the keywords “adjacent”, “*in vitro*”, and “spine” in combination. This revealed a total of 247 articles. The terms were chosen generically to avoid restriction of the search screen. Each article was screened by title, abstract or, if necessary, full text. Only original articles statistically comparing *in vitro* effects of varying surgical treatments on neighboring levels in human lumbar spinal specimens were included. Values needed to be provided as absolute data, i.e. not normalized to the motion of the whole specimen. Furthermore, a statistical comparison to the intact condition was required. Twenty-five articles fulfilled these criteria. A review of the references revealed eight additional studies, leading to a total of 33 studies.

## Basic concepts

The term “adjacent level effects” (ALE) defines all effects of a surgical treatment on adjacent intervertebral segments, immediately neighboring or more distant to the treatment site (modified from Panjabi 2007 [12]). The observed effects involve changes in kinematics and/or alterations in load transfer in the discs and the facet joints.

## Testing protocols

To reveal ALEs *in vitro*, diverse test protocols have been used that differ mainly in the way the movement of a specimen is introduced or controlled. To objectively evaluate the literature, a basic knowledge of the different methods is crucial. Hence, the three approaches adopted for quasistatic testing are presented in the following paragraphs:

1. Flexibility protocol
2. Stiffness protocol
3. Hybrid protocol

With the flexibility protocol, a predefined load is applied to the free end of spinal specimens, while the other end is rigidly fixed [13]. The resulting displacements are recorded. The load applied to the intact specimen is maintained in all subsequent testing conditions.

With the stiffness protocol, a predefined displacement is applied to the free end of the specimen, while the other end is rigidly fixed [13]. The resulting loads (i.e. forces and moments) and intervertebral motions are measured. The displacement in the primary motion direction is maintained in all testing conditions.

The hybrid protocol represents a combination of the flexibility and stiffness protocols and was exclusively designed to investigate ALEs in vitro. Published in 2007 by Panjabi [12], the hybrid protocol gained widespread popularity. As reference, the total ROM of the intact specimen is measured under application of appropriate pure moments. Subsequently, the specimen is surgically treated and the construct is moved under moment control until the ROM of the intact specimen is reached. Intervertebral specimen kinematics and/or loads may be compared as outcome variables.

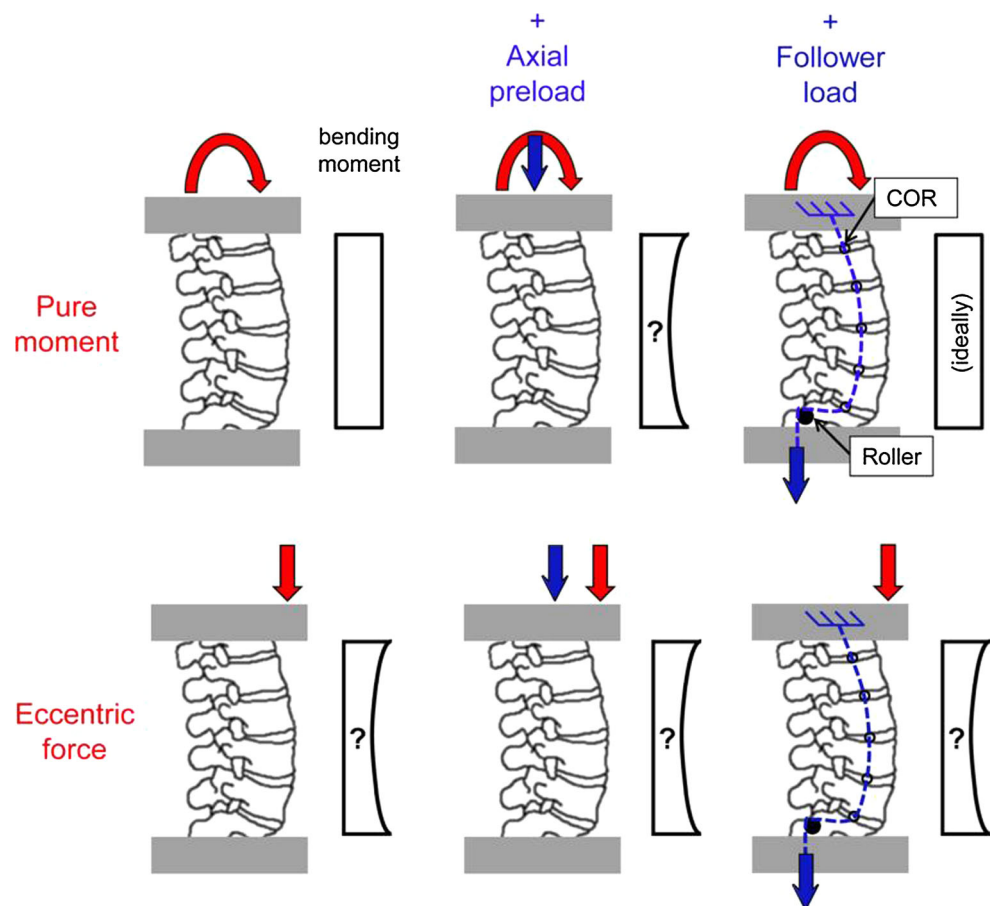
## Loading conditions

Secondary to the test protocol, the choice of appropriate loads or displacements plays an essential role for the extent of ALEs, as well as the reproducibility of data. The primary loads or displacements are used to move the specimen, whereas additional compressive loads (axial preload or follower load [14]) that are intended to simulate physiologic compression may modify this motion.

Today, the application of pure moments is accepted as the ‘gold standard’ [13, 15]. When pure moments are applied, the loading along the length of the specimen is always of constant magnitude (i.e. the moment applied to the segment of interest is known) (Fig. 1). This ensures high reproducibility and comparability of data.

Bending of a specimen can also be induced by an eccentric compressive force. The resulting moment can be calculated by multiplying the lever arm (i.e. distance of the compressive force to the center of rotation (COR) of each intervertebral segment) by the compressive force. As long as the specimen is straight, the lever arm at each cross section is of the same length, evoking the same moment at

**Fig. 1** The illustration shows the distribution of bending moment along the specimen, dependent on the manner of load application. In the two lines, the effect of the two principal conceptions of primary load application (pure moment and eccentric force) on the resulting bending moment is demonstrated. These loads are applied to move the spine. In the columns, the effect of an additional preload or ideal follower load on the bending moment is demonstrated. The first line represents the pure load cases. These compressive secondary loads are considered to achieve a more physiologic loading condition of the specimen. The cable of the follower load is rigidly fixed at the uppermost vertebra and ideally passes through the center of rotation (COR) of each vertebra (concept adapted from Panjabi, 1988 [13])



each spinal segment. Flexing or tilting the specimen changes the lever arm at each segment, resulting in altered, variable moments. Furthermore, it is known that the COR migrates when the specimen is moving [16]. In this case, the determination of the resulting loading along the length of the specimen becomes even more complex (Fig. 1).

Infrequently, a shear force perpendicular to the cranial vertebra was used to move the specimen [17, 18]. Due to the uncommonness of this approach in recent years, it is not taken into further consideration.

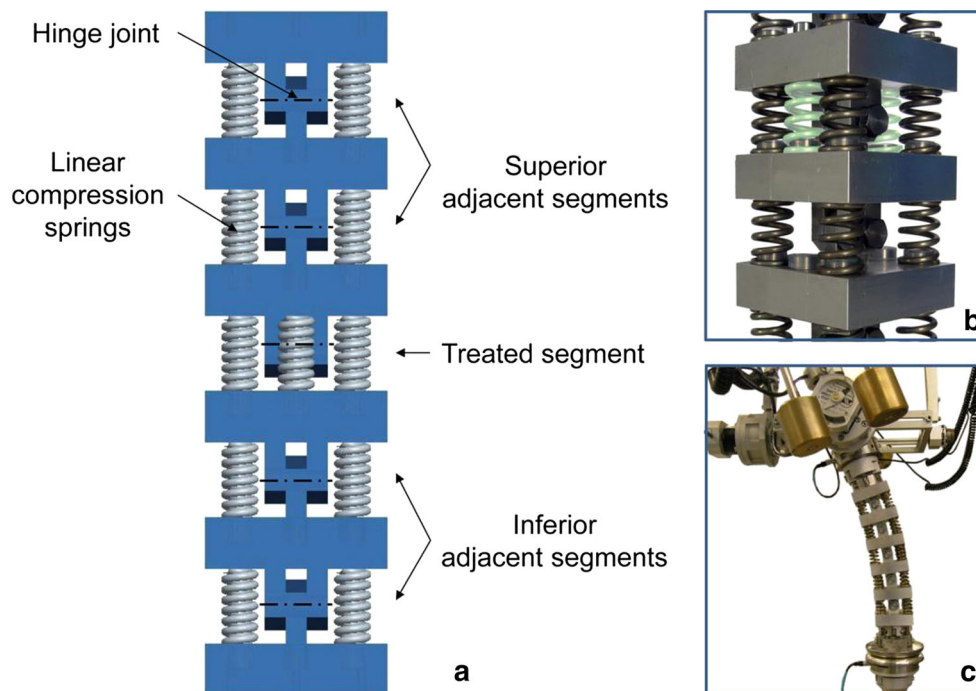
An additional axial preload can be used to simulate physiologic compression of the specimen. As long as the axial preload passes through the COR of each vertebral level, no additional bending moment is produced. However, this criterion can only be fulfilled when the specimen is straight. For this reason, longer specimens with natural kyphosis or lordosis start to bend or buckle at relatively low axial preloads, thereby complicating this testing approach [19, 20].

The so-called “follower load”, suggested by Patwardhan et al. [14], is used to achieve a more physiologic compression of the specimen compared to a simple axial preload. In contrast to an axial preload, the path of the follower load is ideally guided through the COR of each motion segment, leading to a pure compression in each segment without

undesired superimposed load components (Fig. 1). This method increases the load-carrying capacity of the specimen.

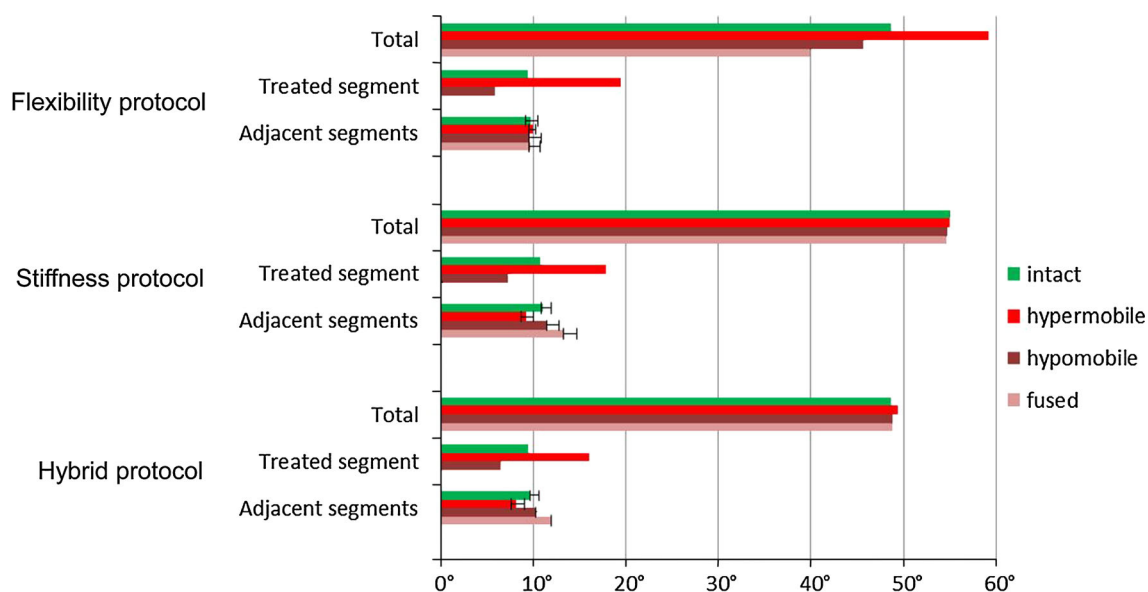
### Influence of the testing protocols on the kinematics of adjacent segments—a mechanical model

To demonstrate the general characteristics of the basic quasi-static testing protocols discussed in this review article in a simplified manner, a mechanical model with five linkages of the same stiffness at each segment was developed (Fig. 2). To keep the model easily comprehensible, it only accounts for motion in one principal plane and does not mimic the three-dimensional and coupled motion of the spine. Four defined stiffnesses of the “treated” level were simulated by varying the number of linear compression springs at each side of the segment (i.e. one for hypermobile, two for intact, or three for hypomobile). This ensured a symmetric behavior of the model. Fusion was achieved by screwing two rigid plates to the front and back of the “treated” segment. The main advantage of the model is that defined stiffness properties can be simulated without influencing the stiffness of the adjacent segments, enabling one to compare different loading protocols in a standardized manner. The model was consecutively subjected to the flexibility, stiffness, and hybrid protocols



**Fig. 2** a CAD-model (Pro/ENGINEER Wildfire 4.0, PTC, Needham, USA) of the mechanical model for evaluation of the three flexibility-, stiffness-, and hybrid-testing protocols. Solid bodies are connected by hinge joints. The stiffness in each segment can be adjusted by varying the number of linear compression springs or by rigidly screwing a plate to the front and back. In the “treated” segment, the stiffness was changed by varying the number of springs to mimic intact (two

springs at each side), hypermobile (one spring at each side), hypomobile (3 springs at each side), and fused conditions (rigidly screwed). In the picture, the hypomobile condition is shown. **b** Detailed view of the model. The springs in *light green* show how the stiffness of the treated segment was adjusted to simulate the different conditions. In this case, the hypomobile case is shown. **c** Model in maximal deflection in the universal spine tester [50]



**Fig. 3** ROM results for the three flexibility-, stiffness-, and hybrid-testing protocols. A pure moment of  $\pm 7.5$  Nm was used for the flexibility protocol, as well as for the intact condition of the hybrid protocol. For the stiffness protocol, a total ROM of  $55^\circ$  was targeted. *Green bars* represent the intact condition; all *red bars* represent the

results when the stiffness of the treated segments was altered. The ROM of all four adjacent segments (immediately neighboring and one segment more distant) was almost identical and is therefore expressed as median value. The *error bars* represent the maximal and minimal values

under the application of pure moments. Three retroreflective markers were mounted on each “vertebral body” of the model. Motion was captured using 6 infrared cameras (Vicon MX13, Vicon, Oxford, UK) with a resolution of  $1280 \times 1024$  pixel. The resulting intersegmental and total ROM was evaluated for each test protocol.

## Results

In the following section, the results of the mechanical spine model are presented first because they form an important basis to understand the results of the published in vitro studies. Subsequently, the findings of the literature review are shown and compared to the findings of the mechanical model.

### Influence of the testing protocols on the kinematics of adjacent segments—a mechanical model

Using the flexibility protocol, the mechanical model clearly demonstrated that the stiffness of the “treated” segment does not have an effect on the ROM of the adjacent segments (Fig. 3). The total ROM hence was reduced by the amount of motion restriction of the “treated” segment.

With the stiffness protocol, the total ROM did not change, which is in accordance with the definition of this protocol. Therefore, the increase or decrease in ROM at the “treated” level was equally redistributed among the four

adjacent levels (Fig. 3). The required applied moment therefore had to decrease or increase proportionally.

For the hybrid protocol, the segmental motions were, as expected, again equally distributed the same way as for the stiffness protocol (Fig. 3). The only difference is that for the stiffness protocol the target ROM was arbitrarily defined, whereas for the hybrid protocol, it is determined by the individual total ROM of the intact specimen.

### Review of in vitro papers

The surgical treatments reported in biomechanical in vitro studies investigating ALEs can be categorized into fusion procedures (Table 1), TDR (Table 2), and dynamic implants (Table 3).

The parameters most frequently investigated were ROM and IDP, whereas parameters like neutral and elastic zone (NZ and EZ), center of rotation (COR), strains in bony structures, loading and motion pattern of the facet joints, as well as applied load (in case of the hybrid and stiffness protocol) were analyzed rarely.

### Fusion procedures

When the flexibility protocol was used with pure moments, most studies did not find a consistent significant impact of the fusion procedure on the adjacent segments, independent of which fusion technique was used [21–27]. This is in

**Table 1** Short summary of the gross ALEs found in biomechanical articles after spinal fusion (for more detailed depiction see electronic supplementary)

Test protocol	Primary load	Secondary load	Parameter	ALEs			
				Decrease	Unchanged	Increase	No tendency
Flexibility	Pure moment	–	ROM	5 [21–24, 26, 27]	1 [47]	1 [46]	
			EZ	1 [22]			
			NZ	2 [22, 23]			2 [27, 46]
			IDP	1 [25]			
			w/axial preload	1 [28]			
	Eccentric force	–	ROM	1 [30]			1 [29]
			IDP	1 [30]			
			Lamina strain			1 [30]	
			w/axial preload	–			
			w/follower load	–			
Stiffness	Pure moment	–	–				
			w/axial preload	–			
			w/follower load	–			
	Eccentric force	–	ROM				1 [32]
			IDP	1 [33]			2 [31, 32]
			w/axial preload	–			
Hybrid	Pure moment	–	ROM			7 [34–38, 50, 51]	1 [39]
			IDP			1 [39]	
			Applied moment			1 [37]	
			w/follower load	ROM	1 [34]	3 [34, 35, 38]	

The table summarizes the effects of different fusion procedures on the adjacent segments. The effects for one parameter of one single study are summarized to represent tendencies. When no clear tendency was found, the studies were assigned to the category “No tendency”. In the first column, the three different protocols are discriminated. The flexibility- and stiffness protocol can be used with different loads, whereas the hybrid protocol is defined to be solely used with pure moments, eventually supplemented by a “follower load”. For this reason, the hybrid protocol is only shown with these two options. Primary loads move the specimen, whereas secondary loads may modify this motion. The category “Parameter” includes all effects in the adjacent levels, which were investigated for the respective protocol and load combination. Rows with no parameter show that no study included in this review used this combination

ALEs adjacent level effects, ROM range of motion, EZ elastic zone, NZ neutral zone, IDP intradiscal pressure

accordance with the results of our mechanical model (Fig. 3).

In one study included in this article, the pure moment was combined with an axial preload. Similarly to most studies applying pure moments, Cheng et al. [28] did not find significant changes in ROM at the first cranial adjacent segment after single-level posterior fusion with and without BAK cage.

When the flexibility protocol was used in combination with an eccentric force, the results were less homogeneous than with pure moments. Akamaru et al. [29] investigated the effect of implant alignment in a 360° construct. For the hypolordotic and neutral alignment, a significant increase in ROM at the cranial level was found, whereas the first caudal segment did not show significant alterations. An opposite trend with an unchanged ROM at the cranial segment and a significant increase at the caudal level was

seen for the hyperlordotic instrumentation. Contrarily, Umehara et al. [30] did not find significant changes in ROM and IDP caused by a neutral or hypolordotic instrumentation, while the lamina strain was significantly increased.

All studies using the stiffness protocol included in this review applied an eccentric force to move the specimen. In the majority of studies investigating IDP or ROM, either an increase or unchanged results were found [31–33].

With the hybrid protocol using long specimens, Panjabi et al. [34–36] predominantly found significant increases and in few cases no changes in ROM in several adjacent segments above and below a posterior fusion. Using short specimens, Molz et al. [37] and Strube et al. [38] showed that the applied load had to be increased significantly to reproduce the ROM of the intact specimen after posterior fixation.

**Table 2** Short summary of the gross ALEs found in biomechanical articles after TDR (for more detailed depiction see electronic supplementary)

Test protocol	Primary load	Secondary load	Parameter	ALEs			
				Decrease	Unchanged	Increase	No tendency
Flexibility	Pure moment	–	–				
		w/axial preload	–				
		w/follower load	–				
	Eccentric force	–	–				
		w/axial preload	–				
		w/follower load	–				
Stiffness	Pure moment	–	–				
		w/axial preload	–				
		w/follower load	–				
	Eccentric force	–	–				
		w/axial preload	–				
		w/follower load	–				
Hybrid	Pure moment	–	ROM		3 [34, 35, 39]		1 [40]
			IDP		1 [39]		1 [40]
			COR		1 [40]		
		w/follower load	ROM		2 [34, 35]		

The table summarizes the effects of TDR on the adjacent segments. The effects for one parameter of one single study are summarized to represent tendencies. When no clear tendency was found, the studies were assigned to the category “No tendency”. In the first column, the three different protocols are discriminated. The flexibility- and stiffness protocol can be used with different loads, whereas the hybrid protocol is defined to be solely used with pure moments, eventually supplemented by a “follower load”. For this reason, the hybrid protocol is only shown with these two options. Primary loads move the specimen, whereas secondary loads may modify this motion. The category “Parameter” includes all effects in the adjacent levels, which were investigated for the respective protocol and load combination. Rows with no parameter show that no study included in this review used this combination

ALEs adjacent level effects, ROM range of motion, EZ elastic zone, NZ neutral zone; IDP intradiscal pressure

### Total disc replacement

Studies investigating ALEs after TDR are rare compared to fusion procedures. All studies meeting the inclusion criteria found that the absolute ROM [34, 35, 39, 40], NZ [40] and IDP [39] of the adjacent segments was unchanged. Furthermore, in contrast to the fusion procedure, TDR preserved the location of the COR at the first cranial segment [40].

### Dynamic implants

The studies investigating ALEs with dynamic implants can be divided into two subgroups: 8 studies investigated the effect of pedicle-screw-based systems such as Dynesys® or StabilimaxNZ®, 5 studies investigated interspinous distraction devices (ISDD) such as X-Stop® spacer, Coflex®, and Aspen ISA. Comparable to TDR, the majority of these studies did not find a general impact on absolute adjacent levels’ ROM [23, 26, 28, 36, 41, 42], NZ [23], IDP [25, 43], facet pressure [44], and facet force [44].

### Discussion

Testing the effect of surgical treatments in vitro offers the opportunity to investigate characteristics of implants in conjunction with its natural mechanical environment. However, for investigation of ASD, no conclusive consensus exists regarding test protocol and load application.

### Explanations for contradictory results of in vitro biomechanical studies

This review on in vitro biomechanical studies investigating ALEs revealed a wide variety of methodological approaches. Principally, based on the differing assumptions on the patients’ postoperative behavior embedded in the protocols, discrepancies in ALEs between these protocols can be expected. However, the review showed that even when the same protocol and load application was used, the results varied in some cases. These differences might be explained by the following issues:

**Table 3** Short summary of the gross ALEs found in biomechanical articles after dynamic instrumentation (for more detailed depiction see electronic supplementary)

Test protocol	Primary load	Secondary load	Parameter	ALEs				
				Decrease	Unchanged	Increase	No tendency	
Flexibility	Pure moment	–	ROM	4 [21, 23, 26, 41]	1 [47]			
			NZ					
			IDP					
	Eccentric force	–	w/axial preload	ROM	1 [42]			
				IDP	1 [43]			
			w/follower load	ROM			1 [47]	
Stiffness	Pure moment	–						
			w/axial preload					
			w/follower load					
	Eccentric force	–						
			w/axial preload					
			w/follower load					
Hybrid	Pure moment	–	ROM	1 [36]		1 [38]		
			Applied moment			1 [38]		
			ROM	1 [36]		2 [38, 51]		
			w/follower load	Applied moment		1 [38]		

The table summarizes the effects of dynamic instrumentation on the adjacent segments. The effects for one parameter of one single study are summarized to represent tendencies. When no clear tendency was found, the studies were assigned to the category “No tendency”. In the first column, the three different protocols are discriminated. The flexibility- and stiffness protocol can be used with different loads, whereas the hybrid protocol is defined to be solely used with pure moments, eventually supplemented by a “follower load”. For this reason, the hybrid protocol is only shown with these two options. Primary loads move the specimen, whereas secondary loads may modify this motion. The category “Parameter” includes all effects in the adjacent levels, which were investigated for the respective protocol and load combination. Rows with no parameter show that no study included in this review used this combination

ALEs adjacent level effects, ROM range of motion, EZ elastic zone, NZ neutral zone, IDP intradiscal pressure

### Testing apparatus

Generally, the spine testers differed in the way the load was generated and applied to the spine. Eccentric loads and shear forces were most commonly generated in a modified materials testing machine or by tensioning cables affixed to the spine with dead weights. To apply pure moments, three basic constructions were identified among the studies: two systems using cables and pulleys and one system applied pure moments via stepper motors. Cable-driven systems were either integrated into materials testing machines as established by Crawford et al. [45] or custom-built with loads generated by dead weights or pneumatic cylinders tensioning the cables. Theoretically, all these apparatus have the ability to apply pure moments. When the flexibility method is used with pure moments, isolated alterations of the stiffness of certain segments do not affect adjacent levels (Fig. 3). However, several studies found partly inconsistent or unexpected results in the adjacent segments [27, 46, 47]. As long as the procedure does not

influence the integrity of structures attached to adjacent segments (e.g. ligaments spanning multiple levels or joint capsules), the only explanation for consistent trends in kinematic changes in the adjacent segments is a deviation from the pure moment condition. As shown by Panjabi in 2007 [12], the cables of pulley-systems need to be readjusted after each increment of motion to be parallel and perpendicular to the uppermost vertebra. If this is not or improperly done, impure moments with undesired forces will result.

### Load application

The second potential key factor for differing results is the choice of loads applied to the specimen. When pure moments are used, the results in the untreated segments are independent of the length and condition of the specimens. This likely explains why the findings for this load case were most homogeneous among the different load scenarios (i.e. eccentric force or pure moments supplemented by



an axial preload or follower load). When the loads are supplemented by an axial preload, the loading situation of the specimen becomes more complex (Fig. 1), whereas a follower load theoretically does not have adverse effects on the pure moment condition.

However, even within the group of studies combining pure moments with a vertical preload or a follower load, variations in the set-ups can lead to substantially different findings. For vertical preloads, it was shown that resulting artefact loads and movements highly depend on the constraints of the preload path [48]. To achieve a pure compression without artefact moments, the path of the follower load needs to be guided through the COR of each spinal segment. However, when the path is adjusted in neutral standing position and the specimen is moving, the migration of the COR inevitably leads to artefact moments, potentially influencing the findings in the adjacent segments. In summary, *in vitro* biomechanical studies investigating ALEs require careful interpretation, not only with regard to the test protocols, but also concerning the apparatus and manner of load application.

### Limitations of the test protocols

The use of pure moments allows a high reproducibility and comparability; the reason why it is internationally considered the “gold standard” [15]. The hybrid protocol was developed to be solely used with pure moments, eventually supplemented by a compressive follower load [12]. To reach the preoperative total ROM, fusion inevitably causes the adjacent segments to compensate for the motion restriction of the treated segment by an adaptive hypermobility (Fig. 3). This entails some major limitations.

First, the moment applied to the specimen increases proportionally to the additional adjacent segment motion. Hence, a stiff implant forces adjacent segments to compensate more than a mobile device. This means that the results of this method are highly predictable.

Second, the motion distribution among adjacent segments is directly related to their stiffness. Due to the non-linear mechanical behavior of the spine, ROM is not increasing linearly with the loading of a segment. Close to the neutral position of the spine, it exhibits low stiffness and can be moved without applying significant loads. When the specimen is further moved, it gradually stiffens [15]. Hence, the ROM of an inherently stiff adjacent segment will increase less than the motion of a mobile segment. This explains why the fraction of hypermobility may vary among adjacent segments despite all segments experiencing the same load.

Another difficulty of this method is that, when testing a short spinal specimen after fusion, the motion reduction is redistributed to only few segments with correspondingly

high moments. Strube et al. [38] reported that with a two-level rigid instrumentation with two open segments, a moment of up to 35 Nm was reached, which brings high potential to structurally damage the specimen or any bone-implant interfaces. Additionally, results become more pronounced with reducing the number of remaining open segments. Unfortunately, no consensus was found on standardization of this issue yet. Therefore, special care has to be taken on the use of specimens with adequate length to allow redistribution over multiple levels. The same is true when the stiffness protocol is used.

Independent of the test protocol with respective specific assumptions, all *in vitro* studies using spinal specimens can only reflect the directly postoperative state. Therefore, effects of tissue remodeling such as fusion consolidation and scar formation cannot be considered. For the reasons mentioned above, interpretation of biomechanical *in vitro* experiments investigating ALEs requires proper consideration of the test protocol. Otherwise the results of these studies might be misleading.

### Biomechanical premises for the test protocols

*In vitro* biomechanical testing protocols mainly focus on the role of increased stresses due to hypermobility as one of the suspected main causes for fusion-related ASD. These alterations were studied *in vitro* using varying protocols differing in their underlying assumptions on the patients' postoperative motion behavior. These test protocols can be validated by clinical *in vivo* kinematic studies using static and dynamic roentgenographic techniques.

For the flexibility protocol, the loads applied to the spine are of constant magnitude for each test step, independent of the manipulation of the specimen. This represents a scenario where the patient accepts the fusion-related confinements and tries not to overload the spine by applying the same preoperative loads. Consequently, the motion of the whole spine is decreased by the amount of movement restricted at the treated segment when pure moments are used (Fig. 3). In contrast, both hybrid and stiffness protocols assume that a patient does not accept the restricted motion and compensates the motion loss of the fused segment by redistributing the motion to adjacent levels to reach the preoperative limits.

A systematic analysis of *in vivo* kinematic studies by Malakoutian et al. [4] showed a partly inconsistent postoperative motion behavior of the patients. Furthermore, it was pointed out that cranial and caudal adjacent segments responded differently to surgical treatments. In that review, after a fusion procedure, most studies either found an unchanged or increased ROM for the first cranial level, while the more distal cranial segments showed no increase

in flexion–extension ROM. The caudal segment seems to be either unaffected or even experience a decrease in motion. When the ROM of the whole lumbar spine was investigated, most studies reported a significant decrease, indicating a protective adaptation to the fusion-related reduction of motion at the treated segment. When the results of the different test protocols (Fig. 3) are compared to the in vivo ROM, it can be seen that the assumption that the patient moves the same after fusion, which is implemented in the stiffness and the hybrid protocols, appears to be incorrect. For this parameter, the flexibility protocol replicates the postoperative kinematics of patients much better. However, when this protocol is used with pure moments, the differences between the cranial and caudal adjacent segments found in vivo cannot be reproduced.

In conclusion, none of the current test protocols can replicate the in vivo kinematics, putting the findings of the current in vitro biomechanical studies on ASD into question. This raises the question if focusing on pure motion behavior of the patients disregards important factors contributing to the pathogenesis of ASD.

## Conclusions

Three different test protocols are used in current biomechanical in vitro studies investigating ASD. Each protocol produces different biomechanical changes at the adjacent levels to spinal instrumentation and the results are somewhat obvious, based upon the basic principles of each approach. Further, these protocols differ in the assumption on the postoperative motion behavior of the patients. In a parallel review on in vivo kinematics by Malakoutian et al. [4], it was shown that the motion of the lumbar spine in patients tends to decrease after fusion in most studies, demonstrating that the notion that patients move the same after fusion as implemented with the stiffness and hybrid protocol does not appear to be correct. The decrease in motion shown in vivo could be predicted by the flexibility protocol. However, when this protocol is used with pure moments, which is the current “gold standard”, the difference in the kinematic changes between the cranial and caudal adjacent segment cannot be replicated, putting the findings of current in vitro biomechanical literature into question.

**Acknowledgments** We wish to thank the Alexander von Humboldt Foundation for their generous support of this research through a Research Award to Thomas R. Oxland during his sabbatical leave at the University of Ulm.

**Conflict of interest** The authors affirm that they have no financial affiliation or involvement with any commercial organization that has direct financial interest in any matter included in this article.

## References

- Lund T, Oxland TR (2011) Adjacent level disk disease—is it really a fusion disease? *Orthop Clin N Am* 42:529–541
- Park P, Garton HJ, Gala VC et al (2004) Adjacent segment disease after lumbar or lumbosacral fusion: review of the literature. *Spine (Phila Pa 1976)* 29:1938–1944
- Hilibrand AS, Robbins M (2004) Adjacent segment degeneration and adjacent segment disease: the consequences of spinal fusion? *Spine J* 4:190S–194S
- Malakoutian M, Volkheimer D, Street J et al (2015) Do in vivo kinematic studies provide insight into the degeneration of adjacent segments?—a qualitative systematic literature review. *Eur Spine J*. doi:10.1007/s00586-015-3992-0
- Cole T-C, Burkhardt D, Ghosh P et al (1985) Effects of spinal fusion on the proteoglycans of the canine intervertebral disc. *J Orthop Res* 3:277–291
- Bushell GR, Ghosh P, Taylor TKF et al (1978) The effect of spinal fusion on the collagen and proteoglycans of the canine intervertebral disc. *J Surg Res* 25:61–69
- Hoogendoorn RJW, Helder MN, Wuisman PIJM et al (2008) Adjacent segment degeneration—observations in a goat spinal fusion study. *Spine (Phila Pa 1976)* 33:1337–1343
- Phillips FM, Reuben J, Wetzel FT (2002) Intervertebral disc degeneration adjacent to a lumbar fusion—an experimental rabbit model. *J Bone Joint Surg Br* 84-B:289–294
- Wilke H-J, Kettler A, Wenger KH, Claes LE (1997) Anatomy of the sheep spine and its comparison to the human spine. *Anat Rec* 247:542–555
- Alini M, Eisenstein SM, Ito K et al (2008) Are animal models useful for studying human disc disorders/degeneration? *Eur Spine J* 17:2–19
- Reitmaier S, Schmidt H, Ihler R et al (2013) Preliminary investigations on intradiscal pressures during daily activities: an in vivo study using the merino sheep. *PLoS One* 8:e69610
- Panjabi MM (2007) Hybrid multidirectional test method to evaluate spinal adjacent-level effects. *Clin Biomech* 22:257–265
- Panjabi MM (1988) Biomechanical evaluation of spinal fixation devices: I. A conceptual framework. *Spine (Phila Pa 1976)* 13:1129–1134
- Patwardhan AG, Havey RM, Meade KP et al (1999) A follower load increases the load-carrying capacity of the lumbar spine in compression. *Spine (Phila Pa 1976)* 24:1003–1009
- Wilke H-J, Wenger K, Claes L (1998) Testing criteria for spinal implants: recommendations for the standardization of in vitro stability testing of spinal implants. *Eur Spine J* 7:148–154
- Gertzbein SD, Holtby R, Tile M et al (1984) Determination of a locus of instantaneous centers of rotation of the lumbar disc by Moiré fringes: a new technique. *Spine (Phila Pa 1976)* 9:409–413
- Quinnell RC, Stockdale HR (1981) Some experimental observations of the influence of a single lumbar floating fusion on the remaining lumbar spine. *Spine (Phila Pa 1976)* 6:263–267
- Dekutoski MB, Schendel MJ, Ogilvie JW et al (1994) Comparison of in vivo and in vitro adjacent segment motion after lumbar fusion. *Spine (Phila Pa 1976)* 19:1745–1751
- Lucas DB, Bresler B (1961) Stability of the ligamentous spine. Technical Report esr. 11 No. 40, Biomechanics Laboratory, University of California at San Francisco, The Laboratory
- Crisco JJ (1989) The biomechanical stability of the human lumbar spine: experimental and theoretical investigations (doctoral dissertation). CT, Yale University, New Haven
- Pfeiffer M, Hoffman H, Goel VK et al (1997) In vitro testing of a new transpedicular stabilization technique. *Eur Spine J* 6:249–255

22. Bastian L, Lange U, Knop C et al (2001) Evaluation of the mobility of adjacent segments after posterior thoracolumbar fixation: a biomechanical study. *Eur Spine J* 10:295–300
23. Schmoelz W, Huber JF, Nydegger T et al (2003) Dynamic stabilization of the lumbar spine and its effects on adjacent segments: an in vitro experiment. *J Spinal Disord Tech* 16:418–423
24. Moore J, Yoganandan N, Pintar FA et al (2006) Tapered cages in anterior lumbar interbody fusion: biomechanics of segmental reactions. *J Neurosurg Spine* 5:330–335
25. Schmoelz W, Huber JF, Nydegger T et al (2006) Influence of a dynamic stabilisation system on load bearing of a bridged disc: an in vitro study of intradiscal pressure. *Eur Spine J* 15:1276–1285
26. Delank K-S, Gercek E, Kuhn S et al (2010) How does spinal canal decompression and dorsal stabilization affect segmental mobility? A biomechanical study. *Arch Orthop Trauma Surg* 130:285–292
27. Dahl MC, Ellingson AM, Mehta HP et al (2013) The biomechanics of a multilevel lumbar spine hybrid using nucleus replacement in conjunction with fusion. *Spine J* 13:175–183
28. Cheng BC, Gordon J, Cheng J, Welch WC (2007) Immediate biomechanical effects of lumbar posterior dynamic stabilization above a circumferential fusion. *Spine (Phila Pa 1976)* 32:2551–2557
29. Akamaru T, Kawahara N, Yoon TS et al (2003) Adjacent segment motion after a simulated lumbar fusion in different sagittal alignments: a biomechanical analysis. *Spine (Phila Pa 1976)* 28:1560–1566
30. Umehara S, Zindrick MR, Patwardhan AG et al (2000) The biomechanical effect of postoperative hypolordosis in instrumented lumbar fusion on instrumented and adjacent spinal segments. *Spine (Phila Pa 1976)* 25:1617–1624
31. Weinhoffer SL, Guyer RD, Herbert M, Griffith SL (1995) Intradiscal pressure measurements above an instrumented fusion: a cadaveric study. *Spine (Phila Pa 1976)* 20:526–531
32. Chow DHK, Luk KDK, Evans JH, Leong JCY (1996) Effects of short anterior lumbar interbody fusion on biomechanics of neighboring unfused segments. *Spine (Phila Pa 1976)* 21:549–555
33. Cunningham BW, Kotani Y, McNulty PS et al (1997) The effect of spinal destabilization and instrumentation on lumbar intradiscal pressure: an in vitro biomechanical analysis. *Spine (Phila Pa 1976)* 22:2655–2663
34. Panjabi M, Malcolmson G, Teng E et al (2007) Hybrid testing of lumbar CHARITE discs versus fusions. *Spine (Phila Pa 1976)* 32:959–966
35. Panjabi M, Henderson G, Abjornson C, Yue J (2007) Multidirectional testing of one- and two-level ProDisc-L versus simulated fusions. *Spine (Phila Pa 1976)* 32:1311–1319
36. Panjabi MM, Henderson G, James Y, Timm JP (2007) Stabili-maxNZ versus simulated fusion: evaluation of adjacent-level effects. *Eur Spine J* 16:2159–2165
37. Molz FJ, Partin JI, Kirkpatrick JS (2003) The acute effects of posterior fusion instrumentation on kinematics and intradiscal pressure of the human lumbar spine. *J Spinal Disord Tech* 16:171–179
38. Strube P, Tohtz S, Hoff E et al (2010) Dynamic stabilization adjacent to single-level fusion: Part I. Biomechanical effects on lumbar spinal motion. *Eur Spine J* 19:2171–2180
39. Dmitriev AE, Gill NW, Kuklo TR, Rosner MK (2008) Effect of multilevel lumbar disc arthroplasty on the operative- and adjacent-level kinematics and intradiscal pressures: an in vitro human cadaveric assessment. *Spine J* 8:918–925
40. Kikkawa J, Cunningham BW, Shirado O et al (2010) Biomechanical evaluation of a posterolateral lumbar disc arthroplasty device: an in vitro human cadaveric model. *Spine (Phila Pa 1976)* 35:1760–1768
41. Ilharreborde B, Shaw MN, Berglund LJ et al (2011) Biomechanical evaluation of posterior lumbar dynamic stabilization: an in vitro comparison between Universal Clamp and Wallis systems. *Eur Spine J* 20:289–296
42. Lindsey DP, Swanson KE, Fuchs P et al (2003) The effects of an interspinous implant on the kinematics of the instrumented and adjacent levels in the lumbar spine. *Spine (Phila Pa 1976)* 28:2192–2197
43. Swanson KE, Lindsey DP, Hsu KY et al (2003) The effects of an interspinous implant on intervertebral disc pressures. *Spine (Phila Pa 1976)* 28:26–32
44. Wiseman CM, Lindsey DP, Fredrick AD, Yerby SA (2005) The effect of an interspinous process implant on facet loading during extension. *Spine (Phila Pa 1976)* 30:903–907
45. Crawford NR, Brantley AGU, Dickman CA, Koeneman EJ (1995) An apparatus for applying pure nonconstraining moment to spine segments in vitro. *Spine (Phila Pa 1976)* 20:2097–2100
46. Rohlmann A, Neller S, Bergmann G et al (2001) Effect of an internal fixator and a bone graft on intersegmental spinal motion and intradiscal pressure in the adjacent regions. *Eur Spine J* 10:301–308
47. Hartmann F, Dietz SO, Kuhn S et al (2011) Biomechanical comparison of an interspinous device and a rigid stabilization on lumbar adjacent segment range of motion. *Acta Chir Orthop Traumatol Cech* 78:404–409
48. Cripton PA, Bruehlmann SB, Orr TE et al (2000) In vitro axial preload application during spine flexibility testing: towards reduced apparatus-related artefacts. *J Biomech* 33:1559–1568
49. Wilke H-J, Claes L, Schmitt H, Wolf S (1994) A universal spine tester for in vitro experiments with muscle force simulation. *Eur Spine J* 3:91–97
50. Cardoso MJ, Dmitriev AE, Helgeson M et al (2008) Does superior-segment facet violation or laminectomy destabilize the adjacent level in lumbar transpedicular fixation? An in vitro human cadaveric assessment. *Spine (Phila Pa 1976)* 33:2868–2873
51. Tan J-S, Singh S, Zhu Q-A et al (2008) The effect of cement augmentation and extension of posterior instrumentation on stabilization and adjacent level effects in the elderly spine. *Spine (Phila Pa 1976)* 33:2728–2740
52. Ingalhalikar AV, Reddy CG, Lim TH et al (2009) Effect of lumbar total disc arthroplasty on the segmental motion and intradiscal pressure at the adjacent level: an in vitro biomechanical study. *J Neurosurg Spine* 11:715–723
53. Cabello J, Cavanilles-Walker JM, Iborra M et al (2013) The protective role of dynamic stabilization on the adjacent disc to a rigid instrumented level. An in vitro biomechanical analysis. *Arch Orthop Trauma Surg* 133:443–448
54. Yoganandan N, Pintar F, Maiman DJ et al (1993) Kinematics of the lumbar spine following pedicle screw plate fixation. *Spine (Phila Pa 1976)* 18:504–512
55. Ha S-K, Kim S-H, Kim DH et al (2009) Biomechanical study of lumbar spinal arthroplasty with a semi-constrained artificial disc (activ L) in the human cadaveric spine. *J Korean Neurosurg Soc* 45:169–175