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An experimental methodology to measure the effects of intervertebral interventions on the facet biomechanics in situ

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ABSTRACT

Whereas facet joints are recognised as being involved in back pain with high prevalence of early degeneration, the biomechanics of facet joints remain understudied and often overlooked in the evaluation of spinal interventions.

This study aimed to develop a methodology for investigating the biomechanics of lumbar facet joints and applied it to a mock fusion model with posteriorly centred compressive loads.

The proposed methodology involved measuring facet joint biomechanics through synchronized specimen load and displacement measurements, motion capture of the superior facets with 4 K webcams, and pressure mapping through the facet joints. The experimental method was developed using aged ovine lumbar functional units (N = 6).

Results showed that the proposed methodology to measure facet joints biomechanics was accurate (displacement errors below 0.2 mm) and able to capture changes in biomechanics following a mock fusion (with significant differences in all measured displacements). Pressure measurement was challenging due to curvature changes in old ovine tissue which was used for method development but translated successfully to human lumbar tissue. It showed that an aged sheep model is not a good model for posterior spinal biomechanics.

This work specifies a new, accurate, methodology to evaluate facet joint biomechanics in vitro and, uniquely, how they change following a spinal intervention.

1. Introduction

Back pain is a significant global public health concern, leading to substantial disability-adjusted life years (Hartvigsen et al., 2018; Traeger et al., 2019). Facet (zygapophysial) joint degeneration is a primary contributor to back pain, and alongside intervertebral discs, facet joints are crucial for spinal unit mobility and stability (Cavanaugh et al., 2006; Dreyer and Dreyfuss, 1996; Gellhorn et al., 2013).

Spinal fusion is still the state of the art procedure for various spinal disorders (Lehr et al., 2021). Despite its well-established purpose, fusion presents challenges and a wide range of outcomes (Du et al., 2022), including symptomatic facet joint osteoarthritis (O'Leary et al., 2018).

However, the biomechanics of facet joints remain understudied and often overlooked in the experimental evaluation of spinal interventions (Musso et al., 2022). Similarly, when computational simulations are used in such an evaluation, they rely on little validation of outcomes related to the facet joints (Mengoni, 2021). This is due in part because data and methods are lacking (Gupta et al., 2023; Jaumard et al., 2011; Yang and King, 1984).

The aim of the study was to develop an in vitro methodology for biomechanical evaluation of the facet joints in situ, able to measure the effects of spinal fusion on lumbar facet joints through synchronisation of motion analysis and pressure mapping during mechanical loading. With no large animal model for facet joints biomechanics but good comparability reported in the sheep (Easley et al., 2008; Latif, 2011; Wang et al., 2018; Wilke et al., 1997), a model used of old ovine tissue for degenerated discs (Hegewald et al., 2015; Wang et al., 2018; Wilke et al., 1997) was chosen to develop the methodology.

2. Materials and methods

An experimental approach was developed, built on previous work in ovine cervical spinal tissue (Mengoni et al., 2016), to measure facet displacement and load share between facet and intervertebral joints in a synchronised manner. As an exemplar intervention, it was used in a longitudinal study with a mock-up fusion procedure and axial loading of

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lumbar functional spinal units (FSUs). The methodology was developed using old ovine lumbar tissue (N = 6) then translated to one human lumbar FSU as proof of principle.

2.1. Sample preparation

Six lumbar functional spinal units were excised from three mature (> 6 years old) ovine spines obtained from the control arm of an *in vivo* study (Versus Arthritis, Reference 22031) (Fig. 1a). In addition, one human lumbar L2-3 FSU was used (male, 92 yr.) in accordance with ethical approval (Yorkshire and the Humber REC number 15/YH/0096) as a proof of principle that the methodology developed with ovine tissue can be translated for human tissue.

For both types of tissues, all muscle tissue and ligaments were removed from the samples and the bone was exposed on the rear of the inferior articular facets of the superior vertebrae, taking care not to pierce the facet joint capsules. The vertebrae were set in poly-methyl methacrylate (PMMA) end caps to enable flat surface testing. The samples were imaged at an isotropic resolution of 82 μm using a μCT scanner (XtremeCT, Scanco Medical AG, Switzerland) to visualise the curvature within the facet joints. Samples were stored overnight wrapped in phosphate buffer solution soaked gauze at 5 °C.

Following biomechanical evaluation in their pre-fusion state (described in section 2.2), the FSU samples were processed to replicate a fused clinical state with an analogue of fusing the vertebral bodies.

For the ovine FSUs, the IVD was removed with a posterior approach (Fig. 1b), keeping the inter-vertebral distance fixed, and the resulting space was filled with PMMA cement (Fig. 1c).

For the human FSU, to replicate a more clinical approach of a lateral lumbar interbody fusion (LLIF) cage procedure, a partial nucleotomy was carried out. A lateral incision was made to the annulus approximately 20 mm width × 8 mm height and the nuclear material was excised through this area to create a void within the IVD. The void was then filled with PMMA cement.

The samples were then tested in their post-fusion state with the same biomechanical protocol.

2.2. Biomechanical evaluation

2.2.1. Mechanical testing

The FSUs were loaded in compression in a materials testing machine (3365, Instron, USA with a loading cell of 5 kN) as shown in Fig. 2. Cyclic pre-loading from 20-200 N for five cycles was applied prior to the start of measurement. Displacement-controlled compression experiments were carried out at 1 mm/min up to 950 N (representative of ovine physiological loads) via a ball bearing housed within a flat plate to allow natural rotations, with the sample initially in a neutral position (Costi et al., 2021; Little et al., 2010; Mengoni et al., 2016). The centre of load application was about 15 mm posterior to the geometrical centre of the FSU to ensure engagement of facet joints during compressive loading.

For human tissue, cycling pre-loading from 20-300 N for five cycles was applied prior to the start of the measurement. The maximum load was 1,100 N, representative of high load (Costi et al., 2021; Kuo et al., 2010; Schäfer et al., 2023).

2.2.2. Motion tracking

Two orthogonally placed 4 K, 8MP, manual focus webcams with 3840 × 2160 resolution at 30 FPS (Adesso CyberTrack 6S, Adesso, USA, Fig. 2a) were used to measure the displacement of key facet joint features identified by colour markers. Two markers were placed on each of the superior facets and one marker was placed laterally on the upper vertebral body, posterior to the load application axis (Fig. 2c). An additional reference marker was placed on the upper cement cap. One webcam was positioned posteriorly, perpendicular to the coronal plane, to track the markers positioned on the facet joints and one webcam was positioned laterally, perpendicular to the sagittal plane, to track the markers positioned on the upper vertebral body and the cement endcap. To increase contrast for the coloured markers, a dark blue background sheet was placed behind the testing setup.

Video files (.mp4 format) of each sample during mechanical testing were cropped to reduce Video size (Microsoft ClipChamp, Microsoft, USA). The displacement of each marker was measured using a colour tracking script inspired from work on meniscus motion (Pounds, 2023). Each marker was tracked separately using a combination of colour detection, blob analysis and tracking loop fitting, to identify and isolate the marker from the background on every frame of the Video file (in-house script written with MATLABs R2022a, The Mathworks Inc., Natick, MA,US).

First, a colour thresholder (Image Processing Toolbox) was used to detect and threshold the colour of the marker through hue, saturation and value (HSV) values. The thresholded images were binarized to highlight any missing regions of the marker, with the threshold value corrected to cover the missing regions if necessary, and to create a detection region mask. A blob analysis (Computer Vision Toolbox) was then used to create a region of interest that matched the mask and track it on every frame of the Video. Marker displacements were computed as the changes in lateral and axial positions between each frame. The diameter of each marker, measured using a vernier callipers, was used to transform displacement in pixel to a distance in mm.

The displacement from the upper cement cap marker was used to assess the accuracy of the motion capture, assuming that the axial displacement of that marker should match the crosshead displacement.

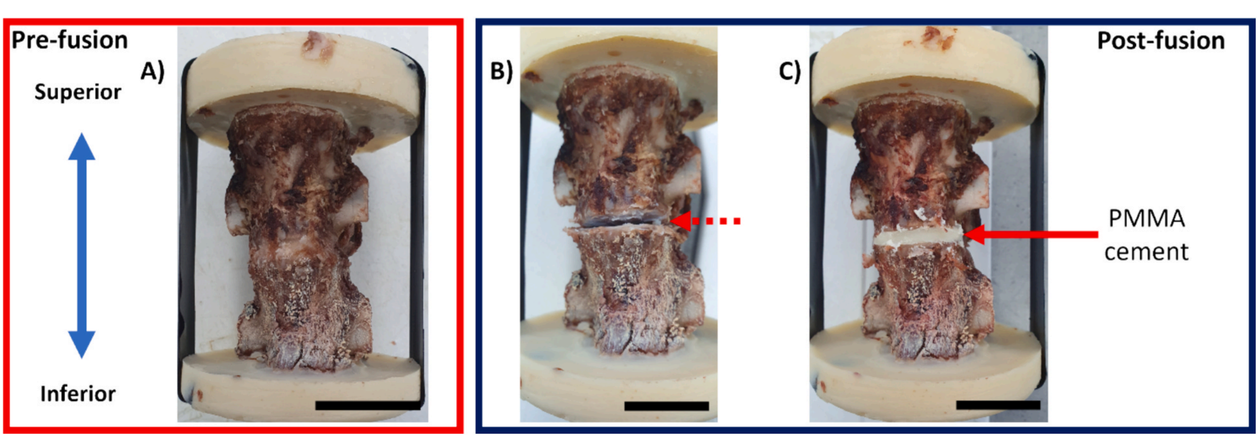

Fig. 1. Photographic images of the preparation process of ovine samples (anterior view), with superior (upper) and inferior (lower) levels.A) Pre-fusion sample where the ends of the vertebral bodies have been capped with PMMA endcaps. B) Nucleotomy of ovine sample. C) PMMA fusion of vertebral bodies. Scale bar = 35 mm.

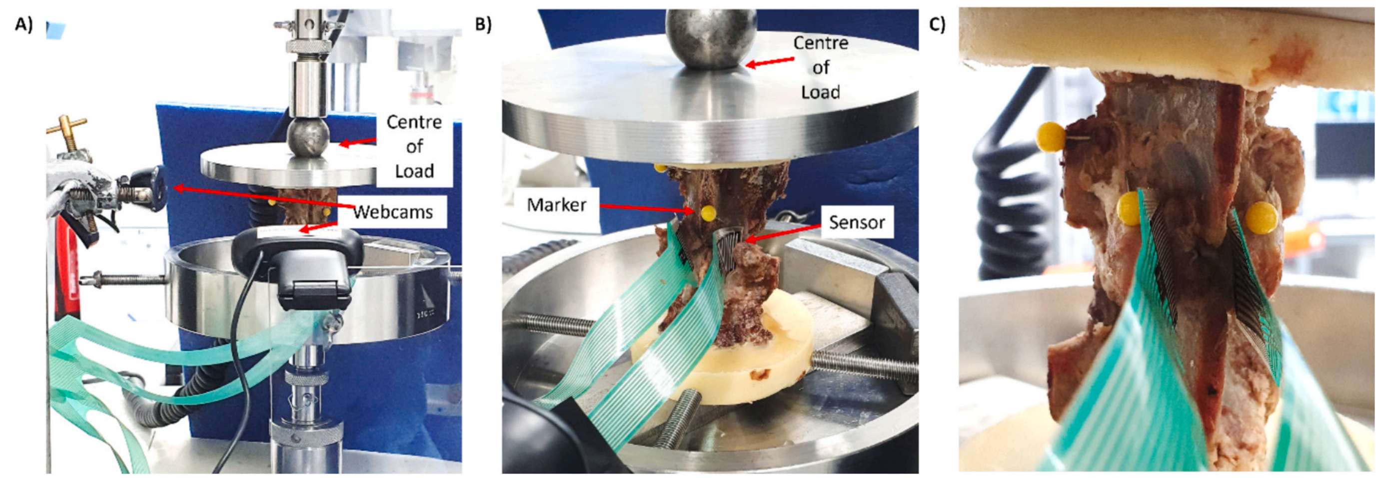

Fig. 2. Photographic images of the experimental set-up to simultaneously measure: i) compressive displacement of the FSU, ii) displacement of facet joints and iii) transfer of load through the facet joints. A-B) Images of setups showing fixtures, centre of load, markers and webcam positions. C) Close-up of setup showing marker positions.

2.2.3. Normal load through the facet joints

Load transfer measurement and pressure mapping between the surfaces of the superior and inferior facets during displacement were carried out using a flexible pressure sensor (Medical Sensor 6900 series, Tekscan, USA). The sensors were calibrated up to either 950 N (ovine tissue) or 1,100 N (human tissue) using a sensitivity range of 0 – 5 kPa (iScan, Tekscan, USA).

Immediately prior to testing in the pre-fusion state, the ligamentous facet joint capsules were carefully cut using a scalpel to allow separation. The sensors were then placed between the ascending and descending surfaces of each facet. Contact pressure at each sensel was recorded throughout mechanical testing. Normal load through each facet joint was computed as the integral of the pressure values over the sensel area, with a measurement error estimated at ± 0.05 N / sensel.

2.3. Data analysis

Load-displacement data was fitted to a bilinear curve (using the crosshead displacement) to extract the toe-region stiffness (N/mm), the stiffness transition point (mm) and the elastic stiffness (N/mm). Goodness-of-fit data was used to assess whether a bilinear assumption was a good approximation of the experimental data.

The toe-region stiffness, stiffness transition, elastic stiffness, normal load through the facets and facet displacements in the axial direction were compared between the pre- and post-fusion states using paired Student's t-tests ($\alpha = 0.05$) after the normally distributed nature of the data was assessed with a Shapiro–Wilk test, using statistical software (Graphpad Prism 9.5.1, GraphPad Software Inc., San Diego, CA). Finally, the facets displacement computed on images at the end of loading and averaged between both facets of the same specimen was compared to the displacement of the crosshead with a paired Student's t-tests ($\alpha = 0.05$). A Bonferroni correction was applied where required.

3. Results

All raw and processed experimental data, including Video frames and CT images, are available through the University of Leeds Data Repository (Warren and Mengoni, 2024).

3.1. Ovine model

3.1.1. FSU behaviour

The in vitro load–displacement showed significantly different behaviours between pre- and post-fusion states (Fig. 3), with less variability between samples post-fusion than pre-fusion.

A bilinear fit for each load–displacement data (Table 1) was a very good approximation ($R^2 \geq 0.997$), except for sample 2 post fusion ($R^2 = 0.993$) which demonstrated very low force for a displacement up to about 0.5 mm before exhibiting a bilinear behaviour (Fig. 3B). Pre-fusion data exhibited a similar toe-region stiffness (350 ± 280 N/mm, average ± standard deviation) compared to post-fusion data (450 ± 210 N/mm). The transition point was significantly ($p = 0.030$) larger in

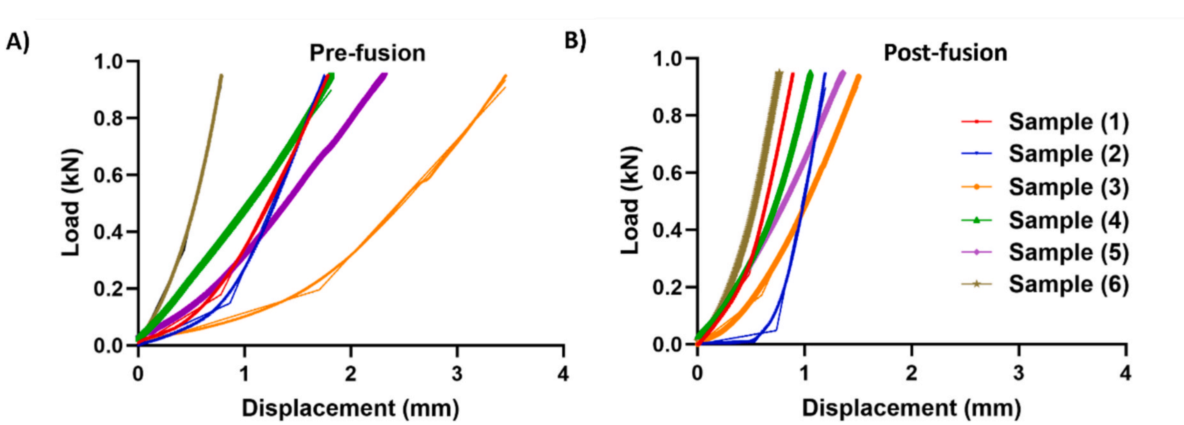

Fig. 3. Load-displacement data of FSU samples in pre- and post-fusion states. Bilinear curves are shown. A) Pre-fusion data. B) Post-fusion data.

Table 1
Stiffness values from bilinear curve fitting analysis of load–displacement data of samples. Statistical analysis was paired student *t*-test (* and # =p < 0.05, ns = not significant). T-R = Toe-region, GoF = Goodness of Fit. Note: Sample 4 lacks parameters for transition point and elastic stiffnesses due to linear displacement-load data.

Sample	T-R Stiffness (Pre) (N/mm)	T-R Stiffness (Post) (N/mm)	Stiffness Transition (Pre) (mm)	Stiffness Transition(Post) (mm)	Elastic Stiffness (Pre) (N/mm)	Elastic Stiffness (Post) (N/mm)	GoF (Pre) R^2	GoF (Post) R^2
1	230	510	0.78	0.48	740	1,680	0.998	0.998
2	170	70	0.86	0.74	880	1,860	0.997	0.993
3	120	290	1.70	0.60	410	810	0.998	0.997
4	500	600	–	–	–	–	0.998	0.999
5	290	570	0.86	0.68	480	820	0.999	0.997
6	780	680	0.43	0.40	1,680	1,810	0.998	0.997
Sig.	ns	ns	*	*	#	#		

the pre-fusion state (0.93 ± 0.42 mm) compared to the post-fusion state (0.58 ± 0.12 mm). The elastic stiffness was significantly lower (p = 0.031) in the pre-fusion state (840 ± 450 N/mm) compared to the post-fusion state (1,400 ± 480 N/mm).

3.1.2. Motion tracking

The difference in the displacement measured from tracking the cement endcap marker, compared to the displacement measurement from the crosshead, was 0.07 ± 0.14 mm.

Displacement measurement of the facet joints at the end of loading showed significant (p = 0.022) reduction from pre- to post-fusion state (Fig. 4). Prior to fusion, the facet joint exhibited a displacement of 1.81 ± 1.19 mm (average ± standard deviation). This displacement was similar (p = 0.858) to the crosshead displacement measured during pre-fusion state testing (1.77 ± 0.82 mm). The average facet joint displacement decreased to 0.86 ± 0.19 mm, while crosshead displacement decreased to 1.23 ± 0.34 mm. After fusion, the crosshead and average facet joint displacements were significantly different (p = 0.0002). Lateral marker motion, which was present in the pre-fusion samples with a displacement of 0.83 ± 0.27 mm (average ± standard deviation), showed a significant decrease (p = 0.023) in the post-fusion state, with a displacement of 0.65 ± 0.25 mm.

The obtained motion pre- and post-fusion was a compression-extension (lateral marker displacement smaller than the facet markers) with the degree of extension decreasing post-fusion.

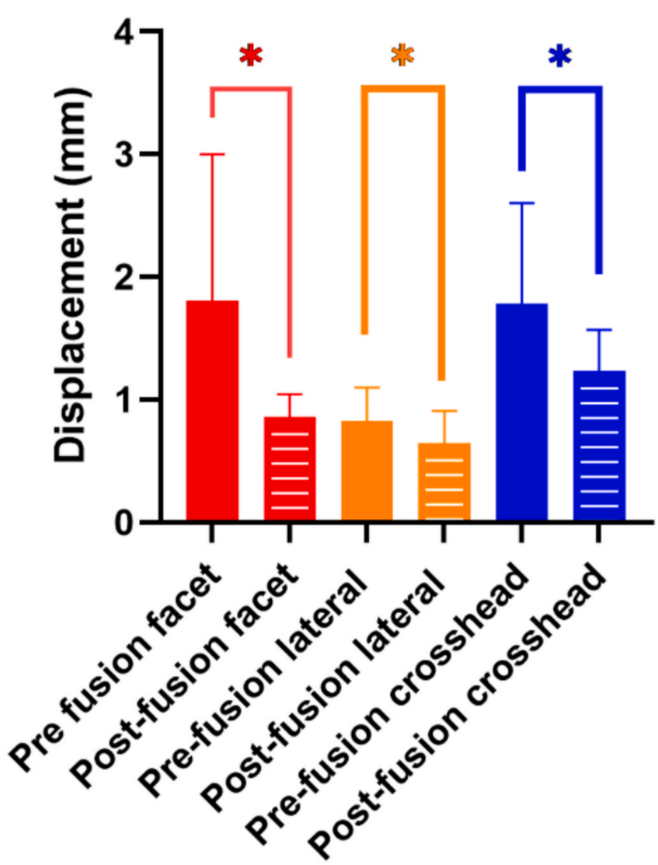

Fig. 4. Displacement of average facet, lateral motion of vertebral body and Instron crosshead of each sample in pre- and post-fusion state. Error bars: mean ± S.D (n = 6), * = p < 0.05.

3.1.3. Load through the facet joints and CT image analysis

The attempt to measure load transfer through the facet joints during compressive loading was prevented by morphological characteristics observed in the old ovine lumbar facet joints. Analysis of CT images of the functional spinal units (FSUs) revealed distinctive facet joint morphologies. Specifically, both facet joints in each sample exhibited either large single curvature of the facet surfaces or a change in curvature polarity of the inferior facet joint (change from concave to convex within the first three millimetres), as depicted in Fig. 5.

3.2. Human tissue model

The adaptation of the developed methodology for ovine tissue to human tissue was successful, with added capacity to measure normal load through the joints.

3.2.1. Compressive displacement

The load–displacement curves of the human FSU were different

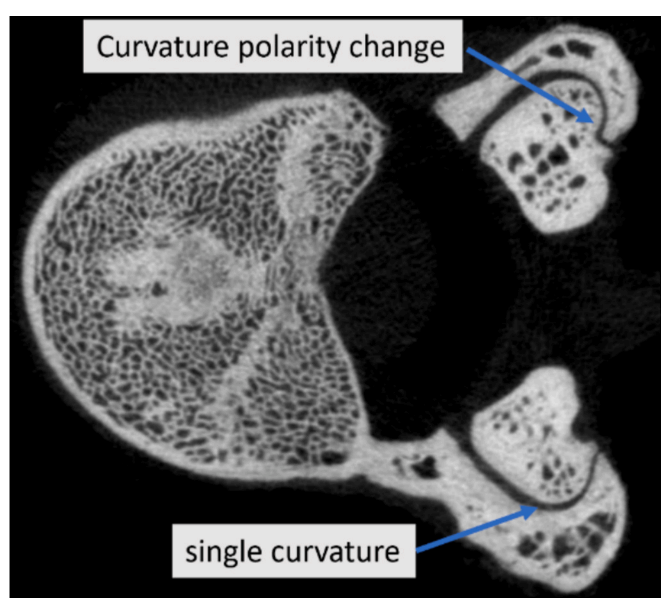

Fig. 5. Superior view of a CT micrograph of an example FSU sample, highlighting the potential single or double polarity curvature of the facet joint.

between the pre- and post-fusion state (Fig. 6a). A lower initial stiffness was measured for the pre-fusion state (80 N/mm) compared to the post-fusion state (130 N/mm). A larger stiffness transition was measured in the pre-fusion state (1.93 mm) compared to the post-fusion state (1.24 mm). A comparable elastic stiffness was measured for the pre-fusion and post-fusion states (790 N/mm).

3.2.2. Motion tracking

A reduction in displacement of the facet joint, the lateral vertebral body and the crosshead was witnessed following fusion, respectively from 2.60 mm, 3.03 mm and 2.99 mm to 1.93 mm, 1.31 mm and 2.30 mm (Fig. 6b). These equated to a reduction of 26 % for facet joint displacement, 57 % for lateral motion and 23 % for crosshead displacement. The motion moved from being almost pure compression pre-fusion to a compression-extension motion post-fusion.

3.2.3. Load transfer analysis

The load transfer analysis proved possible for human lumbar facets, with correct insertion of pressure sensors into the facet joint space obtained thanks to the smooth curvature of the human lumbar facets.

A normal load of 224.8 N was measured through the facet joints in the pre-fusion state compared to 149.8 N in the post-fusion state (Fig. 6c). This equated to a reduction of 33 % in measurable load being transferred through the facet joints between post- and pre-fusion states.

4. Discussion

In this study, an experimental testing methodology was developed, initially tested with ovine lumbar FSUs and then a proof of principle human lumbar FSU was demonstrated. It is to the author's knowledge the first study that successfully measures directly and synchronously several aspects of the biomechanics of the human facet joint (Adams and W. C. Hutton, 1980; Gupta et al., 2023; Ivicsics et al., 2014; Yang and King, 1984).

4.1. Methodology

The accuracy of our measurement method is crucial for validating the biomechanical methodology. Our motion tracking setup for markers on the FSU achieved an accuracy below 0.2 mm, for displacements of 1.8 mm, demonstrating good precision in detecting subtle displacements and alignments. However, this method is limited to planar movements, excluding out-of-plane kinematics. Capturing (as done in this work) and analysing separately the left and right facets independently can provide better understanding of the FSUs behaviour under various conditions, such as flexion, extension, and lateral bending. 3D motion can also be derived by combining information in different planes.

The absence of surrounding tissues in our setup, such as ligaments, muscles, and adjacent vertebrae, means the observed mechanical responses may not fully represent in vivo conditions. These tissues contribute significantly to spinal stability and load distribution and would need to be included to derive clinical information (Bonnel and Dimeglio, 2020; Muto et al., 2017; Rawls and Fisher, 2010). As such, the developed testing setup can provide information which can be useful in a preclinical context of device evaluation rather than to derive clinical guidelines.

The mock fusion process, using PMMA cement, provided changes in motion that could be expected following fusion, with shorter toe-regions and less extension of the FSU (Pradeep and Pal, 2023). As such, it was considered a successful fusion process (Reid et al., 2019; Rushton et al., 2012), with the methodology used able to capture significant paired differences.

The methodology used in this study involved only applied axial compression as a static load, with free flexion–extension rotations, without control of tissue hydration. This is not necessarily representative of the FSU range of motion and natural environment (Bartynski et al., 2021; Ghoshal and McCarthy, 2020). However, the range of measurement developed in this work can be adapted to other testing conditions. The main challenge would be motion tracking when testing in a fluid bath for which appropriate diffraction and refraction correction methods would need to be developed.

It is important to highlight that the ovine tissue outcomes in this work should not be interpreted for relevance of the biomechanical changes to human tissue. Ovine tissue was used for methodological development to ascertain that the methodology was able to produce significant differences in measures of interest and hence be used with human tissue in future studies.

4.2. Ovine tissue

The inability to directly measure the load transfer through the ovine facet joints highlights the issues around anatomical differences between animal and human tissue (Hegewald et al., 2015; Wilke et al., 1997). There is sparse literature on the morphology of ovine facet joints, however what is available did not allow us to anticipate the double curvature found in our tissue. Most studies using young sheep showed ovine thoracolumbar (Easley et al., 2008; Latif, 2011) or thoracic (Wilke

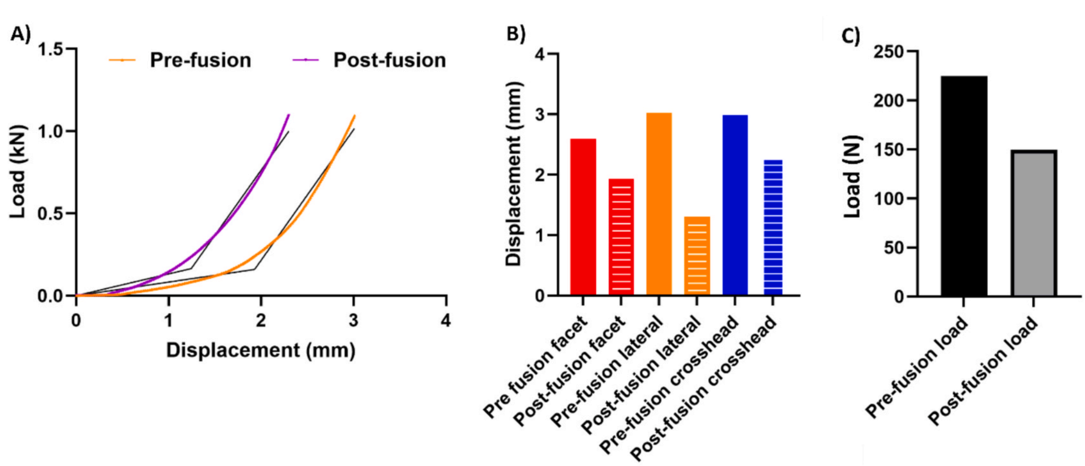

Fig. 6. A) Load-displacement curves of human FSU sample in pre- and post-fusion states. Bilinear curve analysis lines are shown. B) Displacement of human facet, lateral motion of vertebral body and Instron crosshead of each sample in pre- and post-fusion state. C) Normal load transfer through facet joints in pre- and post-fusion state.

et al., 1997) facets with simple curvatures as in the healthy lumbar human joints and with smaller curvature than the corresponding cervical facet joints which are almost flat. One study (Wang et al., 2018), using sheep of a similar age to our study to produce high level of degeneration by immobilisation, had facet joints showing simple curvature before immobilisation whilst double curvature, as observed in our study, occurred only after prolonged immobilisation of the spine. It appears that the older ovine tissue used in this study is different and not as suitable as expected to assess the facet joints contact, which may be related to the specific breeds used in our study and in Wang et al., 2018 or to variability in how the spine ages in the sheep. The lack of pressure data meant that it was not possible to correlate the changes in displacement and behaviour under axial compression to the load transferred through the facet joints.

In both pre- and post-fusion biomechanical tests, the elastic stiffness values for the ovine FSUs were comparable to those reported in the literature for compressive loads. In comparison to maximum compressive stiffness values reported of 1,300 N/mm (range 800 – 1,300 N/mm) or 1,974 N/mm(range 1,766 – 1,974 /mm) (respectively by Costi et al., 2021and Pelletier et al., 2016) our maximum elastic stiffness value was 1,860 N/mm (range 410 – 1,860 N/mm). The lower end of the range in this study, below that of the literature, is likely due to the applied motion which is not simple compression but also has an extension component, hence with axial stiffness not necessarily directly comparable to previous studies.

For the ovine tissue, a significant difference between the pre- and post-fusion biomechanics was observed across all the measurables in this study. The mock fusion resulted in a lager toe-region stiffness, shorter transition point and larger elastic stiffness. This reflects the changes created by the mock fusion, resulting in smaller motions and a more linear behaviour representative of a hard solid than through the softer, non-linear, natural intervertebral disc tissue (Meng et al., 2021; Rabau et al., 2020; Salzmann et al., 2017). This is aligned with previous work (Wangsawatwong et al., 2021, Li et al., 2024) showing that after fusion, the overall range of motion in all axes was reduced, with reduction reported between 20 and 30 %.

4.3. Human tissue

The translation of the methodology to a human tissue sample was successful with only minor alterations to the sample preparation, such as increased PMMA endcaps height and a more clinically representative fusion analogue. Importantly, the simple curvature of the facet joint allowed for pressure sensors to be inserted and contact loads to be measured.

The change in behaviours between the pre- and post-fusion states observed in the ovine tissue model were similarly observed in the single human FSU sample, with a lower displacement at the end of the toe-region and higher linear stiffness post fusion. The change of motion due to the mock fusion indicated that the reduced compliance of the FSUs was compensated by more rotations. However, this did not result in more load transferred through the facet joints.

5. Conclusions

This study successfully developed a methodology which allows to evaluate quantitatively the biomechanical changes following interventions, focusing on facet biomechanics for the first time. A successful mock fusion process was developed which demonstrated that changes in the biomechanical behaviour was measurable with statistical significance. However, challenges were evident in measuring load transfer through facet joints due to morphological variations in aged sheep, which seem to have more variable morphology than in human tissue making it a poor model for degenerated facet joints. The method was successfully translated for one human lumbar sample, with successful load transfer measurement.

Overall, the new experimental methodology provides a successful method to measure the facet joint biomechanics in vitro, opening a path to conduct further experimental testing in human tissue to assess the effect of interventions on the facet joints.

CRediT authorship contribution statement

James P Warren: . **Emily Bomphrey:** Writing – review & editing, Investigation, Formal analysis. **Marlène Mengoni:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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