Published in final edited form as:
Neurosurgery. 2010 April ; 66(4): 722–727. doi:10.1227/01.NEU.0000367616.49291.9F.

Biomechanical, Biochemical, and Histological Characterization of Canine Lumbar Facet Joint Cartilage

Benjamin D. Elder, Ph.D.[1], **Krishanthan Vigneswaran, B.S.**[1,2], **Kyriacos A. Athanasiou, Ph.D., P.E.**[1], and **Daniel H. Kim, M.D.**[1,2,*]

[1]Department of Bioengineering, Rice University Houston, Texas

[2]Department of Neurosurgery, Baylor College of Medicine Houston, Texas

Abstract

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 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 four lumbar spinal segments obtained from skeletally mature canines. In each specimen, articular cartilage was obtained from the superior surface of the L3–L4 and L4–L5 facet joints. Creep indentation was used to determine the compressive biomechanical properties, while uniaxial tensile testing yielded the Young's modulus and ultimate tensile strength of the tissue. Additionally, biochemical assessments included determinations of cellularity, GAG content, and collagen content, as well as ELISAs for collagen I and II production. Finally, histological characterization included H&E staining, as well as staining for collagen and GAG distributions.

Results—The mean ± standard deviation values were determined. There were no differences between the two 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±1.7%. Additionally, the cells/WW ratio was $6.26\pm2.66 \times 10^4$ cells/mg and the cells/DW ratio was $2.51\pm1.21 \times 10^5$ 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's modulus was 10.08±8.07 MPa, and the ultimate tensile strength was 4.44±2.40 MPa.

Conclusions—To the best of our 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.

Keywords

Facet joint; cartilage; biomechanical testing; tissue engineering; collagen

[*]Corresponding Author: Daniel H. Kim, Department of Neurosurgery, 1709 Dryden, Suite 750, Houston, TX 77030, Phone: (713) 798-4696, Fax: (713) 798-3739, dhkim@bcm.edu.

INTRODUCTION

Lower back pain (LBP) has arisen as the second most common reason for a physician visit in the U.S., with a lifetime prevalence of 11–84%.[28] LBP places a significant burden on the healthcare system, as $100–200 billion is 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 morbidity, as reviewed in detail previously.[17]

The 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 from 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 in the L3–L4 and L4–L5 facet levels, with a lifetime prevalence of 72% and 79%, respectively.[11] The predominant clinical treatments include intra-articular facet joint injections, medial branch nerve blocks, as well as radiofrequency neurotomy; however, these treatments generally result in only short-term pain relief, as detailed in a prior review.[5]

As 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–L4 and L4–L5 levels. These levels were selected as they represent the most prevalent areas of facet joint arthritis, and would therefore likely represent the predominant areas requiring a replacement tissue.

MATERIALS AND METHODS

Specimen Preparation

Cartilage was harvested from the superior surface of L3–L4 and L4–L5 facet joint cartilage of 2–4 year-old male mongrel dogs (Figs. 1a, b), euthanized at the end of research studies not involving the musculoskeletal system or spine. No animals had clinical evidence of facet joint osteoarthritis or disc disease. Each facet joint was opened proximally using a hack saw. The cartilage was separated from the subchondral bone with a scalpel. Histologic 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. Following harvest, 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 one freeze-thaw cycle.

Histology

Samples were frozen and sectioned at 14 μm. A safranin-O/fast green stain[24,25] was used to examine GAG distribution. Picrosirius red was used for the examination of collagen content. An H&E stain was used to examine the cellular distribution.

Quantitative Biochemistry

The wet weight of the sample was recorded, after which the samples were frozen overnight and lyophilized for 72 hrs. After recording the dry weight, the sample was re-suspended 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 for 5 days. Following digestion with pepsin, 0.1 mL pancreatic elastase (Sigma) was added in 1x TBS, and mixed at 4°C for 3 days. The DNA content was measured with a Picogreen® Cell Proliferation Assay Kit (Molecular Probes, Eugene, OR). Total sulfated GAG was measured using the Blyscan Glycosaminoglycan Assay kit (Biocolor), based on 1,9-dimethylmethylene blue binding.[7,23] Following hydrolysis with 2 N NaOH for 20 min at 110°C, total collagen content was assessed with a chloramine-T hydroxyproline assay.[29] ELISAs for collagen type I and for collagen type II were performed according to the manufacturer's protocol (Chondrex, Redmond, WA).

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 were allowed to creep until equilibrium, as previously described.[14] These conditions resulted in test strains ranging from 3–8%. Using the analytical solution for the axisymmetric Boussinesq problem with Papkovich 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's 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, Canton, MA) 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's modulus was calculated from the linear region of the stress-strain curve, and the ultimate tensile strength (UTS) was the maximum stress during the test.

Statistical Analysis

All samples were assessed biochemically and biomechanically (n=4). For each assessment, a Student's t-test was used to determine statistical differences ($p<0.05$). If no significant difference was found, the two samples from each animal were averaged together, and the average and standard deviation was then calculated for these four averaged values. All data are reported as mean ± s.d.

RESULTS

Gross Appearance and Histology

There was no difference between L3–L4 and L4–L5 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–L4 and L4–L5 in

hydration, with values of 74.8±2.7 % and 74.5±1.0 %, respectively. The hydration averaged over both levels was 74.7±1.7 %.

Samples from both levels stained positively for collagen (Fig. 2a) and GAG (Fig. 2b) throughout their thickness. Additionally, the H&E stain (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 Biochemistry

There was no difference in cellularity between L3–L4 and L4–L5 cartilage, with cells/WW values of $5.59\pm2.48 \times 10^4$ and $6.94\pm3.14 \times 10^4$ cells/mg, and cells/DW values of $2.27\pm1.13 \times 10^5$ and $2.76\pm1.36 \times 10^5$ cells/mg, respectively (Fig. 3). Averaged over both levels, the cells/WW ratio was $6.26\pm2.66 \times 10^4$ and the cells/DW ratio was $2.51\pm1.21 \times 10^5$ cells/mg. There was no difference in collagen content between the two 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–L4 and L4–L5 cartilage, respectively (Fig. 4). Averaging over both levels, the collagen/WW was 0.168±0.026 and collagen/DW was 0.681±0.154 mg/mg. There was no difference in GAG content between the two 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 the L3–L4 and L4–L5 tissue, respectively (Fig. 5). Averaged over both levels, the GAG/WW was 0.038±0.013 and 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 two 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–L4 and L4–L5 tissue, respectively. Averaged over both levels, the collagen II/WW was 0.134±0.016 and 0.544±0.123 mg/mg.

Biomechanical Evaluation

There was no difference in aggregate modulus between the L3–L4 and L4–L5 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's ratio or permeability between the two levels, with ranges of 0.263–0.275 and $2.88–3.82 \times 10^{-15}$ $m^4/N{\cdot}s$.

There was no difference in Young's modulus between the L3–L4 and L4–L5 tissue, with values of 9.92±10.93 and 10.25±6.48 MPa, respectively (Fig. 7). Averaged over both levels, the Young's modulus was 10.08±8.07 MPa. Additionally, there was no difference in UTS between the L3–L4 and L4–L5 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 reporting 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, in order to yield functional design criteria for future tissue engineering studies. It was demonstrated that L3–L4 and L4–L5 cartilage had equivalent properties, thus indicating similar functional requirements at both levels.

A histological examination of the facet cartilage tissue demonstrated the typical zonal characteristics of hyaline articular cartilage, although some subtle differences with other

tissues were apparent. For instance, the deeper zone of the tissue demonstrated somewhat of a 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] thus suggesting that facet joint cartilage may be less weight-bearing than cartilage tissue in other joints. This is supported by the tissue's relatively high Poisson's ratio, which is 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 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, was significantly higher 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 the 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 content. As reviewed previously,[15] articular cartilage generally has a GAG/WW of 5–10%, and a collagen/WW of approximately 15%. The GAG/WW at 3.5–4.1% in this study correlated with the relatively low compressive properties, while the collagen/WW at 16.1–16.9% correlated with the relatively high tensile properties. The collagen II/WW at 13.4% was slightly lower than the total collagen/WW at 16.8%. This discrepancy has been observed previously and may be due to the significant differences between the assays to measure these parameters, as collagen II content is assessed with an ELISA while total collagen is assessed with a hydroxyproline assay. Moreover, it is possible that the total collagen assay measures other types of collagen, likely present in small amounts in the tissue, including collagen VI, IX, and XI, as well as elastin. Additionally, facet cartilage appeared to be in the range of the thickness of the cartilage of other joints, as hip cartilage has a range of 0.38–0.60 mm, knee cartilage has a range of 0.52–0.90 mm, and shoulder cartilage has a range of 0.47–0.71 mm.

This study utilized a skeletally mature canine model to study 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 as it has been used extensively in prior studies on 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 the two species.

A limited number of studies have been performed to compare the cartilage between the canine and human. In a prior study, the biomechanical properties of knee joint cartilage from canines and humans were assessed at multiple locations, and compared with data from 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, an additional study demonstrated that there were no significant 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 carry over to facet joint cartilage. Therefore, the canine data presented in this manuscript may 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 ranging from 5–10% and collagen/WW values exceeding 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's modulus exceeding 2 MPa.[9]

CONCLUSIONS

As tissue engineering efforts strive to replicate or approach the functional properties of native tissue, it is crucial to know the precise characteristics of facet joint cartilage to enable tissue regeneration. The articular cartilage of other joints has been extensively characterized; however, prior to this study, the functional properties of facet joint cartilage were unknown. Although, additional studies will ideally compare these canine results to those of 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.

Acknowledgments

The authors would like to acknowledge funding from NIAMS R01 AR053286, as well as support from the National Institutes of Health under NIH Grant No. 5T32 GM008362. Additionally, we would like to acknowledge Jesse Rios for help in procuring the canine lumbar spine tissue.

References

1. Athanasiou KA, Agarwal A, Dzida FJ. Comparative study of the intrinsic mechanical properties of the human acetabular and femoral head cartilage. J Orthop Res. 1994; 12:340–349. [PubMed: 8207587]
2. Athanasiou KA, Agarwal A, Muffoletto A, Dzida FJ, Constantinides G, Clem M. Biomechanical properties of hip cartilage in experimental animal models. Clin Orthop Relat Res. 1995:254–266. [PubMed: 7634715]
3. Athanasiou KA, Rosenwasser MP, Buckwalter JA, Malinin TI, Mow VC. Interspecies comparisons of in situ intrinsic mechanical properties of distal femoral cartilage. J Orthop Res. 1991; 9:330–340. [PubMed: 2010837]
4. Aufderheide AC, Athanasiou KA. Assessment of a bovine co-culture, scaffold-free method for growing meniscus-shaped constructs. Tissue Eng. 2007; 13:2195–2205. [PubMed: 17630876]

5. Boswell MV, Colson JD, Sehgal N, Dunbar EE, Epter R. A systematic review of therapeutic facet joint interventions in chronic spinal pain. Pain Physician. 2007; 10:229–253. [PubMed: 17256032]
6. Breit S. Functional adaptations of facet geometry in the canine thoracolumbar and lumbar spine (Th10-L6). Ann Anat. 2002; 184:379–385. [PubMed: 12201048]
7. Brown AN, Kim BS, Alsberg E, Mooney DJ. Combining chondrocytes and smooth muscle cells to engineer hybrid soft tissue constructs. Tissue Eng. 2000; 6:297–305. [PubMed: 10992427]
8. Buttermann GR, Schendel MJ, Kahmann RD, Lewis JL, Bradford DS. In vivo facet joint loading of the canine lumbar spine. Spine. 1992; 17:81–92. [PubMed: 1531558]
9. Elder BD, Athanasiou KA. Synergistic and additive effects of hydrostatic pressure and growth factors on tissue formation. PLoS ONE. 2008; 3:e2341. [PubMed: 18523560]
10. Elder BD, Athanasiou KA. Systematic assessment of growth factor treatment on biochemical and biomechanical properties of engineered articular cartilage constructs. Osteoarthritis Cartilage. 2008
11. Eubanks JD, Lee MJ, Cassinelli E, Ahn NU. Prevalence of lumbar facet arthrosis and its relationship to age, sex, and race: an anatomic study of cadaveric specimens. Spine. 2007; 32:2058–2062. [PubMed: 17762805]
12. Haher TR, O'Brien M, Dryer JW, Nucci R, Zipnick R, Leone DJ. The role of the lumbar facet joints in spinal stability. Identification of alternative paths of loading. Spine. 1994; 19:2667–2670. discussion 2671. [PubMed: 7899961]
13. Hayes WC, Keer LM, Herrmann G, Mockros LF. A mathematical analysis for indentation tests of articular cartilage. J Biomech. 1972; 5:541–551. [PubMed: 4667277]
14. Hu JC, Athanasiou KA. A self-assembling process in articular cartilage tissue engineering. Tissue Eng. 2006; 12:969–979. [PubMed: 16674308]
15. Hu, JC.; Athanasiou, KA. Structure and Function of Articular Cartilage. In: An, YH.; Martin, KL., editors. Handbook of Histology Methods for Bone and Cartilage. 1. Totowa, NJ: Humana Press Inc; 2003. p. 73-95.
16. Kahmann RD, Buttermann GR, Lewis JL, Bradford DS. Facet loads in the canine lumbar spine before and after disc alteration. Spine. 1990; 15:971–978. [PubMed: 2259991]
17. Kalichman L, Hunter DJ. Lumbar facet joint osteoarthritis: a review. Semin Arthritis Rheum. 2007; 37:69–80. [PubMed: 17379279]
18. Katz JN. Lumbar disc disorders and low-back pain: socioeconomic factors and consequences. J Bone Joint Surg Am. 2006; 88 (Suppl 2):21–24. [PubMed: 16595438]
19. Korvick D, Athanasiou K. Variations in the mechanical properties of cartilage from the canine scapulohumeral joint. Am J Vet Res. 1997; 58:949–953. [PubMed: 9284997]
20. Lim TH, Goel VK, Weinstein JN, Kong W. Stress analysis of a canine spinal motion segment using the finite element technique. J Biomech. 1994; 27:1259–1269. [PubMed: 7962013]
21. Liu GT, Lavery LA, Schenck RC Jr, Lanctot DR, Zhu CF, Athanasiou KA. Human articular cartilage biomechanics of the second metatarsal intermediate cuneiform joint. J Foot Ankle Surg. 1997; 36:367–374. [PubMed: 9356916]
22. Mow VC, Kuei SC, Lai WM, Armstrong CG. Biphasic creep and stress relaxation of articular cartilage in compression: Theory and experiments. J Biomech Eng. 1980; 102:73–84. [PubMed: 7382457]
23. Pietila K, Kantomaa T, Pirttiniemi P, Poikela A. Comparison of amounts and properties of collagen and proteoglycans in condylar, costal and nasal cartilages. Cells Tissues Organs. 1999; 164:30–36. [PubMed: 10940671]
24. Rosenberg L. Chemical basis for the histological use of safranin O in the study of articular cartilage. J Bone Joint Surg Am. 1971:69–82. [PubMed: 4250366]
25. Shimizu M, Minakuchi K, Kaji S, Koga J. Chondrocyte migration to fibronectin, type I collagen, and type II collagen. Cell Struct Funct. 1997; 22:309–315. [PubMed: 9248994]
26. Sneddon I. The relaxation between load and penetration in the axisymmetric Boussinesq problem for a punch of arbitrary profile. Int J Eng Sci. 1965:47–57.

27. Takeuchi T, Abumi K, Shono Y, Oda I, Kaneda K. Biomechanical role of the intervertebral disc and costovertebral joint in stability of the thoracic spine. A canine model study. Spine. 1999; 24:1414–1420. [PubMed: 10423785]

28. Walker BF. The prevalence of low back pain: a systematic review of the literature from 1966 to 1998. J Spinal Disord. 2000; 13:205–217. [PubMed: 10872758]

29. Woessner JF Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys. 1961; 93:440–447. [PubMed: 13786180]

30. Yang KH, King AI. Mechanism of facet load transmission as a hypothesis for low-back pain. Spine. 1984; 9:557–565. [PubMed: 6238423]

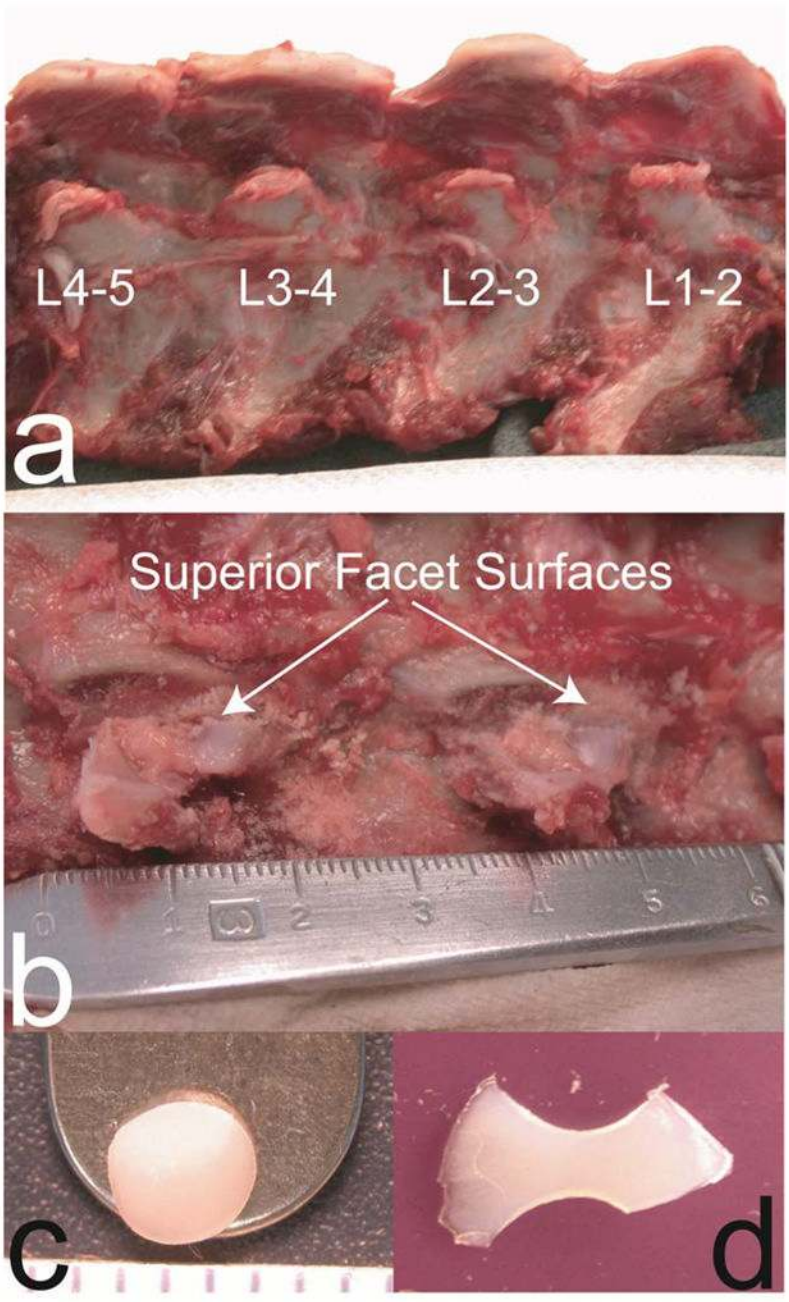

Fig. 1.
Gross morphological properties of (a) lumbar spine prior to dissection, (b) superior facet surfaces following dissection, (c) 3 mm sample for compressive testing, and (d) dog-bone shaped sample for tensile testing.

Fig. 2.

Histological staining, representative of both lumbar spine levels. 10x original magnification. (a) Picrosirius red and (b) safranin-O staining demonstrate extensive production of collagen and GAG, respectively. (c) H&E stain.

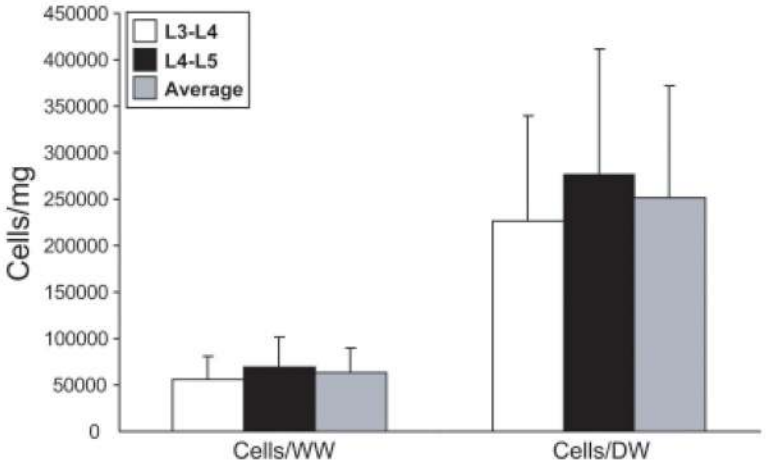

Fig. 3.
Tissue cellularity per mg of tissue.

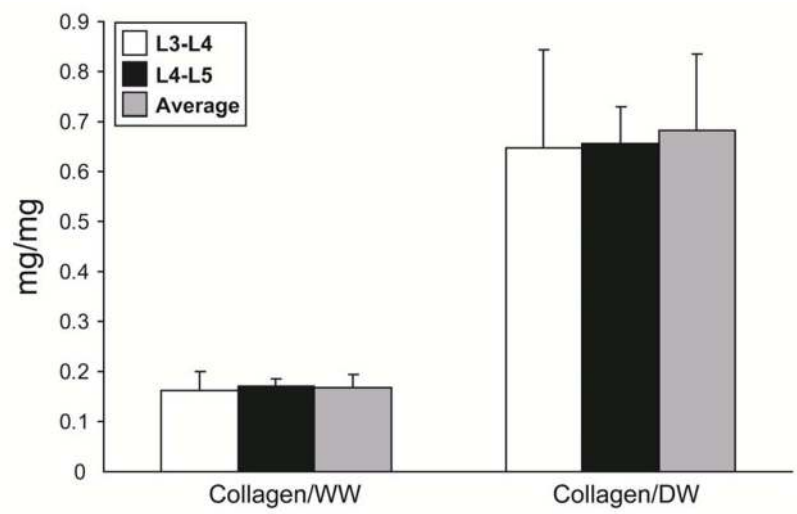

Fig. 4.
Collagen content per mg of tissue.

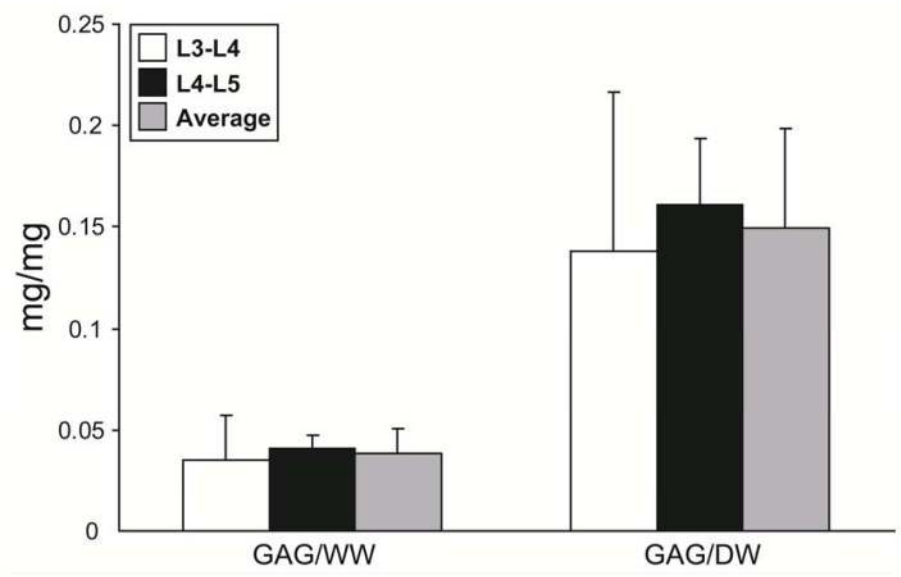

Fig. 5.
GAG content per mg of tissue.

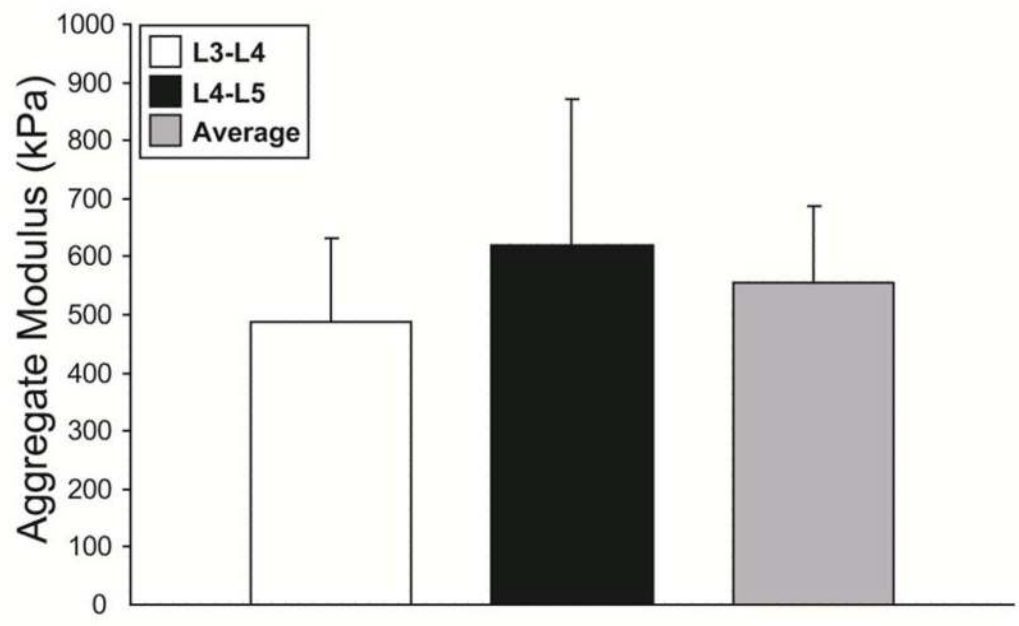

Fig. 6.
Facet cartilage compressive properties.

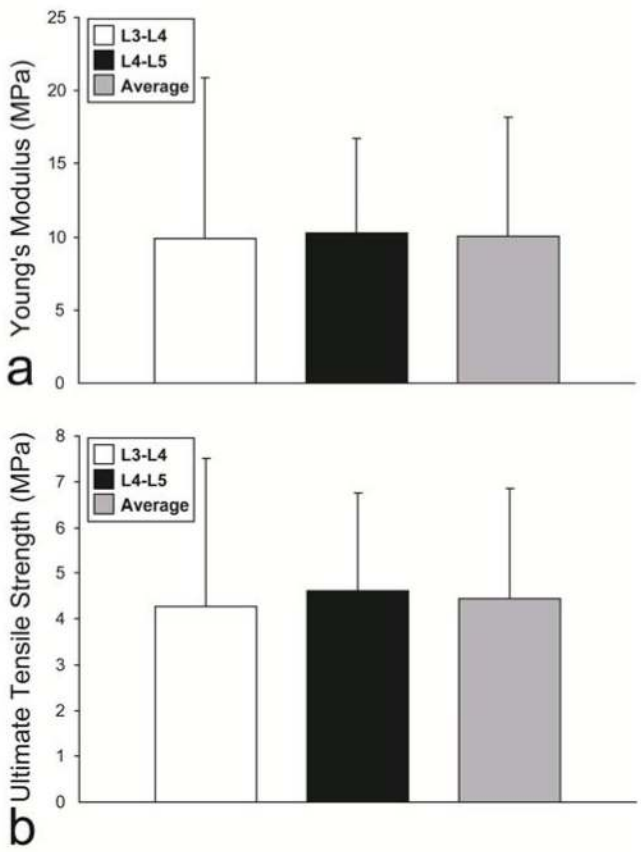

Fig. 7.
Facet cartilage tensile properties. (a) Young's modulus and (b) ultimate tensile strength.