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Characterization of facet joint cartilage properties in the human and interspecies comparisons

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ABSTRACT

The facet joint, a synovial joint located on the posterior-lateral spine, is highly susceptible to degenerative changes and plays a significant role in back-related morbidities. Despite its significance, the facet is rarely studied and thus current treatment strategies are lacking. This study aimed to characterize, for the first time, the properties of human, pig, monkey, and rabbit lumbar facet cartilage providing much-needed design criteria for tissue engineering approaches. In this study, where possible, the facet's morphological, histological, mechanical, and biochemical properties were evaluated. Comparisons between the properties of the inferior and superior facet surfaces, as well as among spinal levels were performed within each species. In addition, interspecies comparisons of the properties were determined. The human facet joint was found to be degenerated; 100% of joint surfaces showed signs of pathology and approximately 71% of these were considered to be grade 4. Joint morphology varied among species, demonstrating that despite the mini-pig facet being closest to the human in terms of width and length, it was far more curved than the human or any of the other species. No notable differences were found in the mini-pig, monkey, and rabbit mechanical and biochemical properties, suggesting that these species, despite morphological differences, may serve as suitable animal models for studying structure-function relationships of the human facet joint. The characterization data reported in this study may increase our understanding of this ill-described joint as well as provide the foundation for the development of new treatments such as tissue engineering.

Statement of Significance

This work provides the first comprehensive description of the properties of lumbar facet joint cartilage. Importantly, this work establishes that histological, biochemical, and mechanical properties are comparable between bipedal and quadrupedal animals, helping to guide future selection of appropriate animal models. This work also suggests that the human facet joint is highly susceptible to pathology. The mechanical properties of facet cartilage, found to be inferior to those of other synovial joints, provide a greater understanding of the joint's structure-function relationships as well as the potential etiology of facet joint pathology. Lastly, this work will serve as the foundation for the development of much-needed facet joint treatments, especially those based on tissue engineering approaches.

1. Introduction

Zygapophyseal joints, frequently referred to as facet joints, are highly susceptible to the development of osteoarthritis (OA) [1]. These diarthrodial joints, located on the posterior-lateral spine, work with the intervertebral disc to transmit loads experienced by the spine while facilitating appropriate motion of the vertebrae (Fig. 1A). Depending on the nature of the spinal movement, the facet joints have been reported to carry up to 25% of the total spine compressive loads [2]. To compensate for a loss in structural integrity of a pathological intervertebral disc, the proportion of load borne by the facet joints can more than double [3]. Loading and abnormal loading of these joints can lead to the development of osteoarthritis.

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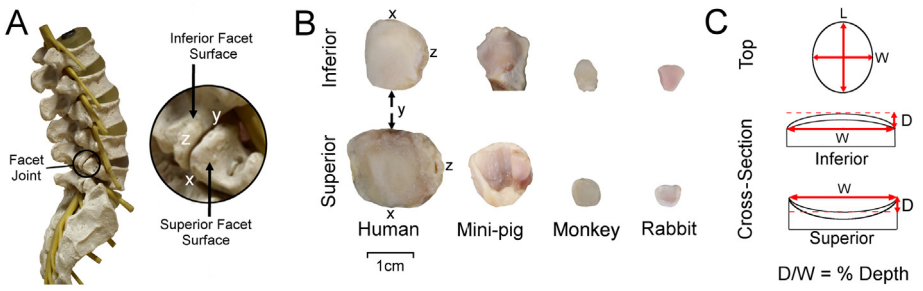

Fig. 1. Illustration of the facet joint anatomy and the gross morphological measurements of the facet joint surface. A) Location of the facet joint on the lumbar spine. Symbols x, y, and z denote the orientation of the facet joint in situ; this corresponds to the orientation of the joint once it is excised, as illustrated in (B). B) Example scaled images of the opposing inferior and superior facet joint surfaces excised between spinal levels L4 and L5 from the four species examined. C) Top view of the facet joint surface. The left to right and top to bottom arrows denote where the measurements of the width (W) and length (L) were taken for all joint surfaces, respectively. Measurements were taken at the widest and longest regions of the tissue. A cross-sectional view shows the arrows that correspond to the regions from which the W was measured for both the convex inferior and concave superior facet surfaces. A dashed line was constructed such that it ran parallel to the arrow denoting W and also through the lowest or highest point on the facet articular surface. The depth (D) is the perpendicular distance between the dashed line and the arrow. The percentage depth was calculated by normalizing D to W.

Facet OA is a universal finding in people over the age of 60 years old and affects approximately 60% of adults over the age of 30 years old [1]. Typical radiographic features of OA include joint space narrowing due to cartilage thinning, development of osteophytes and subchondral cysts, hypertrophy of the articular process, and subchondral bone sclerosis [4]. In a CT scan study of an older population (mean age 67 years old), the prevalence of moderate and severe lumbar facet joint OA was found to be approximately 86% and 50%, respectively [5]. Degeneration is most commonly found at the lower levels of the lumbar spine, i.e., L4-L5 and L5-S1; however, all spinal levels are susceptible [1,6–10].

Degeneration of the facet joint is known to play a significant role in back-related morbidities [11]. Advanced degeneration and concomitant hypertrophy of the facet joint can reduce the spinal canal size and impinge spinal neural elements, causing degenerative spinal stenosis. Spinal stenosis is the most frequently cited reason for lumbar spine surgery in the United States [12,13]. A similar trend is emerging in some European countries [14,15]. Facet joint pathology can also contribute to degenerative spondylolisthesis, where one vertebra translates with respect to the other. This condition occurs in 13.6% of the adult population [16] and contributes to both back pain and leg pain as the spinal cord or nerve roots are squeezed. In short, the facet joint is a significant source of back-related pathology.

In addition to its role in the aforementioned back-related morbidities, the facet joint is also thought to be the locus of low back pain on its own [17]. Similar to other synovial joints, the way by which pain manifests itself within the joint's structure is not well understood. However, the development of OA of this highly innervated joint has long been implicated as a potential cause of pain [5]. Despite the difficulties associated with diagnosis, the facet joints are estimated to be responsible for approximately 38% of chronic pain felt in the lower back [18]. Low back pain is currently the number one contributor to global disability [19] and is estimated to affect approximately 40% of people in their lifetime [20]. Although the prevalence of low back pain is highest between the ages of 40 and 80 years old, [20] young athletes have 3 to 5 times higher prevalence rates when compared to a general age-related population [21]. The debilitating nature of this disease has a huge impact on both the nation's health and health care system, at a total cost of approximately $200 billion per year [22,23]. Unfortunately, according to the latest global burden of disease report, the scale of the problem remains unchanged from 1990 to 2013. Furthermore, due to an aging population, low back pain has been predicted to increase in the coming years [19].

Due to the almost avascular and acellular nature of facet cartilage, it is unable to repair itself; pain alleviation is heavily dependent on medical treatment. Currently available, non-invasive treatment options only offer short-term relief. Treatments such as radiofrequency denervation, medial branch blocks, and intra-articular injections may reduce the symptoms temporarily but cannot provide a long term solution to the problem [24]. In cases of degenerative spinal stenosis and spondylolisthesis, surgical removal of the joints is often the only option. Removal of the facet joints can result in spinal instability necessitating fusion of the entire spinal segment. Like a domino effect, spinal fusion, in turn, is related to adjacent segment disease that encompasses a host of symptoms including hypertrophic facet arthritis in the neighboring vertebral segments [25]. Without suitable therapeutics, tissue engineering of the facet cartilage may serve as an attractive solution for long term motion-preserving pain management.

To date, there has only been one attempt to tissue engineer facet cartilage [26]. The paucity of work may primarily be due to the lack of published data detailing the characteristics of this tissue. In order to successfully engineer facet cartilage, it is critical that appropriate design criteria are established, which will ultimately provide the framework for the regeneration of a functional tissue replacement. Currently, there exist no experimental studies that characterize the biomechanical, biochemical, and histological properties of human cartilage, and only a few detailing the characteristics of animal facet cartilage [27,28]. With regard to the latter, the spines of quadrupeds receive different loading patterns when compared to bipeds, furthering the necessity for comparing the facet joints of humans and animals to develop suitable non-primate animal models.

Toward the long-term objective of tissue engineering facet cartilage replacements, the objectives of this study are 1) to characterize human lumbar facet cartilage and to compare it to mini-pig, monkey, and rabbit lumbar facet cartilage, using morphological, histological, biochemical, and biomechanical methods where appropriate, and 2) to compare properties according to anatomical location (i.e., spinal level and surface type) within and across species. The lumbar region of the spine was selected to study as it is associated with a high degree of pathology and is a popular target of therapeutics aimed to alleviate low back pain.

2. Materials and methods

2.1. Specimens

Human spines (n = 7, 4 female and 3 male) were obtained from Science Care and MedCure (see Table 1 for details). None of the human specimens were noted to have any known musculoskeletal pathology. Animal facet joints were harvested from the spines of

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Table 1
Patient details of human lumbar spine samples.

Spine	Age	Gender	BMI*
1	52	Female	14.5
2	71	Male	14.4
3	75	Female	48.2
4	77	Female	26.2
5	66	Male	29.3
6	80	Female	26.6
7	41	Male	20.7

* BMI was measured at the time of death and therefore may not be representative of patient's true BMI.

Yucatan mini-pigs (n = 6, all aged approximately 18 months old and male), New Zealand White rabbits (n = 5, all aged 6–8 months old and male) and Rhesus Macaque monkeys (n = 3, aged 1 (male), 6 (male), and 12 (female) years old). All animals used for this study were euthanized as part of other research studies unrelated to the musculoskeletal system. Furthermore, all cadaveric and animal facet tissues were harvested for use in this study only and were not subjected to any testing other than what is outlined in this study. Spines were isolated within 24 h of death and were frozen at −20 °C. Lumbar facet joints were accessed proximally; all muscle and soft tissue was removed from the spine, and the intervertebral disc and the facet capsule were carefully severed using a scalpel allowing the facet joints to be easily disarticulated. The facet surfaces along with the underlying bone were removed from the lumbar spine using an oscillating saw. Joints were washed with phosphate buffered saline (PBS), photographed, and wrapped in gauze soaked in PBS containing protease inhibitor (10 mM N-ethylmaleimide and 1 mM phenylmethylsulfonyl fluoride, Sigma) and frozen at −20 °C until further analysis. The length and width of each joint surface for all examined spines were measured photographically as illustrated in Fig. 1B and C. The percentage depth [29] was also calculated for the facet joints of one representative spine for each species by making a cross-sectional cut in the joint and taking measurements according to Fig. 1C. The human facet surfaces were also graded by a spine surgeon according to the International Cartilage Repair Society (ICRS) scale (Appendix A: Supplementary data 1) [30]. The average severity index was calculated for each human facet joint surface by averaging the value of the assigned grade (0 to 4) among examined spines. Mechanical testing and biochemical analyses were performed on facet joints harvested from the right and left sides of each spine, respectively. The number of facet surfaces and spinal levels examined, as well as the characterization methods they were subjected to, are found in Table 2.

2.2. Histology

Samples were fixed in 10% neutral-buffered formalin for 4 to 5 days, rinsed thoroughly in water, and decalcified using 10% formic acid. Samples were embedded in paraffin and sectioned at 6 μm. Sections were stained with hematoxylin and eosin (H&E), safranin-O, and picrosirius red as previously described [31].

2.3. Biochemistry

Facet cartilage sections were removed from the facet joints using a scalpel and weighed (wet weight). Tissues were frozen at −20 °C for ~24 h, lyophilized for ~48 h, and the dry weight was measured. Tissues were digested in 125 mg/ml papain (Sigma-Aldrich) in a phosphate buffer (2 mM N-acetyl cysteine (Sigma-Aldrich) and 2 mM EDTA) for 18 h at 60 °C. Following digestion, DNA and GAG content were measured using Picogreen Cell Proliferation Assay kit (Life Technologies) and Blyscan Glycosaminoglycan Assay kit (Biocolor), respectively. Total collagen was determined using a chloramine-T hydroxyproline assay and Sircol collagen standard (Biocolor), following hydrolysis with 2 N NaOH for 20 min at 110 °C.

2.4. Creep indentation testing

Facet joints were tested under compression creep indentation testing as previously described [32]. All cartilage samples were tested on the bone, and the thickness of the cartilage at the site of testing was determined using a thickness probe [33]. The central region of the articular surface was tested, or, for the mini-pig samples where the surface was curved, the flattest region of the tissue was selected for testing. Care was taken to ensure the same regions on opposing surfaces were tested for all joint surface pairs. Samples were submerged in PBS for at least 15 min prior to testing. A 0.5 mm indenter tip was used with a tare load of 0.075 g and a test load of 0.25 g to achieve strains in the range of 2–12%. Following testing, a semi-analytical, semi-numerical, linear biphasic model was used to approximate the aggregate modulus, shear modulus, and permeability.

2.5. Statistical analysis

Student *t*-tests were performed to compare the biomechanical and biochemical properties between the inferior and superior facet joint surfaces, for all spinal levels of the mini-pig, monkey, and rabbit spines. One way ANOVA with Tukey's *post hoc* test were used to assess biomechanical and biochemical differences among spinal levels and in the case of biomechanical properties across species (mini-pig, monkey, and rabbit). Student *t*-tests were performed to assess differences between the biochemical properties of the mini-pig and rabbit. Regarding the physical dimensions of the facet surfaces, one way ANOVA followed by Tukey's *post hoc* test were used to compare the dimensions of the inferior facet joint at each spinal level and also the superior facet joints at each spinal level for each species (human, mini-pig, rabbit, and monkey). Student *t*-tests were used to compare average dimensions of the inferior facet versus the superior facet for each species and one way ANOVA followed by Tukey's *post hoc* test were used to compare

Table 2
Interspecies sample numbers and methods used to characterize the facet cartilage.

Species	No.	Spinal levels	Joint surfaces	Morphological measurements	Compression indentation testing	Biochemical analysis	Histological analysis
Human	7	5 (L1-S1)	77	Yes	No**	Yes	Yes
Mini-pig	6	6 (L1-S1)	78	Yes	Yes	Yes	Yes
Monkey	3	5 (L1-L5)*	30	Yes	Yes	No***	Yes
Rabbit	5	8 (L1-S1)	85	Yes	Yes	Yes	Yes

* Joints at level L5/S1 were not examined for the monkey due to restricted access to spinal material.
** Human facet cartilage was pathological thus, compression indentation testing could not be performed on this tissue.
*** The Rhesus Macaque is a well-known carrier of herpes B virus. Biochemical analysis was not performed on this cartilage due to the increase in associated handling and subsequent increase in risk to the user.

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dimensions across species. For all statistical tests, a p value of less than 0.05 indicated statistical significance. In figures displaying quantitative results, groups marked by different letters are statistically different. All data are presented as means ± standard deviations.

3. Results

3.1. Gross morphology

Grading of the human facet cartilage morphology revealed that of the 154 joints examined, all (100%) showed evidence of degradation. A representative image of each human facet joint grade is illustrated in Fig. 2A and several more example images can be seen in Appendix A: Supplementary data 2. In the most severe cases, the facet cartilage had completely degraded leaving the entire underlying bone surface exposed. Other observations, included the replacement of cartilage with fibrous like tissue. In general the cartilage surface morphology was a yellow or reddish color, showing varying degrees of fibrillation, fissuring, flaking, and surface erosion that tended to span the entire surface of the joint.

All joints examined demonstrated varying degrees of degeneration and according to the ICRS scale, 72.1%, 22.7%, 3.2%, 1.9%, and 0% were considered to be grade 4, grade 3, grade 2, grade 1, and grade 0, respectively (Fig. 2B). With the exception of a single joint surface, five out of the seven examined cadavers (ages 52, 71, 75, 77, 80 years old) had facet joint surfaces graded 3 or above. The youngest cadaver (age 41 years old) had the most amount of facet joint surfaces graded 2 or below (total of 4 surfaces), followed by the 66 year old cadaver that had a total of 3 joint surfaces graded 2 or below. Comparing the severity of degradation between the inferior and superior joint surfaces (Fig. 2C) showed that in general, the superior facet joint surfaces received a higher average score than the inferior facet joint surfaces. In addition, although all levels of the spine were affected by degeneration, the superior joint surfaces located at spinal levels L4 and L5 were found to have the highest frequency of grade 4 joints.

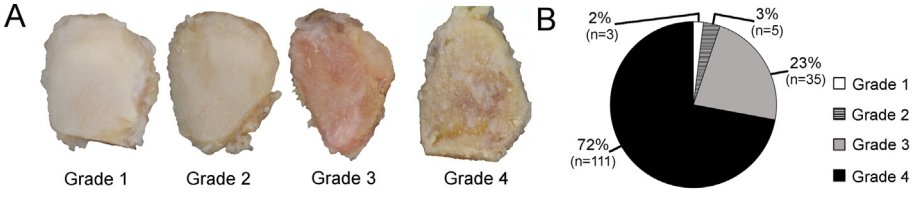
The gross morphology and average physical dimensions of the facet joints harvested from each species are represented in Fig. 1B and Fig. 3, respectively. In general, the inferior surface was found to have a more convex shape whereas the superior surface was more concave. The average length and width of the human, mini-pig, monkey, and rabbit inferior facet joints were 16.32 ± 1.95 mm and 13.91 ± 1.42 mm, 10.14 ± 2.15 mm and 7.98 ± 0.91 mm, 5.58 ± 0.70 mm and 5.43 ± 0.41 mm, and 4.75 ± 0.48 mm and 4.26 ± 0.45 mm, respectively. In terms of the superior facet dimensions, the average length and width of the human, mini-pig, monkey, and rabbit joints was 13.07 ± 1.78 mm and 16.52 ± 2.74 mm, 9.33 ± 1.34 mm and 9.63 ± 1.21 mm, 5.27 ± 0.71 mm and 5.27 ± 0.50 mm, and 3.37 ± 0.36 mm and 4.87 ± 0.52 mm. The inferior surfaces of the rabbit and the human were found to be significantly longer than the superior surfaces. Also, the superior facet joint was significantly wider in the mini-pig and rabbit, when compared to the inferior facet joint. The human facet joint average length and width for both the inferior and superior surface were significantly greater than any other species. Although the average mini-pig's facet dimensions were significantly smaller than the human, they were significantly larger than the monkey and rabbit, whose dimensions were similar.

Examining the differences in the joint's dimensions at each spinal level shows that the human facet tended to increase in both width and length for both the inferior and superior facet surfaces in the lower lumbar levels (Appendix A: Supplementary data 3). Also, the mini-pig had significantly longer inferior surface at spinal level S1 than the other spinal levels and the superior surface at spinal level S1 was also longer than the superior surfaces at spinal levels L2, L4, L5, and L6. In addition the width of the mini-pig's superior surface at spinal level S1 was significantly wider than the superior surfaces at spinal levels L1 to L4. In general, the length and width of the inferior and superior surfaces from spinal level to spinal level were not found to change significantly for the monkey and rabbit.

The average percentage depth measured for the inferior and superior surfaces of the human, mini-pig, monkey, and rabbit was 15.86 ± 2.54% and 12.89 ± 2.10%, 56.74 ± 9.65% and 72.25 ± 11.50%, 10.00 ± 3.48% and 8.46 ± 1.49%, and 14.02 ± 3.58% and 8.71 ± 3.69%, respectively (Fig. 3C). The inferior surface was found to be significantly more curved than the superior surface for the rabbit and the reverse was found to be true for the mini-pig.

3.2. Histology

A histological representation of the deleterious effects of OA of human facet cartilage is depicted in Fig. 4A. H&E staining of OA changes through grades 1, 2, 3, and 4 highlighted the complete disruption to the cartilage structure. Specifically, in grade 1, despite some minor fibrillation, the surface was largely intact and the middle and deep zones of the tissue were well preserved. In grade 2, greater surface discontinuity and disruption of the structure was observed that was propagated to the middle zone of the tissue. In grade 3, the presence of multiple vertical fissures that almost stretched to the deep zone of the tissue as well as severe surface erosion and loss of cartilage was apparent. Finally, in grade 4, com-



C

Frequency												
Surface	L1-S	L1-I	L2-S	L2-I	L3-S	L3-I	L4-S	L4-I	L5-S	L5-I	S1	Total
Grade 0	0	0	0	0	0	0	0	0	0	0	0	0
Grade 1	1	0	0	0	0	1	0	1	0	0	0	3
Grade 2	0	1	0	2	0	0	0	0	0	2	0	5
Grade 3	4	5	2	4	3	2	1	6	1	3	4	35
Grade 4	9	8	12	8	11	11	13	7	13	9	10	111
Severity Index												
	3.50 ±0.85	3.50 ±0.65	3.86 ±0.36	3.43 ±0.76	3.79 ±0.43	3.64 ±0.84	3.93 ±0.27	3.36 ±0.84	3.93 ±0.27	3.5 ±0.76	3.7 ±0.47	

Fig. 2. Human facet joint pathology grading. A) Example images of facet cartilage grades 1, 2, 3, and 4. B) Pie chart representing the distribution of grades in the examined population. C) Table describing the frequency of occurrence of grades 1 – 4 for each superior (S) and inferior (I) facet surface at each spinal level. The severity index (please see text for definition) calculated for the facet surfaces at each spinal level demonstrates that human facet cartilage is severely degenerated.

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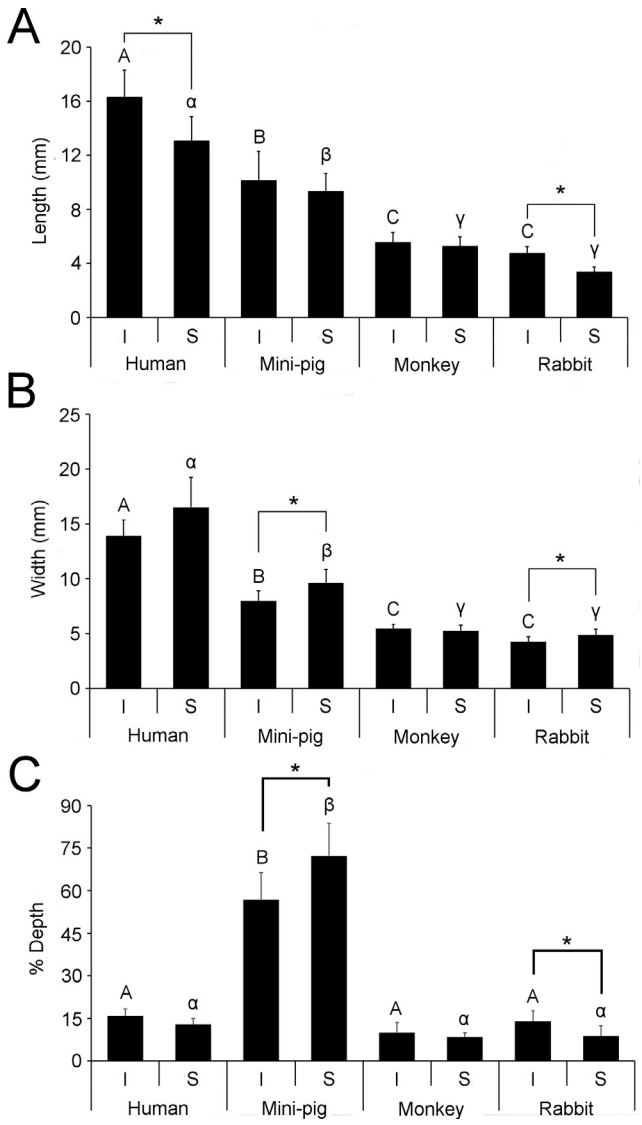

Fig. 3. Interspecies comparison of the average A) length B) width and C) percentage depth of the inferior (I) and superior (S) facet surfaces. The human facet joint is longer and wider than the other species examined and the mini-pig has a more curved facet surface compared to other species which were relatively flat. Differences between I and S in terms of the average length, width, and percentage depth within species were analyzed using a Student *t*-test and groups connected by an asterisk are significantly different. An interspecies comparison of the average dimensions of I and S facet surfaces was assessed using a one way ANOVA, followed by a Tukey's *post hoc* test, and groups not connected by the same letter or symbol are significantly different. To note, the average values for the percentage depth are based on the joints from one spine however, the average length and width are representative of 7 humans, 6 mini-pig, 3 monkey, and 5 rabbit spines.

plete denudation of unmineralized cartilage was observed leaving only calcified cartilage and/or bone present. In addition, cellular arrangement and density appear relatively normal in grade 1; however, a rapid decline in both of these metrics was observed as the disease progressed. Similarly, the rapid loss in sulfated GAG was demonstrated as the joint becomes more diseased, represented by the decrease in positive safranin-O staining from grade 1 through to grade 4.

The results of staining of the mini-pig, monkey, and rabbit facet cartilage with H&E, safranin-O, and picrosirius red are illustrated in Fig. 4B. H&E staining across all animals revealed cells that were smaller and flatter in the superficial region when compared to the intermediate and deep zones of the tissue where cells were observed to be more round and organized in a columnar fashion. All animals had positive safranin-O staining for sulfated GAGs and it appeared more intense in the middle and deep zones in comparison to the superficial zone and also highlighted a GAG-rich territorial and interterritorial matrix. A similar staining pattern and intensity was observed for the staining of collagen with picrosirius red across all animals.

3.3. Biochemistry

The GAG, collagen, and DNA content, all normalized to wet weight, were compared between the ICRS grades for each human facet joint surface (Table 3). Considering the uneven group numbers, statistical comparisons were not deemed appropriate. A similar pattern for both GAG and collagen content was observed across grades. Both properties decreased between grade 1 and 2, partially recovered between grade 2 and 3, and were finally observed to decline again between grade 3 and grade 4. The DNA content was observed to decrease between grade 1 and 2, however it steadily increased between grades 2, 3, and 4 and was considered the highest for grade 4.

In general, Student *t*-tests comparing the GAG, collagen, and DNA content, all normalized to wet weight, between opposing joint surfaces of the mini-pig and rabbit found that they were not significantly different (Appendix A: Supplementary data 4). The GAG/ww, collagen/ww, and DNA/ww content in the mini-pig and rabbit were not statistically different between spinal levels (Fig. 5). However, when the GAG content was averaged across all spinal levels, the overall GAG/ww was found to be higher in the mini-pig than in the rabbit (4.2 ± 0.4% versus 2.4 ± 0.4%). The average measured values of collagen/ww across all spinal levels were 15.77 ± 1.0% and 16.62 ± 1.2% for the mini-pig and rabbit, respectively and these values were not found to be statistically different. The averaged DNA content across spinal levels for each species was found to be higher in the rabbit than in the mini-pig (0.034 ± 0.003% versus 0.030 ± 0.003%).

3.4. Mechanical testing

In general, Student *t*-test's comparing the thickness, shear modulus, aggregate modulus, and permeability between opposing inferior and superior joint surfaces of the mini-pig, monkey, and rabbit found that they were not significantly different (Appendix A: Supplementary data 4). Furthermore, the average measured thickness, shear modulus, aggregate modulus, and permeability between spinal levels were not found to significantly different within the same species (Fig. 6). When averaged across all spinal levels the average values for the thickness, shear modulus, aggregate modulus and permeability were 0.37 ± 0.02 mm, 71.49 ± 4.92 kPa, 174.49 ± 19.64 kPa, $4.87 \pm 0.74 \times 10^{-15}$ m^4/Ns, for the mini-pig, 0.35 ± 0.01 mm, 60.82 ± 14.76 kPa, 161.17 ± 37.41 kPa, $6.27 \pm 2.71 \times 10^{-15}$ m^4/Ns, for the monkey and 0.29 ± 0.01 mm, 54.95 ± 6.39 kPa, 158.95 ± 20.22 kPa, $5.95 \pm 1.33 \times 10^{-15}$ m^4/Ns, for the rabbit. The averaged thickness and shear modulus across all levels was found to be significantly lower in the rabbit cartilage compared to the mini-pig cartilage (0.37 ± 0.02 mm versus 0.29 ± 0.01 mm and 71.49 ± 4.92 kPa versus 54.95 ± 6.39 kPa).

4. Discussion

Due to the dearth of information regarding the facet joint, as its first objective, this study sought to provide much-needed characterization data of human lumbar facet cartilage and compare to various species. Toward the second objective, facet joint properties were compared with respect to spinal level and joint surface (i.e.,

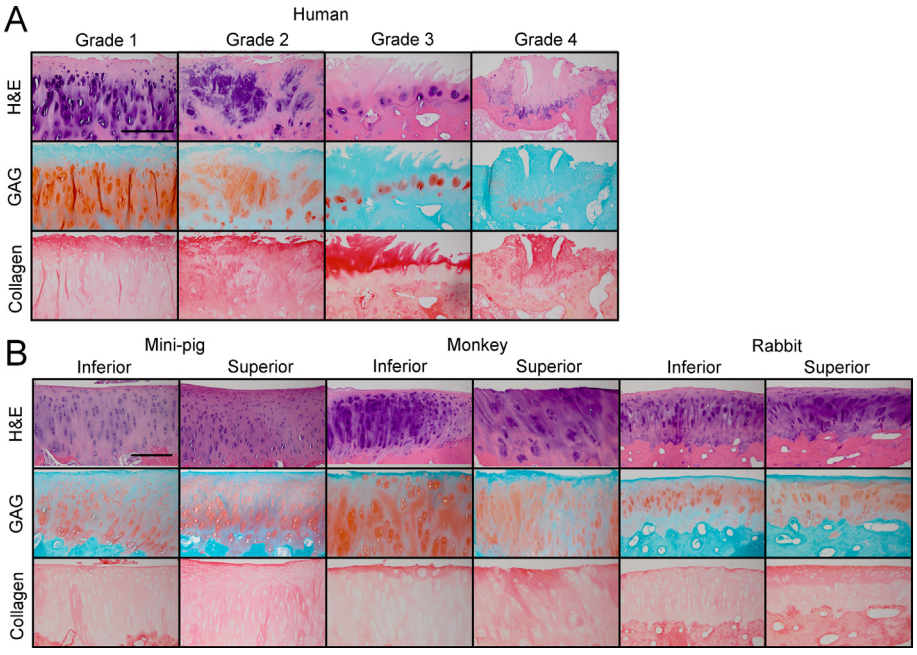

Fig. 4. Facet joint histology. Hematoxylin and eosin (H&E), safranin-O, and picrosirius red stain were used to highlight the structure, GAG, and collagen content of the facet cartilage, respectively. A) An interspecies comparison of the inferior and superior facet joint surfaces revealed that the structure is rich in GAG and collagen and has an architecture characteristic of articular cartilage. Scale bar = 500 μm. B) Histological staining of pathological human facet joint illustrated the gradual breakdown in structure and loss of GAG and collagen as the disease progressed. Scale bar = 200 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Biochemical properties of human facet cartilage as a function of degeneration severity.

Grade	1	2	3	4
Number of samples	n = 3	n = 5	n = 22	n = 75
GAG/ww (%)	2.6 ± 1.4	1.2 ± 0.3	1.4 ± 0.7	1.1 ± 0.7
Collagen/ww (%)	21.6 ± 6.4	14.5 ± 2.4	16.0 ± 2.6	15.6 ± 2.6
DNA/ww (%)	0.07 ± 0.03	0.05 ± 0.02	0.11 ± 0.06	0.12 ± 0.10

inferior versus superior) within and across species. Guided by prior literature in other joints, it was hypothesized that the characterization data would reveal differences between a) the opposing inferior and superior facet joint surfaces and b) joints of differing spinal levels. It was also hypothesized that these trends would persist across all examined species, although differing magnitudes were expected, some more similar than others to properties obtained for the human facet cartilage. Contrary to the hypothesis, results showed that biochemical and biomechanical properties were similar among spinal levels and between the inferior and superior facet joint surfaces. This finding was consistent among species, however, an unexpected high percentage of pathology was found in the human facet joints precluding in depth characterization and comparison to other animal models. Of the 154 human facet joints surfaces analyzed, 146 displayed cartilage degeneration corresponding to ICRS grades 3 or 4. This significant finding thus allowed us to provide characterization of human facet cartilage pathology as a function of ICRS grade. This study provides data that may eventually improve our understanding of functional deterioration of the three-joint complex overall. This investigation also serves as the first source of comprehensive design criteria toward the design of a replacement facet joint and also offers insights into the selection of an appropriate animal model.

The prevalence and degree of degeneration in the human lumbar facet joint were staggeringly high. Advanced pathology was found in all but 8 of the examined joint surfaces. The finding of widespread pathology was consistent with previous findings where pathology was also observed in the majority of facet surfaces. [34] [35] Since these other studies employed their own scoring scale, the calculated severity index, denoted by averaging the scores assigned to a particular surface among examined spines (Fig. 2C), was different across this and other studies. For reference, the three scoring scales are presented in Appendix A: Supplementary data 1. Of the scores assigned to surfaces from spinal levels L1 to S1 in a previous study, [34] the lowest severity index was found at L1 inferior and L3 superior (both 2.45), and the highest was found at S1 (2.92). In addition, a similar study found that scoring of facet surfaces from spinal levels L1 to L5 revealed that the superior surface of L1 exhibited the lowest score (2.80), and that the superior and inferior surfaces of L5 exhibited the highest score (3.70) [35]. In the current study, the lowest score was found for L4 inferior (3.36); and the highest score was found for L4 superior and L5 superior (3.93) (Fig. 2C). In summary, our study reported a similar prevalence in pathology but a higher degree of severity compared to other studies.

Considering the evaluation of human facet joints here and in other studies, it is clear these joints are particularly prone to degeneration. However, establishing a relationship between the cause and effect is difficult without being able to compare pathological to healthy facet cartilage. In this and other studies, healthy facet cartilage was seldom seen. This may in part be due to the fact that the mean age of the patients examined in these studies were 66 (this study), 76, [34] and 88 [35]. In this study, even a 41 year old donor's facet joints were found to be heavily diseased. Further-

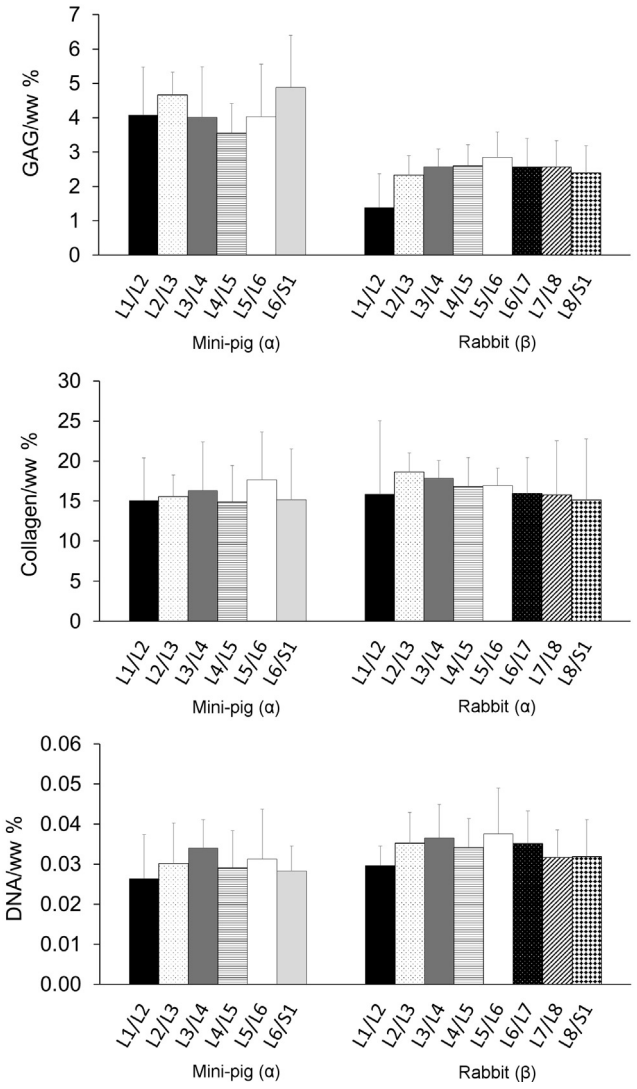

Fig. 5. Comparison of the biochemical properties of facet cartilage among spinal levels within one species and also among species. A one way ANOVA determined that there were no differences in biochemical properties of facet cartilage among spinal levels for either the mini-pig or the rabbit. Student *t*-tests were performed to detect differences in the average biochemical properties between species and species not connected by the same letter (α, β) are different. Overall, the mini-pig exhibited higher GAG content but lower DNA content than the rabbit.

more, literature estimates that 60% of people over 30 years old show evidence of degeneration and facet OA has been reported to develop as young as 15 years old [1,36]. Ideally, to compare the facet joint properties of animals and humans, the samples should be age-matched, therefore, necessitating the use of a young, yet skeletally mature population. Future work needs to access a significant number of spines from multiple ages to ascertain the age at which significant degeneration develops in the facet joint.

In the human facet joints that were characterized, the GAG and collagen contents of relatively healthy cartilage (grade 1) are comparable to those of normal human articular cartilage found elsewhere in the body (3–6% and 12–24% GAG and collagen contents, respectively). These biochemical components are also comparable to the facet cartilage of the rabbit and mini-pig reported here. The overall GAG and collagen content of facet cartilage decreased as a result of degeneration (Table 3). In comparison to the amount of GAG loss, the amount of collagen loss was less. Also the apparent increase in these properties between grades 2 and 3 may be interpreted as the tissue's attempt at repair.[37,38] The cellularity of the cartilage was also observed to increase in the later stages of disease. Loss of GAG and collagen content, and an increase in cellularity are all consistent with the progression of OA in cartilages of other joints.[39] This work shows that human facet cartilage has similar biochemical properties to those of other species and other human synovial joints. It also demonstrates that human facet cartilage follows the well-characterized progression of OA, suggesting that therapies under development for other cartilages may also hold promise for the facet.

In general, the shape of the opposing inferior and superior facet joint surfaces differ with the superior surface tending to be concave and the inferior surface tending to be convex. The extent to which these surfaces are concave and convex differs greatly among species, with the mini-pig facet joint having a remarkably larger percentage depth (similar to a smaller radius of curvature) compared to the other species (Fig. 3). The radius of curvature has previously been linked to spinal kinematics and, specifically, to the spine's ability to rotate axially and to translate. It has been found that a smaller radius of curvature is associated with a reduced range of axial rotation [29]. The degree of axial rotation and flexion/extension has previously been measured in the porcine spine and found to be significantly less than in the human spine [40]. Thus, these data suggest that the mini-pig facet joint mostly allows for articulation in a constrained fashion. Data showing that curvatures among rabbit, monkey, and human are similar concur with the descriptions of similar ranges of axial rotation among these species [41,42]. It is known that the gait of the rabbit and the monkey are different to that of the human; the rabbit and monkey swing their hind legs forward under the torso and then propel them backwards into an extended position. This may account for the small differences in the facet joint curvature observed between species. It should be noted that the degree of flexion and extension is higher for the rabbit than the human suggesting that perhaps facet radii of curvature are better correlated with rotation than flexion/extension. In terms of selecting an animal model, the dramatic differences between the mini-pig and the human facet joint should be considered. The shape of the facet, which is often intimately linked to joint biomechanics, is an important factor, and the data here suggest that the monkey or even the rabbit model may be most similar to human in terms of shape.

Interestingly, the mechanical and biochemical properties between opposing inferior and superior joint surfaces were not found be different for the animals examined. Dramatic differences in opposing articulating surfaces in other joints have been reported previously. In a study characterizing the bovine ankle joint, the tibial plafond exhibited 3-fold higher tensile properties and 2-fold higher compressive and shear moduli compared with its articulating talar dome [43]. This disparity was hypothesized as the reason for increased rates of pathology found in the talar dome compared to the tibial plafond. Within the human facet literature, there is conflict with regard to disposition of pathology, since some report a higher incidence of degeneration in the inferior facet surface [44] while others have concluded that the superior facet joint surface is more susceptible to pathology [45]. The biomechanical properties of human facet cartilage were not collected in this study due to the high incidences of pathology, and it remains unclear if disparities exist across the articulating surfaces of human facet joints. However, the absence of disparities in the animal data suggests that pathology should not be preferentially observed in either inferior or superior surfaces. Indeed, for human facet joints examined here, only a slight preference for the superior facet joint in terms of rate and severity of pathology could be found between joint surfaces. These findings may help explain why similar amounts of evidence currently exist for pathology of the inferior or superior surfaces.

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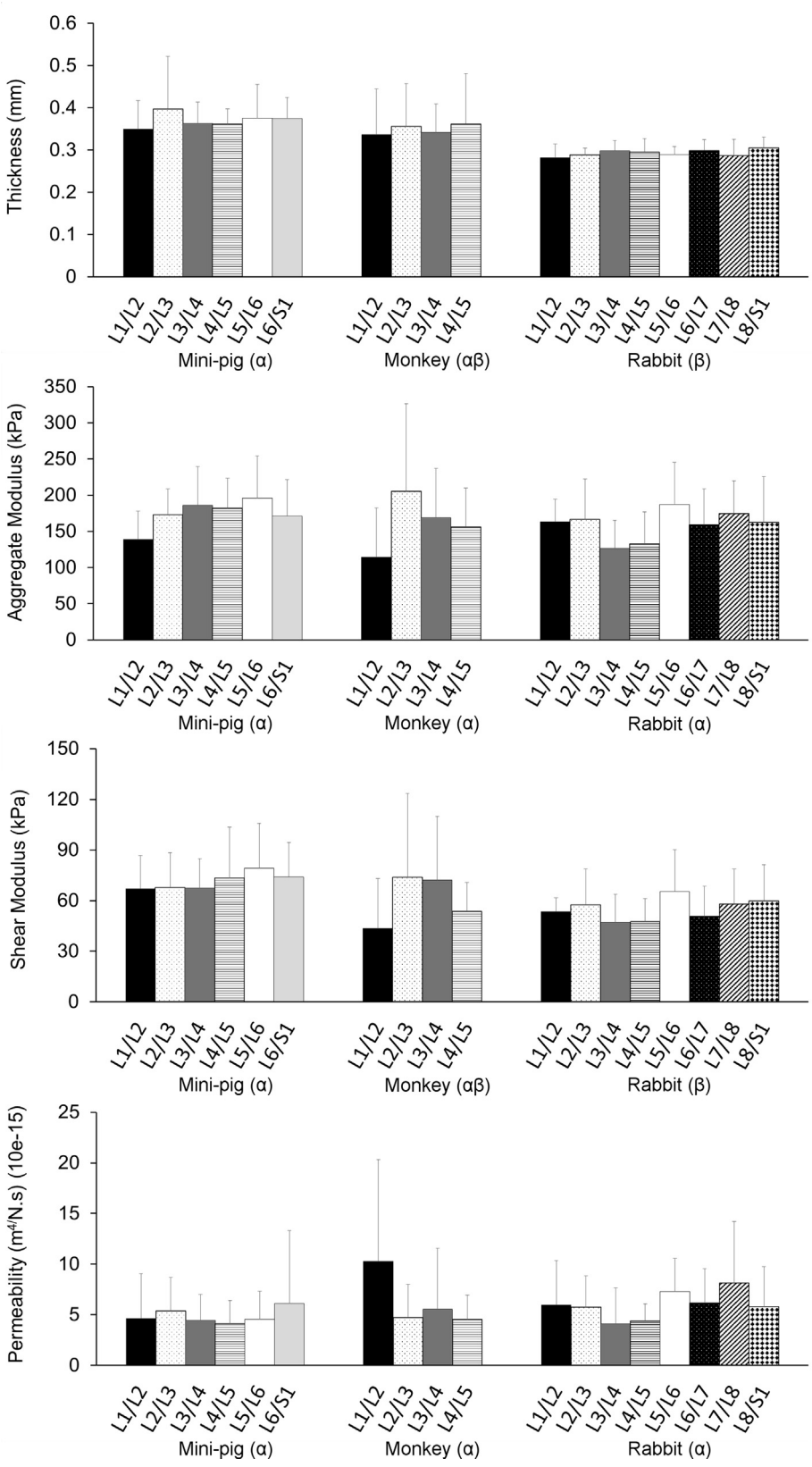

Fig. 6. Comparison of the mechanical properties of facet cartilage among spinal levels within one species and also among species. One way ANOVAs determined that there were no differences in mechanical properties of facet cartilage among spinal levels for the mini-pig, monkey, or rabbit. One way ANOVAs, followed by Tukey's *post hoc* test, were also performed to detect differences in the average mechanical properties among species and species not connected by the same symbol (α, β) are different. Overall, the mini-pig facet cartilage was found to be thicker and have a higher shear modulus than the rabbit.

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The properties of canine intervertebral disc have been shown to differ according to the lumbar spinal level, [46] a pattern that was expected to be mirrored in the facet joint. However, biochemical and biomechanical properties of the animal facet joints were not found to differ according to the spinal level. This finding is interesting considering the aforementioned changes in the disc properties as well as reported changes in the range of spinal motion according to spinal level that was observed for both humans and a wide variety of animals [47]. This suggests that different functional properties should be observed in accordance with the way the cartilage is loaded. Despite this, similar to the findings of our study, it was also reported elsewhere that there were no significant differences in biomechanical and biochemical properties between the canine facet joints at spinal levels L4 and L5 [48]. Though the properties are similar across all spinal levels, the lower lumbar facet joints are usually the most problematic and the target of both therapeutic and surgical treatments. Taken together with the epidemiological literature, data collected here suggest that facet pathology is not likely due to inherent cartilage properties. Rather, it is likely the differences in loading experienced in these lower joints, or, perhaps, the high susceptibility of disc degradation at these spinal levels, that render the lower lumber facet joints targets of treatment.

The reported compressive stiffness of facet cartilage, though not different for the mini-pig, rabbit, and monkey, is substantially lower than in other joints within the same species. For example, the aggregate modulus values of the rabbit and monkey (cynomolgus) knee cartilage have been reported to be approximately 600 kPa and 700 kPa, respectively, [32] versus an average of approximately 159 and 161 kPa for the facet cartilage reported in this study. The compressive stiffness of the healthy human facet cartilage was not measured due to the presence of disease, however, given this trend in other species, it is likely that it, too, possesses a lower aggregate modulus than cartilage of the human knee joint, which has an aggregate modulus of approximately 607 kPa [32]. Taken together, the findings of this interspecies study suggest that the facet joint may not be subjected to high compressive loads *in vivo* compared to other joints of the body. This is consistent with literature reporting that the intervertebral disc is primarily responsible for supporting the compressive loads of the spine, [49] and, therefore, the facet joints may be shielded from high compressive loads. This finding may help to further elucidate the role of the facet joint and its contribution to spinal biomechanics and also supports the idea that quadrupeds may be suitable animal models. The expectation is that the bipedal model would be the gold standard animal model for studying the facet joint; however, in terms of facet mechanical properties, the rabbit and mini-pig may also be suitable.

The design criteria for tissue engineering a facet joint replacement may be more easily attainable than other joints, such as the knee, considering the results of this study. The facet joint has a small surface area in comparison to other articular surfaces [32]. Therefore, it may be possible to tissue engineer the entire surface of the facet joint, which would avoid any of the well-known integration issues between the native and engineered cartilage. Furthermore, the facet cartilage is thinner than cartilage of other joints, and, therefore, a full thickness replacement would not be as challenging to engineer. Since both the compressive loads borne by facet joints and the compressive stiffness of facet cartilage are lower than other joints, a biomimetic cartilage replacement may be more readily achieved. The fact that the properties of facet cartilage are not different between the opposing joint surfaces or between the spinal levels is also advantageous since this negates the need to design site-specific replacements. For example, using a scaffoldless approach, self-assembled cartilage constructs have been formed with compressive aggregate modulus values ranging from 100–400 kPa, [43,50–52] shear modulus of 45–76 kPa, [43] and thickness between 260 and 950 μm [52,53]. Furthermore, self-assembled neocartilage has biochemical properties akin to the facet joint, possessing 2–5% GAG/ww [43,52,54] and 15–20% collagen/ww [52]. Given that engineered cartilage already exhibits similar properties as native facet cartilage, engineering the entire facet joint may be a worthy aspiration in providing novel therapeutics to this oft-degenerated joint.

In conclusion, this study will serve as the first database of human and interspecies facet joint properties. The data reported here will help to further elucidate the role of the facet joint in spinal biomechanics as well as increase our general understanding of this ill-described joint. This study also provides valuable information regarding the selection of an appropriate animal model. The methods reported here could be used to assess the potential of other large animal models such as sheep and goats. The universal finding of degeneration in examined human facet joints further underscores the importance of this joint and its potential contribution to back-related morbidities. Importantly, this work adds to the literature by providing morphological, histological, biomechanical, and biochemical data for bipedal and quadrupedal animals which will aid in future determinations of suitable animal models as well as new treatment modalities.

Disclosures

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actbio.2017.03.017.

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