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ABSTRACT

The temporomandibular joint (TMJ) disc plays a critical role in normal function of the joint, and many disorders of the TMJ are a result of disc dysfunction. Previous quantitative TMJ characterization studies examined either the human or a specific animal model, but no single study has compared different species, in the belief that differences in joint morphology, function, and diet would be reflected in the material properties of the disc. In this study, we examined topographical biochemical (collagen, glycosaminoglycan, and DNA content) and biomechanical (tensile and compressive) properties of the human TMJ disc, and also discs from the cow, goat, pig, and rabbit. Regional and interspecies variations were identified in all parameters measured, and certain disc characteristics were observed across all species, such as a weak intermediate zone under mediolateral tension. While human discs possessed properties distinct from those of the other species, pig discs were most similar to the human, suggesting that the pig may be a suitable animal model for TMJ bioengineering efforts.

KEY WORDS: TMJ disc, interspecies, animal model, TMJ characterization.

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INTRODUCTION

The temporomandibular joint (TMJ) disc has several important functions, most notably the dissipation and distribution of masticatory loads (Tanaka and van Eijden, 2003). Disorders of the TMJ are widespread, likely afflicting between 5% and 15% of the adult population (Reston and Turkelson, 2003), and approximately 70% of these are associated with displacement of the disc (Farrar and McCarty, 1979). In its normal relationship, the disc is positioned between the mandibular condyle and glenoid fossa of the temporal bone. However, displacement leading to internal derangement can occur, resulting in uncoordinated movements of the disc relative to the joint surfaces (Wong *et al.*, 2006). Previous attempts to replace the disc with alloplastic devices have failed, resulting in further joint degradation (Estabrooks *et al.*, 1990; Henry and Wolford, 1993). Therefore, studies aimed at tissue engineering of the TMJ, and especially the disc, are warranted (Detamore and Athanasiou, 2003a; Detamore *et al.*, 2007).

For the establishment of design criteria for tissue engineering efforts, a thorough characterization of native TMJ disc tissue is necessary. A general characterization of the biochemical composition of the disc can be elucidated from previous examinations. Collagen constitutes approximately 30% of the wet weight (Gage *et al.*, 1995), of which the majority is collagen type I. Total collagen concentration of the porcine disc is highest in the center relative to the lateral region (Almarza *et al.*, 2006). The total concentration of glycosaminoglycans (GAGs) in the TMJ disc is between 0.6 and 10% of the dry weight (Allen and Athanasiou, 2006b). Studies of pig and cow discs indicated that the greatest GAG content is located in the center relative to the periphery (Nakano and Scott, 1996; Almarza *et al.*, 2006), but the opposite trend has been seen in primate discs (Mills *et al.*, 1994). The cells of the TMJ disc reflect a heterogeneous fibrochondrocyte cell population consisting of both fibroblast and chondrocyte-like cells. Regionally, the greatest DNA concentration and cell number have been seen in the medial portion of the pig disc (Almarza *et al.*, 2006), and in the anterior and posterior bands of primate discs (Mills *et al.*, 1994).

Previous biomechanical examinations have highlighted the TMJ disc's anisotropic and heterogeneous properties. Tensile strength and stiffness correlate with local collagen orientation, with greater values present in the central region when tested in the anteroposterior direction relative to the mediolateral direction (Shengyi and Xu, 1991; Beatty *et al.*, 2001; Detamore and Athanasiou, 2003b). Compressive properties vary topographically; the relaxation modulus of the medial region is highest, while the posterior and anterior bands appear to support the highest instantaneous loads (del Pozo *et al.*, 2002; Kim *et al.*, 2003; Allen and Athanasiou, 2005, 2006a). Overall, the disc is 10 to 1000 times softer under compression than it is under tension (Tanaka and van Eijden, 2003).

While these studies have increased the collective knowledge of TMJ disc physiology, no single study has compared biomechanical and biochemical characteristics of the human disc with those of an animal model. Furthermore, there are no studies examining topographical variations or orientation-dependent characteristics. The porcine disc has been identified as the model most appropriate for comparison with the human, based on similarities such as disc size and shape, joint anatomy, masticatory patterns, and omnivorous diet (Berg, 1973; Strom *et al.*, 1986; Bermejo *et al.*, 1993; Springer *et al.*, 2001). Other species examined include cows (Hatton and Swann, 1986; Tanaka *et al.*, 2006), dogs (Shengyi and Xu, 1991; Tanaka *et al.*, 1999), goats (Bifano *et al.*, 1994), rabbits (Kurita *et al.*, 1990; Scapino *et al.*, 1996), rats (Deschner *et al.*, 2007), and sheep (Bosanquet and Goss, 1987). In an effort toward better understanding of the quantitative similarities and differences between and among some of these models, this study compares the regional biochemical and biomechanical properties of the human, cow, goat, pig, and rabbit discs.

MATERIALS & METHODS

Specimen Procurement

Tissue specimens were procured from skeletally mature sources over 1 mo. Goat [8 mos old (m.o.), half male, half female], pig (12 m.o., all female), rabbit (12 m.o., half male, half female), and cow (24 m.o., all female) heads with intact joint capsules were obtained from local abattoirs within hours of death. Human TMJ discs were dissected from seven dentate female cadaver donors of age 63 to 84 yrs (mean age, 73). All tissues were fresh or fresh-frozen (human) and were never fixed. TMJ discs were carefully dissected from their attachments and verified to be grossly normal. Discs were washed in phosphate-buffered saline (PBS), wrapped in gauze soaked with PBS containing protease inhibitors (10 mM N-ethylmaleimide and 1 mM phenylmethylsulfonyl fluoride, Sigma, St. Louis, MO, USA), and frozen at -20°C until being tested.

Biochemical Analysis

For quantitative biochemistry, 6 (rabbit, goat, pig), 5 (cow), or 4 (human) left discs were thawed in PBS and then sectioned into 5 pieces (Fig. 1A). All specimens were blotted dry, weighed to obtain a wet weight, and then lyophilized for 48 hrs. Samples were digested in 1.5 mL of 125 mg/mL papain (Sigma) solution overnight at 60°C . The DNA content of the samples was measured by reaction of DNA with Picrogreen reagent (Invitrogen, Carlsbad, CA, USA). The total amount of sulfated GAG was measured with the use of a dimethylmethylene blue colorimetric assay kit (Biocolor, Newtownabbey, UK). The total collagen content was determined by a hydroxyproline assay, as described previously (Almaraz *et al.*, 2006).

Histology

For topographical histology, a right disc from each species was divided into 5 regions as shown in Fig. 1A. Disc samples were snap-frozen in freezing medium (Triangle Biomedical, Durham,

NC, USA) and cryo-sectioned at $12\ \mu\text{m}$ in the anteroposterior direction. Qualitative analysis of sulfated GAG was conducted by safranin-O/fast green staining.

Tensile Sample Preparation and Testing Procedure

Discs from each species were thawed and cut into 3 regions in either an anteroposterior or mediolateral direction (Fig. 1B). Regions were sectioned in a cryotome to a uniform thickness between 300 and 600 μm , with a width of 1 mm. These sections were taken from the middle zone after removal of the superior surface. Tests were conducted on a materials testing machine (Instron 5565, Canton, MA, USA) in an isotonic saline bath at room temperature. A 0.02-N tare load was applied to the samples, followed by pre-conditioning with 15 cycles of 5% strain at a rate of 10 mm/min. After pre-conditioning, step strains were applied at 5% increments beginning with 10% and up to 40%, with 10 min between steps for stress relaxation. For tensile testing, data were only kept and analyzed from samples which broke in the center. Any specimens that broke at the grips were discarded because we would not be able to calculate accurate material properties. We obtained peak and relaxed moduli by constructing stress vs. strain plots through points of peak and relaxed stresses at each step strain (Detamore and Athanasiou, 2003b).

Compression Sample Preparation and Testing Procedure

Cylindrical tissue plugs were taken from 5 regions of each disc (Fig. 1A) and sectioned such that the superior and inferior surfaces were parallel using a cryotome. During the preparation of compressive specimens, they were frozen to the stage of a cryotome and the top and bottom surfaces of the specimen were sectioned until they were flat and parallel to each other. The unconfined compression testing performed in this study required that the surfaces of the sample be flat and parallel. The final sample thicknesses ranged from 0.8 to 4 mm. Unconfined compression testing was performed on the Instron in a saline bath at room temperature. A 0.02-N tare load was applied to the sample, followed by pre-conditioning with 5% strain for 15 cycles. During the test, 10% step strains were applied from 10% up to 30% strain, with 10-minute intervals between steps for stress relaxation. We obtained values for instantaneous modulus, relaxation modulus, and coefficient of viscosity by fitting data to a viscoelastic solution for a Kelvin solid (Allen and Athanasiou, 2005).

RESULTS

Gross Morphology

Gross morphology and measured dimensions of discs from all 5 species are presented in Figs. 1C and 1D. The pig was the only species that was not significantly different from the human in both mediolateral and anteroposterior dimensions.

Biochemical Analysis

Quantitative biochemical results and two-factor ANOVA analysis are shown in Fig. 2. Numerical data along with one-factor ANOVA analysis within each species can be found in Appendix

Table 3. The mean collagen content normalized to wet weight (ww) was found to vary between 16.5% and 30.1% for all species and regions tested. TMJ discs from the cow showed significantly greater collagen/ww than all other species except the pig (Fig. 2A). Human discs were not statistically different from either the pig or rabbit discs. The PBC displayed significantly greater collagen/ww than IZL, but there were no other statistical differences among the disc regions.

The mean DNA/ww varied between 0.0055% and 0.0358%, showing stark differences in both region and species. Human discs contained significantly less DNA/ww than all of the other species, but there were no other interspecies variations (Fig. 2B). Regionally, ABC and PBC contained significantly more DNA than all 3 regions of the intermediate zone. DNA content was the only biochemical parameter that showed a consistent trend in regional variation within the species (Appendix Table 3).

The mean sulfated GAG/ww content was found to vary between 0.273% and 0.936% for all samples tested. Cow discs contained significantly more GAG/ww than all species except the goat (Fig. 2C). The GAG concentration of the human TMJ discs was significantly different from those of all other species, falling between those of the rabbit and pig discs. While all 3 regions of the intermediate zone possessed significantly more GAG than the anterior band, only IZM contained more GAG than the posterior band.

Histology

Histological staining supported the quantitative biochemical results. Positive safranin-O staining for sulfated GAGs was clearly visible in all samples except the anterior band of the human disc and all regions of the pig disc (Fig. 2D). Hematoxylin staining indicated fewer cells in human samples relative to the other 4 species, and fewer cells in the intermediate zone compared with the bands for the rabbit, goat, and pig specimens.

Tension

Peak and relaxed Young's moduli under tension are presented in Fig. 3. Raw data for tensile properties with one-way and two-way ANOVA analysis are shown in Appendix Table 1. Analysis of intraspecies topographical variation demonstrated significance for all parameters. Topographically, the ML C samples

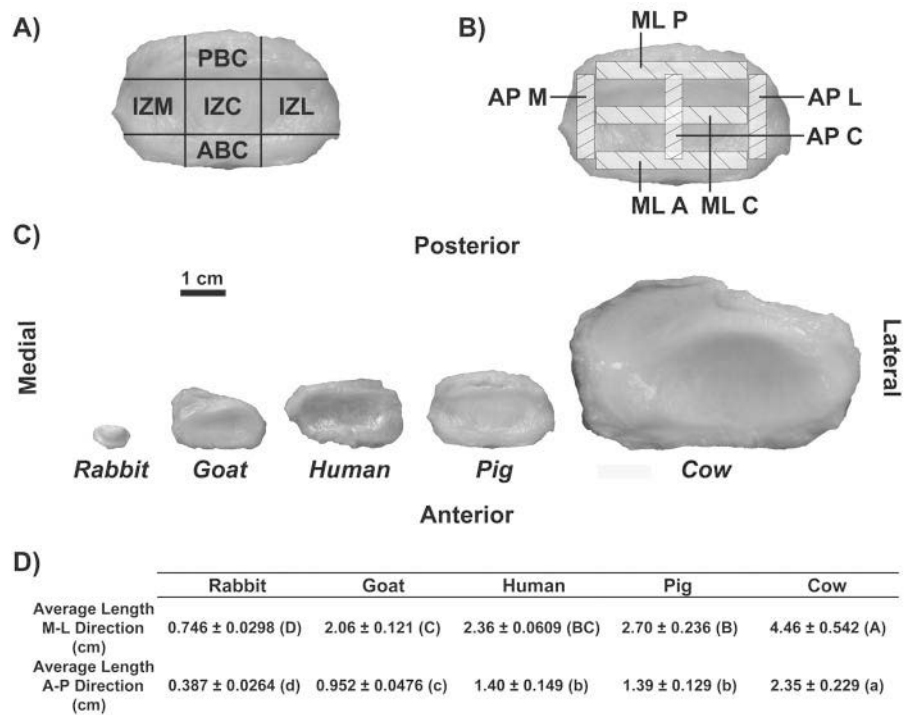


Figure 1. Description of the TMJ disc regions tested in this investigation and gross morphology of the collected discs. (A) Regions of the TMJ disc used for biochemical, histological, and compression testing: posterior band central (PBC), intermediate zone (IZ), medial (IZM), IZ central (IZC), IZ lateral (IZL), and anterior band central (ABC). (B) Regions of the TMJ disc used for tensile testing: mediolateral (ML), posterior (ML P), ML central (ML C), ML anterior (ML A), anteroposterior (AP), medial (AP M), AP central (AP C), AP lateral (AP L). (C) Scaled figure showing the gross morphology of TMJ discs collected from the 5 different species tested. (D) Dimensions of discs collected from each species measured in the mediolateral (M-L) and anteroposterior (A-P) directions. Data are presented as mean ± SD. A one-way ANOVA was conducted on the data from each direction, and animals not connected by the same letter were statistically different from one another. The pig was the only animal with dimensions not significantly different from those of the human in both the A-P and M-L directions.

exhibited weaker and softer behavior than all other groups, though no other regional variation was found (Figs. 3A, 3B). With regard to interspecies variation, human tissue was significantly stiffer and stronger than all other species, while rabbit tissue was softer than all other groups. Strength values ranged from 0.2 MPa for bovine ML C to 13.8 MPa for the human AP C tissue. The human AP C group also displayed the highest peak and relaxed moduli of 51.7 MPa and 34.4 MPa, compared with the bovine ML C values of 0.2 MPa and 0.1 MPa, respectively.

Compression

Instantaneous and equilibrium compressive moduli at 20% strain are shown in Fig. 4. Raw data for compressive assessments with one-way and two-way ANOVA analysis are shown in Appendix Table 2. All parameters increased with advancing strain. The interspecies analysis showed that the bovine, lepore, and caprine tissue had the highest relaxation and instantaneous moduli (Figs. 4A, 4B). Porcine tissue had the lowest moduli overall and was similar to human tissue at 20% and 30% strain for both moduli. Topographically, the bands yielded

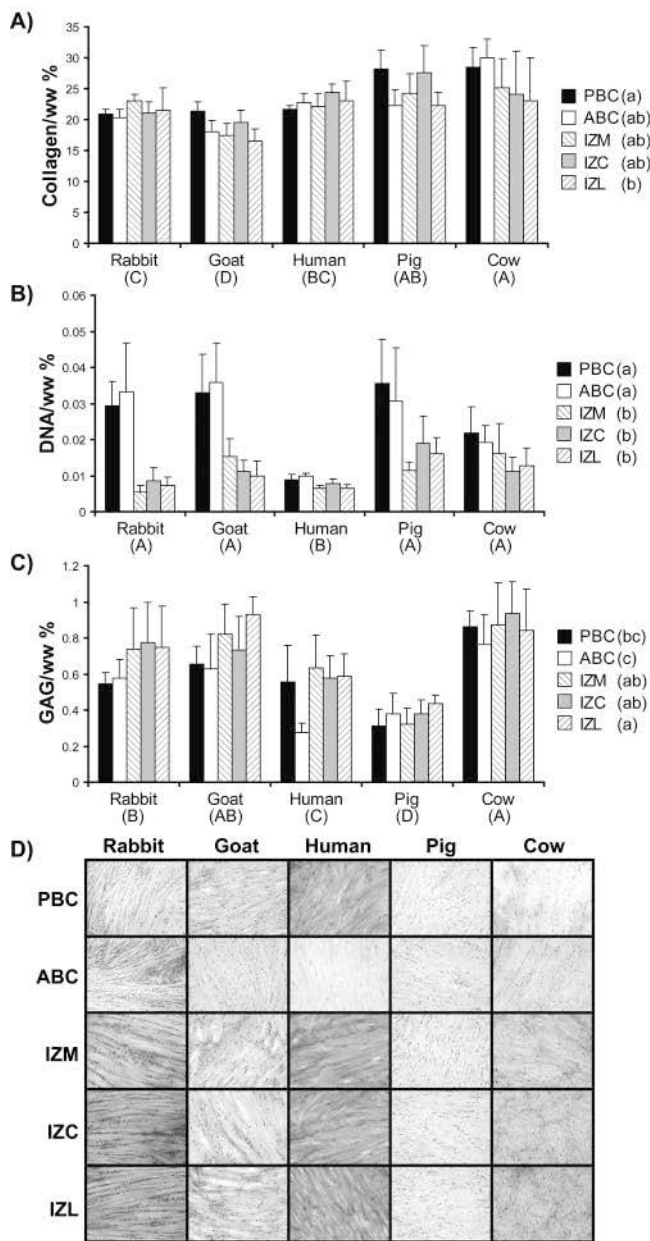


Figure 2. Biochemical and histological analysis. (A-C) Interspecies comparison of the quantitative biochemical content of the TMJ disc. Data were normalized to wet weight and are presented as mean ± SD. A two-way ANOVA is presented with the factors of species and region. Samples not connected by the same letter are statistically different from each other. (A) Total collagen content of human samples was not statistically different from that in pig or rabbit discs. (B) Human discs contained significantly less DNA content than the other species, likely because of age. (C) Sulfated GAG content of human samples fell in between those of the pig and rabbit samples. (D) Safranin-O/fast green staining of sections from the TMJ disc. Positive Safranin-O staining (red to purple) is clear in all samples except human ABC and all regions of the pig disc. Images were taken on a Nikon E600 microscope with a 20X objective. (Note: The complete biochemical dataset is presented online in Appendix Table 3.)

higher instantaneous and relaxed moduli relative to the intermediate zone samples, with the exception of the relaxation modulus of the IZC region. The highest relaxed modulus of 199 kPa

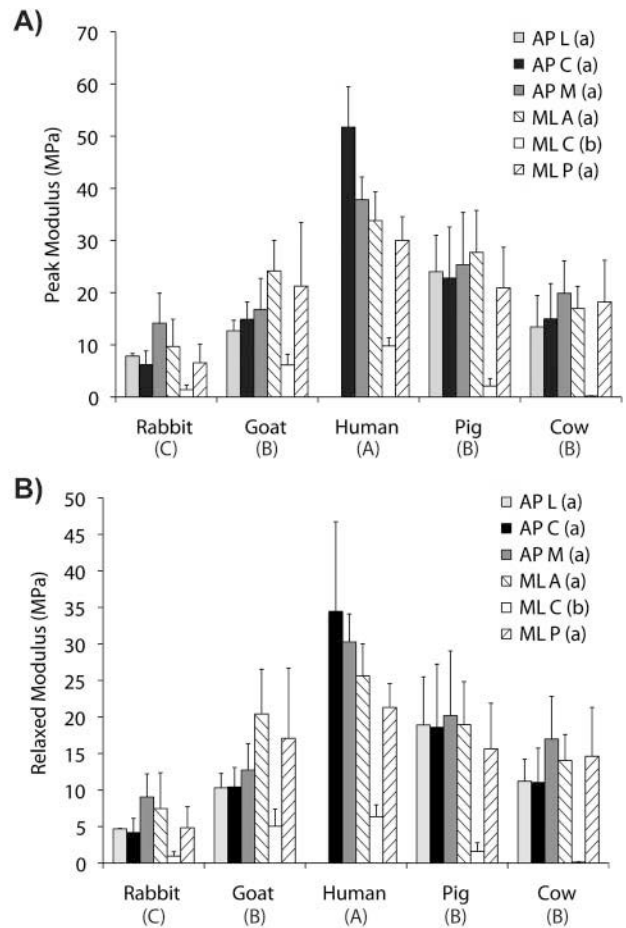


Figure 3. Biomechanical properties under tension. Data are presented as mean ± SD. Data were analyzed by a two-way ANOVA with Tukey's HSD test *post hoc* where appropriate, and groups not connected by the same letter are significantly different ($p < 0.05$). Peak (A) and relaxed (B) Young's moduli under tensile loading. Human tissue was the stiffest among the species, and the central region under mediolateral strain was the most compliant among the regions under both loading conditions. (Note: The complete tensile dataset is presented online in Appendix Table 1.)

was obtained from the bovine PBC samples, while the highest instantaneous modulus of 6.55 MPa was noted from the goat ABC group. The softest tissue was from the lateral region of the pig disc. The band regions of the goat and cow displayed the highest viscosity coefficients, with values between 35 and 40 MPa at 30% strain.

DISCUSSION

While previous studies of the TMJ disc investigated regional variation in biochemical and biomechanical properties, this study is the first to examine these properties across species. Furthermore, this is the first study to quantify both biochemical and biomechanical properties concurrently. The advantage of this study's approach is that animal models could be compared with the human disc by the same testing methods in a consistent environment, mitigating variability associated with comparisons made across different studies. Because of the contradictory

nature of results from prior studies, the results of this investigation do not agree with all prior work, but they are consistent with those of previous studies performed in our group using the pig model (Detamore and Athanasiou, 2003b; Allen and Athanasiou, 2005, 2006a; Detamore *et al.*, 2005; Almarza *et al.*, 2006). The interspecies characterization data collected here will provide valuable design parameters for tissue engineers seeking to recapitulate the properties of the disc *in vitro*, and for those looking to study functional replacements *in vivo*.

Prior studies have indicated that structure-function relationships exist within the porcine TMJ disc (Allen and Athanasiou, 2006b), but now a comprehensive comparison can be made, not only within a single species, but also across species. Sulfated GAG content is frequently related to compressive stiffness, and indeed in this study, the species with the greatest GAG/ww (cow) also had the highest compressive moduli. In contrast, regional variations in GAG content showed no relationship with compressive properties. Instead, the region with the highest total collagen/ww (PBC) also possessed the highest compressive properties, as seen previously for the pig disc (Allen and Athanasiou, 2006a). Although collagen is generally thought to mediate tensile properties, no correlations with total collagen content were observed in this study. As seen previously, tensile properties of the disc depend more on the orientation of collagen than on total content (Detamore and Athanasiou, 2003b). Analysis of these data emphasizes that tissue engineering studies will have to account not only for biochemical content, but also for organization to produce heterogeneous mechanical properties similar to those of native tissue.

Collecting a significant amount of interspecies data within a single study allows for the correlation of disc properties to the greater functional requirements of each animal. It is apparent that some properties of the disc vary with the specific anatomy or diet of each species. For example, the herbivores (cow, goat, and rabbit), whose joint motion is primarily translatory (Herring, 2003), had greater GAG content and compressive properties across all disc regions compared with the omnivores (human and pig), whose motion is both rotatory and translatory. In contrast, some regional variations were constant among species, indicating that certain portions of the disc may have similar functional requirements within all species. For example, the intermediate zone under mediolateral tension was always weaker than other regions. While it is clear that the disc from each species is unique, it does appear that some properties of the disc transcend species.

While this study provides a significant reference for interspecies variations in the TMJ disc, there are a few limitations to the work. Although the human specimens appeared grossly normal, they were derived from cadavers of advanced age, whose lack of TMJ dysfunction could not be verified with medical records. These factors could have affected the measured discal properties. Aging of the TMJ disc has been shown to result in stiffer tissues (Tanaka *et al.*, 2006) with lower cellularity (Minarelli and Liberti, 1997), which matches well with the human data in this study. While this study did examine regional variations, it did not examine every region of the disc, and it measured properties only *in vitro*. Future studies should build on this work by investigating interspecies variations in other important discal

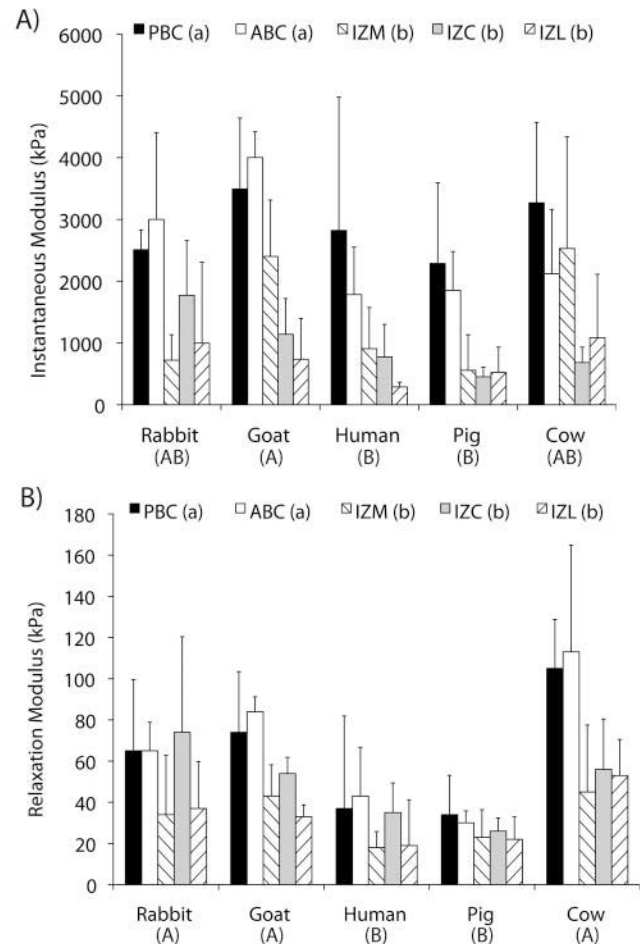


Figure 4. Biomechanical properties under compression at 20% strain. Data are presented as mean \pm SD. Data were analyzed by a two-way ANOVA with Tukey's HSD test *post hoc* where appropriate, and groups not connected by the same letter are significantly different ($p < 0.05$). **(A)** Compressive moduli under instantaneous loading. The porcine, leporine, and bovine tissue yielded values statistically similar to those of the human, while the caprine tissue was stiffer than the human tissue. **(B)** Compressive moduli at equilibrium. Human and pig discs were significantly more compliant than rabbit, goat, and cow discs. The band regions were significantly stiffer than the intermediate zone regions under both instantaneous and relaxed conditions. (Note: The complete compressive dataset is presented online in Appendix Table 2.)

properties, such as cellular populations, as well as imaging of the intact joints. This imaging would allow for both correlation of the measured properties to native movement of the disc and verification that there is no disease present.

A major goal of this study was to quantify the differences and similarities among animal models, and specifically, how these relate to their appropriateness as analogs of the human TMJ disc. When one takes into account all of the parameters tested in this study, it can be argued that the pig disc is most similar to that of the human. This is based on the pig disc demonstrating the most statistical similarities to the human disc, including dimensions, collagen and GAG content, and compressive properties. Therefore, the results of this study point to the pig as the most

appropriate animal model and support results from prior anatomical studies.

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