

Biomechanical, biochemical, and histological characterization of canine lumbar facet joint cartilage

Laboratory investigation

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Object. Tissue engineering appears to be a promising strategy for articular cartilage regeneration as a treatment for facet joint arthritis. Prior to the commencement of tissue engineering approaches, design criteria must be established to determine the required functional properties of the replacement tissue. As characterization of the functional properties of facet joint cartilage has not been performed previously, the objective of this study was to determine the biomechanical, biochemical, and histological properties of facet joint cartilage.

Methods. The *in vitro* testing was conducted using 4 lumbar spinal segments obtained from skeletally mature canines. In each specimen, articular cartilage was obtained from the superior surface of the L3–4 and L4–5 facet joints. Creep indentation was used to determine the compressive biomechanical properties, while uniaxial tensile testing yielded the Young modulus and ultimate tensile strength of the tissue. Additionally, biochemical assessments included determinations of cellularity, glycosaminoglycan (GAG) content, and collagen content, as well as enzyme-linked immunosorbent assays for collagen I and II production. Finally, histological characterization included H & E staining, as well as staining for collagen and GAG distributions.

Results. The means \pm standard deviation values were determined. There were no differences between the 2 spinal levels for any of the assessed properties. Averaged over both levels, the thickness was 0.49 ± 0.10 mm and the hydration was $74.7 \pm 1.7\%$. Additionally, the cells/wet weight (WW) ratio was $6.26 \pm 2.66 \times 10^4$ cells/mg and the cells/dry weight (DW) ratio was $2.51 \pm 1.21 \times 10^5$ cells/mg. The GAG/WW was 0.038 ± 0.013 and the GAG/DW was 0.149 ± 0.049 mg/mg, while the collagen/WW was 0.168 ± 0.026 and collagen/DW was 0.681 ± 0.154 mg/mg. Finally, the aggregate modulus was 554 ± 133 kPa, the Young modulus was 10.08 ± 8.07 MPa, and the ultimate tensile strength was 4.44 ± 2.40 MPa.

Conclusions. To the best of the authors' knowledge, this study is the first to provide a functional characterization of facet joint articular cartilage, thus providing design criteria for future tissue engineering studies.

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KEY WORDS • facet joint • cartilage • biomechanical testing • tissue engineering • collagen

LOWER back pain has arisen as the second most common reason for a physician visit in the US, with a lifetime prevalence of 11–84%.²⁸ Lower back pain places a significant burden on the healthcare system, with \$100–200 billion spent annually on its treatment.¹⁸ As a result of the loads imparted on the facet joints, particularly in the lumbar spine, as well as the joint's dense innervation, the facet joint can be a significant source of disability, as reviewed in detail in a previous study.¹⁷

Facet joints are synovial joints located on the posterolateral region of the vertebrae. The joints provide

stabilization during flexion and extension, and prevent axial rotation of the vertebral unit. It has previously been determined that the facet joint transmits 3–25% of the spinal load normally, and as much as 47% of the load in an arthritic joint,³⁰ resulting in significant compressive and tensile forces applied to the hyaline articular cartilage surface. Additionally, it was demonstrated that facet arthrosis is most prevalent at the L3–4 and L4–5 facet levels, with a lifetime prevalence of 72 and 79%, respectively.¹¹ The predominant clinical treatments include intraarticular facet joint injections, medial branch nerve

Abbreviations used in this paper: DW = dry weight; GAG = glycosaminoglycan; UTS = ultimate tensile strength; WW = wet weight.

This article contains some figures that are displayed in color online but in black and white in the print edition.

blocks, and radiofrequency neurotomy; however, these treatments generally result in only short-term pain relief, as detailed in a prior review.⁵

Because there are no current long-term repairs for facet joint arthritis, tissue engineering appears to be a promising approach for facet joint cartilage regeneration. Typically, biochemical and biomechanical tissue characterization is required to establish design criteria for tissue engineering strategies which aim to form tissue with functional properties approaching those of native tissue. However, studies characterizing the biochemical or biomechanical properties of facet joint cartilage are lacking.

Therefore, the objective of this study was to characterize the functional properties of canine facet joint cartilage to yield design criteria for eventual tissue regeneration. To accomplish this objective, the cellularity, GAG, and collagen content and distribution, as well as compressive and tensile biomechanical properties, were measured at both the L3–4 and L4–5 levels. These levels were selected because they represent the most prevalent areas of facet joint arthritis, and would therefore probably be the predominant areas requiring replacement tissue.

Methods

Specimen Preparation

Cartilage was harvested from the superior surface of L3–4 and L4–5 facet joints of 2–4-year-old male mongrel dogs (Figs. 1a and b) that were killed at the end of research studies not involving the musculoskeletal system or spine. No animal had clinical evidence of facet joint osteoarthritis or disc disease. Each facet joint was opened proximally using a hacksaw. The cartilage was separated from the subchondral bone with a scalpel. Histological staining was used to verify separation of the chondral and bone tissue, and a cryotome was used to verify uniform thickness of the tissue sample prior to biomechanical testing. Tissue from the left side was used for biochemical and histological analyses, while tissue from the right side was used for compressive and tensile biomechanical analyses. After harvesting, the tissue was wrapped in gauze, soaked in normal saline with protease inhibitors (EDTA, benzamidine HCl, *N*-ethylmaleimide, and phenylmethylsulfonylfluoride), and frozen until testing. Each sample was subjected to 1 freeze-thaw cycle.

Histological Testing

Samples were frozen and sectioned at 14 μ m. A safranin-O/fast green stain^{24,25} was used to examine GAG distribution. Picrosirius red was used for the examination of collagen content, and H & E staining was used to examine the cellular distribution.

Quantitative Biochemistry

The WW of the sample was recorded, after which the samples were frozen overnight and lyophilized for 72 hours. After the DW was recorded, the sample was resuspended in 0.8 ml of 0.05 M acetic acid with 0.5 M NaCl and 0.1 ml of a 10 mg/ml pepsin solution (Sigma) at 4°C

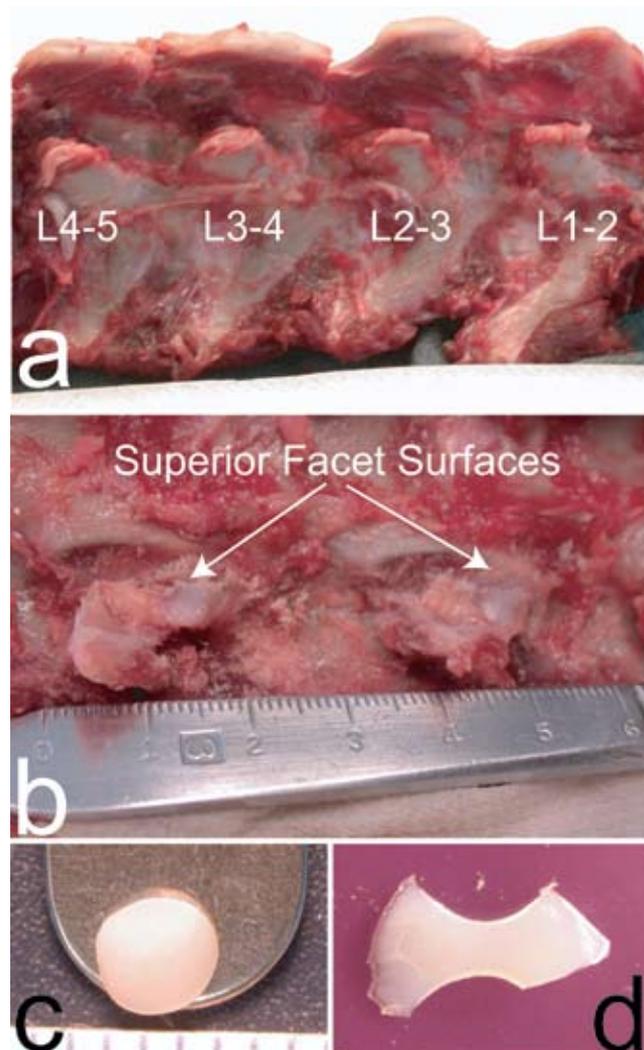


FIG. 1. Photographs showing the gross morphological properties of lumbar spine prior to dissection (a), superior facet surfaces following dissection (b), a 3-mm sample for compressive testing (c), and dog-bone shaped sample for tensile testing (d).

for 5 days. After digestion with pepsin, 0.1 ml pancreatic elastase (Sigma) was added in 1× Tris-HCl buffer solution and mixed at 4°C for 3 days. The DNA content was measured using a Picogreen Cell Proliferation Assay Kit (Molecular Probes). Total sulfated GAG was measured using the Blyscan Glycosaminoglycan Assay kit (Bioscolor), based on 1,9-dimethylmethylene blue binding.^{7,23} After hydrolysis with 2N NaOH for 20 minutes at 110°C, total collagen content was assessed with a chloramine-T hydroxyproline assay.²⁹ Enzyme-linked immunosorbent assays for collagen Types I and II were performed according to the manufacturer's protocol (Chondrex).

Indentation Testing

Compressive properties were evaluated using an automated indentation apparatus on a 3-mm-diameter sample (Fig. 1c).¹ A step mass of 2 g (0.02 N) was applied with a 1-mm flat-ended, porous indenter tip, and specimens

Functional characterization of lumbar facet joint cartilage

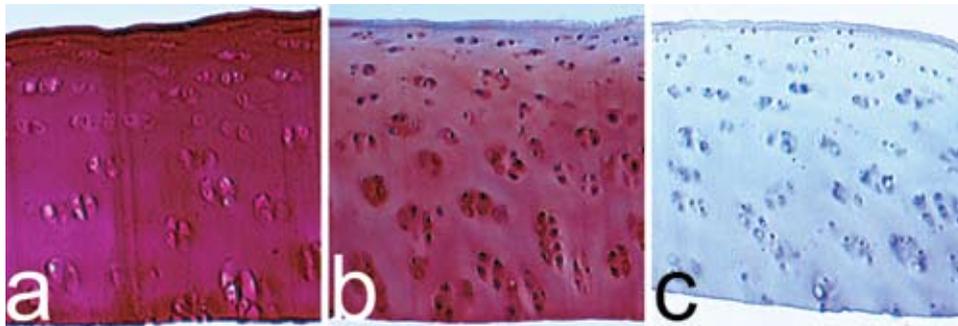


FIG. 2. Photomicrographs of sections representative of both lumbar spine levels. Picrosirius red (a) and safranin-O (b) stains reveal extensive production of collagen and GAG, respectively, and H & E stain (c) demonstrates tissue cellularity. Original magnification $\times 10$

were allowed to creep until equilibrium, as previously described.¹⁴ These conditions resulted in test strains ranging from 3 to 8%. Using the analytical solution for the axisymmetric Boussinesq problem with Papkovitch potential functions, preliminary estimations of the aggregate modulus of the samples were obtained.^{13,26} Using the linear biphasic theory, the intrinsic biomechanical properties of the samples, including aggregate modulus, Poisson ratio, and permeability were calculated.²²

Tensile Testing

Samples were cut into a dog-bone shape (Fig. 1d) and clamped outside of the gauge length. A micrometer was used to obtain gauge length, thickness, and width measurements, as well as to confirm uniform sample thickness throughout the gauge length. Tensile tests were performed using a uniaxial materials testing system (Instron Model 5565) with a 50-N load cell as described previously.⁴ The samples were pulled at a constant strain rate of 0.01 s^{-1} , and all samples broke within the gauge length. The Young modulus was calculated from the linear region of the stress-strain curve, and the UTS was the maximum stress during the test.

Statistical Analysis

All 4 samples were assessed biochemically and biomechanically. For each assessment, the Student t-test was used to determine statistical differences ($p < 0.05$). If no significant difference was found, the 2 samples from each animal were averaged together, and the mean and SD was then calculated for these 4 averaged values. All data are reported as means \pm SDs.

Results

Gross Appearance and Histological Characteristics

There was no difference between L3–4 and L4–5 cartilage in thickness, with values of 0.52 ± 0.10 and 0.46 ± 0.11 mm, respectively. The thickness averaged over both levels was 0.49 ± 0.10 mm. Additionally, there was no difference between L3–4 and L4–5 in hydration, with values of $74.8 \pm 2.7\%$ and $74.5 \pm 1.0\%$, respectively. The hydration averaged over both levels was $74.7 \pm 1.7\%$.

Samples from both levels stained positively for col-

lagen (Fig. 2a) and GAG (Fig. 2b) throughout their thickness. Additionally, H & E staining (Fig. 2c) demonstrated round cells with a columnar orientation in the deep zone of the cartilage, transitioning to more spindle-shaped cells in the superficial region of the tissue.

Quantitative Biochemical Findings

There was no difference in cellularity between L3–4 and L4–5 cartilage, with cells/WW values of $5.59 \pm 2.48 \times 10^4$ and $6.94 \pm 3.14 \times 10^4$ cells/mg, and cells/DW values of $2.27 \pm 1.13 \times 10^5$ and $2.76 \pm 1.36 \times 10^5$ cells/mg, respectively (Fig. 3). Averaged over both levels, the cells/WW ratio was $6.26 \pm 2.66 \times 10^4$ and the cells/DW ratio was $2.51 \pm 1.21 \times 10^5$ cells/mg. There was no difference in collagen content between the 2 levels, with collagen/WW values of 0.161 ± 0.039 and 0.169 ± 0.016 mg/mg, and collagen/DW values of 0.648 ± 0.196 and 0.655 ± 0.075 mg/mg, for the L3–4 and L4–5 cartilage, respectively (Fig. 4). Averaged over both levels, the collagen/WW ratio was 0.168 ± 0.026 and collagen/DW was 0.681 ± 0.154 mg/mg. There was no difference in GAG content between the 2 levels, with GAG/WW values of 0.035 ± 0.022 and 0.041 ± 0.007 mg/mg, and GAG/DW values of 0.138 ± 0.078 and 0.161 ± 0.033 mg/mg for L3–4 and L4–5 tissue, respectively (Fig. 5). Averaged over both levels, the GAG/WW was 0.038 ± 0.013 and

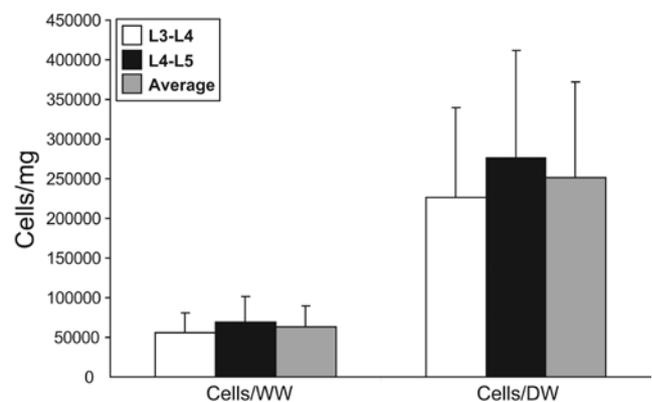


FIG. 3. Bar graph of tissue cellularity per milligram of tissue.

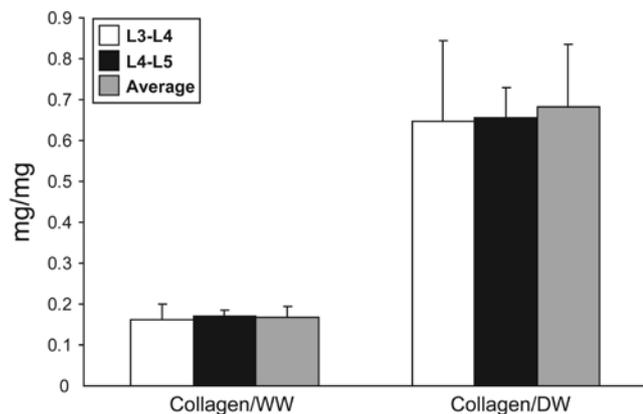


FIG. 4. Bar graph of collagen content (in mg) per milligram of tissue.

the GAG/DW was 0.149 ± 0.049 mg/mg. Furthermore, there was no collagen I production measured in cartilage from either level. Finally, there was no difference in collagen II content between the 2 levels, with collagen II/WW values of 0.123 ± 0.027 and 0.140 ± 0.071 mg/mg, and collagen II/DW values of 0.500 ± 0.168 and 0.543 ± 0.097 mg/mg, for the L3–4 and L4–5 tissue, respectively. Averaged over both levels, the collagen II/WW was 0.134 ± 0.016 and the collagen II/DW was 0.544 ± 0.123 mg/mg.

Biomechanical Evaluation

There was no difference in aggregate modulus between the L3–4 and L4–5 tissue, with values of 488 ± 144 and 620 ± 252 kPa, respectively (Fig. 6). Averaged over both levels, the aggregate modulus was 554 ± 133 kPa. Additionally, there were no differences in Poisson ratio or permeability between the 2 levels, with ranges of 0.263–0.275 and $2.88\text{--}3.82 \times 10^{-15}$ m⁴/N·s, respectively.

There was no difference in Young modulus between the L3–4 and L4–5 tissue, with values of 9.92 ± 10.93 and 10.25 ± 6.48 MPa, respectively (Fig. 7). Averaged over both levels, the Young modulus was 10.08 ± 8.07 MPa. Additionally, there was no difference in UTS between the L3–4 and L4–5 tissue, with values of 4.27 ± 3.23 and 4.61 ± 2.15 MPa, respectively. Averaging over both levels, the UTS was 4.44 ± 2.40 MPa.

Discussion

As we continue our efforts to pursue tissue engineering approaches for facet joint cartilage regeneration, it is apparent that the scant data concerning the characteristics of healthy facet cartilage tissue will be a hindrance. Therefore, the objective of this study was to determine the histological, biochemical, and biomechanical composition of facet joint cartilage to yield functional design criteria for future tissue engineering studies. It was demonstrated that L3–4 and L4–5 cartilage had equivalent properties, thus indicating similar functional requirements at both levels.

Histological examination of the facet cartilage tissue demonstrated the typical zonal characteristics of hyaline

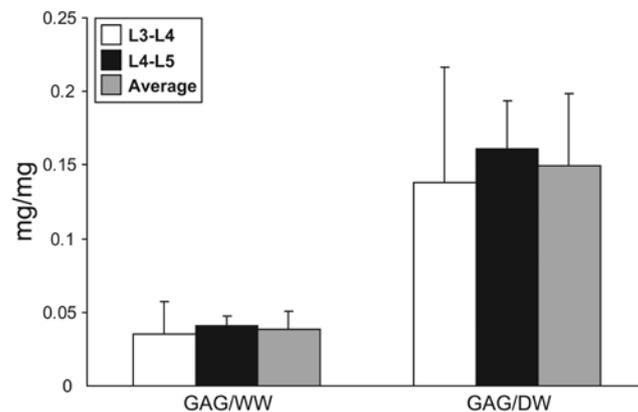


FIG. 5. Bar graph showing GAG content (in mg) per milligram of tissue.

articular cartilage, although some subtle differences from other tissues were apparent. For instance, the deeper zone of the tissue demonstrated a somewhat random chondrocyte orientation, with less of a columnar arrangement than has been noted in other joints. Interestingly, the appearance of the joint is somewhat similar to that of the second metatarsal intermediate cuneiform articulation,²¹ suggesting that facet joint cartilage may be less weight-bearing than cartilage tissue of other joints. This is supported by the tissue's relatively high Poisson ratio (a measure of the tissue's apparent compressibility), although its low permeability suggests that the tissue can still withstand and disperse applied loads.

Although the histological properties of the facet cartilage were slightly different than those observed in other joints, the functional tissue properties are relatively similar to canine articular cartilage from other joints. For instance, canine tissue from the lateral condyle of the knee was found to have an aggregate modulus of 603 ± 237 kPa, and tissue from the patellar groove had an aggregate modulus of 555 ± 144 kPa; however, tissue from the medial condyle had a significantly higher aggregate modulus, at 904 ± 218 kPa.³ Additionally, the compressive properties of the canine shoulder cartilage were similar to those of the facet joint, with aggregate moduli of 710 ± 260 and 670 ± 220 kPa for humeral and glenoid tissue, respectively.¹⁹ However, canine hip cartilage has been shown to possess slightly stiffer compressive properties, with a reported range of 480–1050 kPa, depending on the location.² These differences in material properties may suggest that the facet joint is exposed to lower compressive loads than some regions of the hip or medial condyle; this is expected as the intervertebral disc is believed to protect the facet joint from a significant portion of the compressive load.¹² However, the relatively high tensile properties, at the upper end of those reported for native cartilage tissue, suggest that facet cartilage is still exposed to significant tensile loads.

The biochemical properties of the facet cartilage correlated with the observed biomechanical properties. It is generally believed that the compressive properties of articular cartilage are related to the tissue's GAG content, while the tensile properties are related to the collagen

Functional characterization of lumbar facet joint cartilage

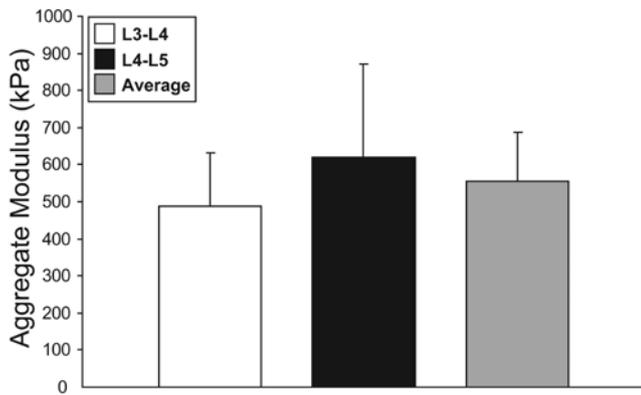


FIG. 6. Bar graph of facet cartilage compressive properties.

content. As reviewed previously,¹⁵ articular cartilage generally has a GAG/WW of 5–10%, and a collagen/WW of ~15%. The GAG/WW of 3.5–4.1% in this study correlated with the relatively low compressive properties, while the collagen/WW of 16.1–16.9% correlated with the relatively high tensile properties. The collagen II/WW of 13.4% was slightly lower than the total collagen/WW of 16.8%. This discrepancy has been observed previously and may be the result of the significant differences between the assays used to measure these parameters, as collagen II content is assessed with an enzyme-linked immunosorbent assay while total collagen is assessed with a hydroxyproline assay. Moreover, it is possible that the total collagen assay measures other types of collagen that are probably present in small amounts in the tissue, including collagen VI, IX, and XI, and elastin. Additionally, facet cartilage appeared to be in the range of the thickness of cartilage of other joints: hip cartilage has a thickness of 0.38–0.60 mm; knee cartilage, 0.52–0.90 mm; and shoulder cartilage, 0.47–0.71 mm.

In the present study we used a skeletally mature canine model to examine the functional properties of facet joint articular cartilage; this model is not entirely representative of the human spine as it represents a quadruped rather than a biped animal, thus presumably resulting in different loading conditions. However, this model was selected because it has been used extensively in prior studies of lumbar spine biomechanics that suggest the canine is an acceptable model.^{6,8,16,20,27} Moreover, Lim et al.²⁰ compared loading conditions between the human and canine lumbar spine and found similar stress distributions and facet contact forces; it was further determined that a pedicle fixation device resulted in similar stress-shielding profiles for both species.

A limited number of studies have been performed comparing cartilage in canines and humans. In a prior study, the biomechanical properties of knee joint cartilage obtained in canines and humans were assessed at multiple locations, and compared to data in bovines, monkeys, and rabbits as well.³ It is important to note that regardless of the location tested, there were no significant differences between the canine and human tissue in terms of compressive stiffness. Furthermore, the authors of an additional study demonstrated that there were no signifi-

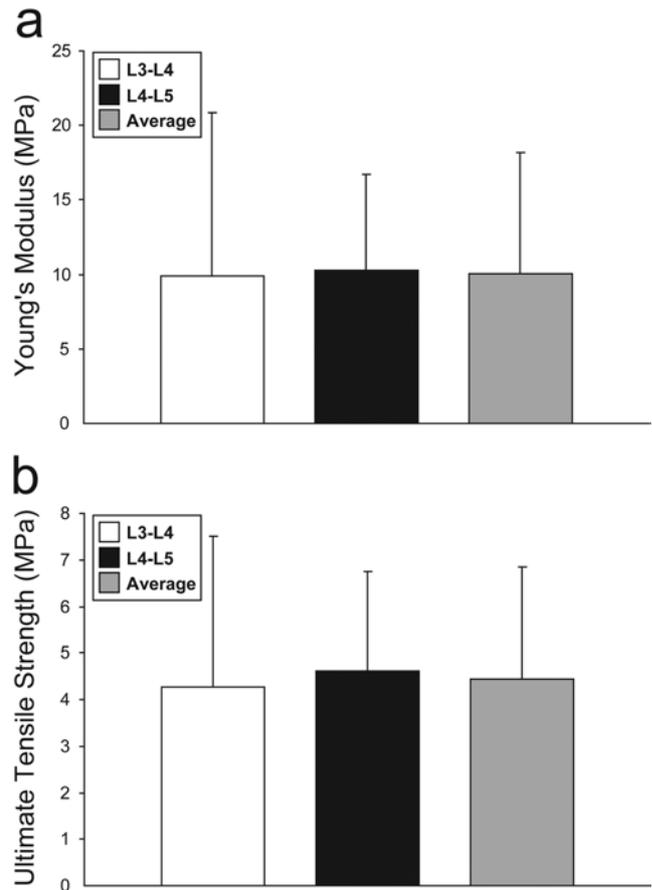


FIG. 7. Bar graphs of facet cartilage tensile properties: Young modulus (a) and UTS (b).

cant differences in compressive stiffness between canine and human femoral head cartilage in the anterior and posterior regions, although a slight difference was noted in the inferior and superior regions.² Although these prior studies were performed in the knee and hip joints, respectively, it is possible that the similarities in cartilage properties extend to facet joint cartilage. The canine data presented here may therefore be representative of human facet cartilage studies, although a characterization of human tissue is needed to definitively identify these functional properties.

The results of this study are especially exciting as they indicate that an engineered articular cartilage replacement tissue with functional properties matching those of the facet joint may be attainable in the near future. For instance, in a prior study using a scaffoldless approach to articular cartilage tissue engineering,⁹ we have already achieved biochemical properties matching those of the facet joint, with GAG/WW values of 5–10% and collagen/WW values > 15%. Additionally, engineered constructs have been created with biomechanical properties approaching those of the facet joint, with an aggregate modulus exceeding 300 kPa,¹⁰ and a Young modulus exceeding 2 MPa.⁹

Conclusions

As tissue engineering efforts strive to replicate or approach the functional properties of native tissue, knowledge of the precise characteristics of facet joint cartilage is crucial to enable tissue regeneration. The articular cartilage of other joints has been extensively characterized; however, prior to the present study, the functional properties of facet joint cartilage were unknown. Although additional studies will ideally compare our results in canines to those in the human lumbar spine to confirm that the tissues share similar properties, the results of this study are important as they establish baseline values for the functional properties of lumbar facet joint cartilage.

Disclosure

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