Jesse Lowe

Department of Bioengineering,
University of Pittsburgh,
Pittsburgh, PA 15260;
Center for Craniofacial Regeneration,
University of Pittsburgh,
Pittsburgh, PA 15260

Rohan Bansal

Center for Craniofacial Regeneration,
University of Pittsburgh,
Pittsburgh, PA 15260

Stephen F. Badylak

Department of Surgery,
University of Pittsburgh,
Pittsburgh, PA 15260;
McGowan Institute for Regenerative Medicine,
University of Pittsburgh,
Pittsburgh, PA 15260

Bryan N. Brown

Department of Bioengineering,
University of Pittsburgh,
Pittsburgh, PA 15260;
McGowan Institute for Regenerative Medicine,
University of Pittsburgh,
Pittsburgh, PA 15260

William L. Chung

Oral and Maxillofacial Surgery,
University of Pittsburgh,
Pittsburgh, PA 15260

Alejandro J. Almaraz¹

Department of Oral Biology,
University of Pittsburgh,
Pittsburgh, PA 15260;
Department of Bioengineering,
University of Pittsburgh,
566 Salk Hall, 3501 Terrace Street,
Pittsburgh, PA 15260;
Center for Craniofacial Regeneration,
University of Pittsburgh,
Pittsburgh, PA 15260;
McGowan Institute for Regenerative Medicine,
University of Pittsburgh,
Pittsburgh, PA 15260
e-mail: aja19@pitt.edu

Properties of the Temporomandibular Joint in Growing Pigs

A subset of temporomandibular joint (TMJ) disorders is attributed to joint degeneration. The pig has been considered the preferred in vivo model for the evaluation of potential therapies for TMJ disorders, and practical considerations such as cost and husbandry issues have favored the use of young, skeletally immature animals. However, the effect of growth on the biochemical and biomechanical properties of the TMJ disk and articulating cartilage has not been examined. The present study investigates the effect of age on the biochemical and biomechanical properties of healthy porcine TMJs at 3, 6, and 9 months of age. DNA, hydroxyproline, and glycosaminoglycan (GAG) content were determined and the disks and condyles were tested in uniaxial unconfined stress relaxation compression from 10% to 30% strain. TMJ disks were further assessed with a tensile test to failure technique, which included the ability to test multiple samples from the same region of an individual disk to minimize the intraspecimen variation. No differences in biochemical properties for the disk or compressive properties at 30% stress relaxation in the disk and condylar cartilage were found. In tension, no differences were observed for peak stress and tensile modulus. The collagen content of the condyle was higher at 9 months than 3 months ($p < 0.05$), and the GAG content was higher at 9 months than 6 months ($p < 0.05$). There was a trend of increased compressive instantaneous modulus with age. As such, age-matched controls for growing pigs are probably appropriate for most parameters measured. [DOI: 10.1115/1.4039624]

Introduction

The temporomandibular joint (TMJ) is a bilateral joint formed by the articulation of the mandibular condyle (mandibular head of the condylar process in pigs) against the glenoid fossa (mandibular fossa of the squamous temporal bone in pigs) and articular eminence of the temporal bone. The fibrocartilaginous TMJ disk is situated between the condyle and fossa and distributes the compressive and tensile forces within the joint during mandibular movements [1–4]. In TMJ cases, where there is severe joint degeneration, and surgery is deemed necessary, the current surgical standard for an irreparable TMJ disk is to perform a

discectomy, usually without autograft replacement, due to previous failures of autograft implantation [5,6]. The resulting absence of an interpositional disk leads to bone on bone contact and pathologic remodeling of the mandibular condyle.

Tissue engineering strategies for TMJ disease have included cell-based and scaffold-based approaches [7–16]. Clinical translation will require preclinical animal studies across both acute and long-term time points. The pig is generally accepted as an appropriate model for large animal studies of the TMJ. While pigs are considered skeletally mature at 18 months of age, most studies are conducted on pigs between 3 and 9 months of age because of cost and husbandry constraints [17]. The rapid rate of growth and the weight of a 135–180 kg pig at 1 year of age make such studies difficult to perform.

Although several studies have examined the biochemical and biomechanical properties of the healthy native articulating tissues

¹Corresponding author.

Manuscript received June 19, 2017; final manuscript received February 23, 2018; published online April 19, 2018. Editor: Beth A. Winkelstein.

of the porcine TMJ, no study has examined the effect of age on these properties under uniform testing parameters [18–38]. The objective of the present study is to determine the effect of age and growth on the biochemical and biomechanical properties of porcine TMJ disks and condylar mandibular cartilage at 3, 6, and 9 months of age.

Materials and Methods

Specimen Procurement. Ten female Yorkshire pig heads from 3, 6, and 9 month old pigs were procured at a local abattoir immediately following sacrifice. The TMJ disks and mandibular condyles were excised within 24 h of collection, wrapped in gauze, and soaked in 0.1 M phosphate buffered saline (PBS). All disks and condyles were assessed morphologically and no signs of degeneration were observed. At this time, the dimensions of each group were also recorded in the anterior-posterior (AP) and mediolateral (ML) directions. Samples were stored at -20°C until testing. Samples were stored for as short as a few days to as long as 2 months prior to testing.

Histology. Disks ($n = 2$ per group) were embedded in the optimal cutting temperature compound (tissue-Tek, Torrance, CA) for 45 min, and then frozen to -80°C . The samples were cryotomed to $10\text{ }\mu\text{m}$ and fixed in cold acetone for 30 min. Samples were then stained with hematoxylin and eosin to visualize cellular distribution and content.

Biochemical Assays. Specimens ($n = 10$ per age group) were collected from the intermediate zone of the TMJ disk and the mandibular condylar cartilage using 4 mm biopsy punches (Fig. 1). Samples were then digested in 0.1 M Papain at 65°C overnight. All assays were performed using this digest. The DNA content was measured using a PicoGreen dsDNA quantification kit (Molecular Probes, Inc., Eugene, OR). The total hydroxyproline content was assessed using a modified protocol that hydrolyzes the hydroxyproline group with 4 N NaOH, followed by reaction with chloramine T and dimethylaminobenzaldehyde that provided for a colorimetric output compared against hydroxyproline standards. The total amount of glycosaminoglycans (GAG) was measured using a 1,9-dimethylmethylene blue colorimetric assay, using chondroitin-4-sulfate as a standard (Biocolor, Wexbury, NY).

Compression Testing. Unconfined compression testing and analysis were performed as described previously ($n = 10$ per group) [39]. Compressive samples of disks and condyles were obtained using 4 mm biopsy punches and allowed to equilibrate in 0.1 M PBS for 1 h. Condyle samples were separated from the

subchondral bone so that only the fibrocartilage was tested. The sample diameter was measured using calipers. An MTS Insight[®] (MTS, Eden Prairie, MN) was used for testing. Specimens were loaded onto a compression platen using a thin film of cyanoacrylate and preloaded to 0.02 N before the platen was removed. This step was also used to determine the thickness of each sample as the distance between the compression platen and the stage at the conclusion of preloading. The water bath was then filled with PBS at 37°C and maintained with a thermocouple throughout testing. The upper platen was lowered to within 0.1 mm of the cross head position determined previously, and a preload of 0.05 N was applied for 30 min to determine the specimen height. The samples then were subjected to ten cycles of preconditioning at 9% strain/min to 10% strain. Following preconditioning, the samples underwent three stress-strain steps between 10% and 30% strain at 10% intervals, with 30 min of wait time between each step.

The peak stress for each strain step was determined using the force obtained and the cross-sectional area of the sample. The instantaneous modulus was determined as the slope of the last 20% of each stress strain curve. Only quasistatic parameters were calculated.

Tensile Testing. Prior to testing, disks were thawed and cut into a dumbbell shape using 8 mm biopsy punches in the intermediate zone of the disk, as shown in Fig. 1. The resulting samples were sectioned in a cryotome to approximately $300\text{ }\mu\text{m}$ by freezing superior surface of the disk to a cryotome platen using optical cutting temperature compound such that the inferior surface of the disk was visible. The cryostat blade cut parallel to the disk surface. Tensile sections were collected in the order of their inferior-superior position. This method allowed for collection of multiple sections ($n = 3\text{--}6$ per disk), enabling within disk variance analysis of tensile properties. Following procurement, samples were allowed to equilibrate for 1 h at room temperature in 0.1 M PBS.

Samples were gripped between 400 grit sandpapers, placed in pneumatic clamps at 45 PSI, and loaded onto an Instron mechanical testing apparatus (Instron 5566). The grip to grip length was measured using a ruler and varied between 8 mm and 16 mm in the anterior-posterior direction. Specimen thickness was measured using a micrometer (an average of $0.3 \pm 0.1\text{ mm}$), and width was determined using calipers (an average of $2.0 \pm 0.3\text{ mm}$). Samples were preloaded to 0.2 N and the load cell was reset to zero. Twenty cycles of preconditioning were applied at a strain rate of 10 mm/min to 5% strain. The upper bound of 1.1 N and lower bound of 0.45 N for the intensity of the first cycle were applied during preconditioning before continuing on with testing; a range determined from preliminary testing showed that measured values for stress and strain were outliers (two standard deviation from the mean) if the load did not fall within these bounds. If less than three samples per disk failed to meet this criteria, that disk was not included in

Fig. 1 Tensile testing sample preparation: (a) 8 mm biopsy punches are used to create a dogbone shape in the intermediate zone of the TMJ disk. Histology and compression samples were taken from the resulting 8 mm biopsy punches. A razor blade is then used to finish the dogbone shape. (b) Final dogbone shape placed into cryotome for sectioning.

Table 1 Dimensions for disks and condyles at 3 months, 6 months, and 9 months in the AP and ML direction. All errors represent standard deviation.

Tissue	Age	AP (cm)	ML (cm)
Disk	3 month	1.2±0.2	2.2±0.3
	6 month	1.5±0.2	2.7±0.1
	9 month	2.8±0.3	2.8±0.3
Condyle	3 month	1.4±0.2	2.0±0.2
	6 month	2.1±0.3	2.7±0.2
	9 month	3.0±0.2	3.0±0.2

analysis. The exclusion criteria, specifically using an upper and lower bound of the first peak of the preconditioning curve, did not show a bias toward any age group. During testing, roughly 10% of 72 samples were excluded because of these upper or lower bounds, and this value was consistent among all three age groups. All disks were tested, with only one sample per disk being excluded 10% of the time. The samples were then pulled to failure at 10mm/min. The sample data were analyzed for peak stress (maximum stress seen before break), tensile modulus (slope of the stress-strain curve from 4% to 8% strain), and clamp to clamp strain.

Statistics. A one-way ANOVA was used to determine differences between age (continuous variable) per disk or condyle for biochemical and biomechanical values, with $p < 0.05$ defined as statistically significant. Tukey's post-hoc testing was used to examine any differences between groups. When comparing disks to condyles, a student's t-test was used with significance at $p < 0.05$. For tensile testing, multiple samples from each individual disk were first averaged together. The resulting values ($n = 7$ per age group) were then used to statistically analyze the data. This removed the effect of varying sample sizes within each disk, and ensured that each disk was weighted equally. All statistical analysis was performed using statistical packages for social sciences (IBM, Armonk, NY). All data are reported as average $\pm$ standard deviation.

Results

Histology. The sizes of each disk and condyle in the AP and ML direction can be seen in Table 1. The pigs grow in a linear fashion, and in general are about 60–90 lb at 3 months, 120–180 lb at 6 months, and 240–360 lb at 9 months.

Histological stains of the TMJ disks at 3, 6, and 9 months in the transverse and sagittal plane can be seen in Fig. 2. A general decrease in overall cellularity occurs with the TMJ disk as age

increases. There also appears to be minor amounts of adipose tissue incorporated into the 3 month disk, which is not observed in later age groups. Collagen fiber organization becomes more aligned in the anterior-posterior direction as age increases.

Biochemistry. The DNA content of the disks and condyles is shown in Fig. 3(a). There were no differences ($p < 0.05$) in DNA content/dry weight (DW) between TMJ disks at 3, 6, and 9 months of age. There were also no differences ($p < 0.05$) in DNA content between condyles at 3, 6, and 9 months of age.

The hydroxyproline content of the disks and condyles is shown in Fig. 3(b). There were no significant differences ($p < 0.05$) in hydroxyproline content/DW between TMJ disks at 3, 6, and 9 months of age. The hydroxyproline content of 3 month condylar cartilage was significantly different ($p < 0.05$) than condylar cartilage at 6 and 9 months of age.

The GAG content of the disks and condyles is shown in Fig. 3(c). There were no differences ($p < 0.05$) in GAG content/DW between TMJ disks at 3, 6, and 9 months of age. The GAG content of 6 month condylar cartilage was significantly different ($p < 0.05$) than condylar cartilage at 9 months of age.

Compression Testing. The compressive stresses of the disks and condyles at each strain step are shown in Table 2. There were no differences ($p < 0.05$) in compressive stress at 30% strain between TMJ disks at 3, 6, and 9 months of age. There were no differences ($p < 0.05$) in compressive stress between condyles at 3, 6, and 9 months of age. The same trend was observed in the 10% and 20% strain steps (Table 2).

The compressive moduli of the disks and condyles at each strain step are shown in Table 2. There were no differences ($p < 0.05$) in instantaneous at 30% strain between TMJ disks at 3, 6, and 9 months of age. There were no differences ($p < 0.05$) in instantaneous modulus between condyles at 3, 6, and 9 months of age. The same trend was observed in the 10% and 20% strain steps (Table 2).

Tensile Testing. To obtain the results for tensile testing, an average stress and modulus was obtained for each individual TMJ disk, consisting of the separate samples obtained for each individual disk. These averaged values for each disk were then aggregated together for each time point, and the average stress and modulus values presented here are the averages of these aggregates. The tensile stress of the disks is shown in Fig. 4(a). There were no differences ($p < 0.05$) in tensile stress between TMJ disks at 3, 6, and 9 months of age. There were also no differences

Fig. 2 Hematoxylin and Eosin staining of MJ disks at 3, 6, and 9 months. Row 1: samples sectioned in the transverse plane. Row 2: samples sectioned in the sagittal plane. All images were taken from the intermediate zone. Scale bar is 100 μ m.

Fig. 3 Biochemistry results for TMJ disks ($n = 10$ per group) and mandibular condyles ($n = 10$ per group). All error bars represent standard deviation. There were no significant differences detected in (a) DNA content. Significant differences were detected for (b) hydroxyproline content, and (c) GAG content between time points for both disks (white bars) and condyles (gray bars).

($p < 0.05$) in tensile modulus (shown in Fig. 4(b)) between disks at 3, 6, and 9 months of age.

Discussion

The results of the present study show that age affects the biochemical properties of mandibular condylar cartilage, but there seemed to be no effects of age on the mechanical properties of the mandibular condylar cartilage. There was, however, a trend of increased compressive instantaneous modulus with age. Furthermore, growth did not seem to impact any of the properties measured for the disk. The lack of differences in the biochemical content from different ages corresponds to a lack of differences in the mechanical properties in the TMJ disk. Since the composition of these major components of the extracellular matrix remains

largely unchanged across time points, it would be expected that the mechanical properties would be similar. The results of mechanical testing also suggest that the collagen deposited by fibrochondrocytes upon formation of the disk is already aligned into a densely packed collagen network by 3 months.

The literature is bereft of studies on the properties of the mandibular condylar cartilage. To our knowledge, this is the first study on the porcine condylar cartilage matrix constituents. Several studies have been performed on the mechanical properties of the mandibular condylar cartilage, but they have either not been stress relaxation studies, or the studies have utilized different strain rates, which does not allow for direct comparisons [27,33,34,37].

For compression of the TMJ disk, a peak stress at a similar stress relaxation strain rate has not been reported, but an instantaneous modulus of 2079 ± 581 MPa has been reported at 30% strain, which is in range with the presented values [23]. The tensile peak stress of 5 MPa was in the range of what has been reported for the porcine TMJ disk at 10 mm/min strain rate (5.98 MPa, 4.3 MPa) [21,23]. The tensile modulus determined here, 35 MPa, was also in range of previously stated values (28.6 MPa, 23.0 MPa).

The utilization of multiple tensile samples per individual disk allowed for average values per disk to be determined, which led to a reduction of overall error observed during testing. We have calculated the within animal variance for tensile modulus to be 13 MPa, from taking the standard deviation of each animal and then average. The animal to animal variance was 17 MPa, as calculated by averaging all the samples per animal, and then taking the standard deviation. As the within animal variance is as high as the animal to animal variance, the test's experimental error is large. This is why we argue that more than once sample per disk needs to be tested because the test is not repeatable. In other words, the experimental error hides the animal to animal variance. We believe that the heterogeneous orientation of the collagen fibers in the TMJ disk is the cause for the high within animal variance [40].

The effect size for this experiment was 0.251, which would have required a sample size of 198 to find a significant difference, assuming a power of 0.8. The computed power from the results obtained during testing was 0.11, which suggests that the research conducted for this study was underpowered. The large sample size

Table 2 Compressive peak stress and modulus results at each strain step for TMJ disks and condyles at each time point ($n = 10$ per group). All errors represent standard deviation.

Strain step	Tissue	Age	Peak stress (kPa)	Instantaneous modulus (kPa)
10%	Disk	3 months	13 ± 11	251 ± 205
		6 months	7 ± 4	126 ± 75
		9 months	6 ± 5	127 ± 94
	Condyle	3 months	6 ± 1	104 ± 23
		6 months	8 ± 4	135 ± 83
		9 months	8 ± 6	126 ± 116
20%	Disk	3 months	61 ± 35	756 ± 366
		6 months	44 ± 24	656 ± 323
		9 months	44 ± 35	640 ± 470
	Condyle	3 months	23 ± 6	263 ± 91
		6 months	27 ± 15	287 ± 147
		9 months	27 ± 19	288 ± 197
30%	Disk	3 months	138 ± 56	1476 ± 487
		6 months	124 ± 55	1568 ± 588
		9 months	112 ± 70	1383 ± 689
	Condyle	3 months	51 ± 16	516 ± 146
		6 months	61 ± 30	580 ± 240
		9 months	58 ± 38	607 ± 380

Fig. 4 Tensile results for TMJ disks ($n = 7$ per group). All error bars represent standard deviation. There were no significant differences detected in (a) tensile peak stress and (b) tensile modulus across age groups.

required by the effect size, however, would not be considered reasonable for the scope of this study. As a result, we can only conclude from the study that there does not appear to be a difference in mechanical properties from growth.

For future studies, while the mechanical and biochemical properties of pigs do not seem to vary with age, the use of 3 month old farm pigs does include an increased regenerative potential when compared to adults. It will be important, therefore, to consider that potential efficacy of any treatment tested in young farm pigs could be attributed to their regenerative potential instead of the success of said treatment. To address this, minipigs could be considered as a viable alternative to overcome the rapid growth and maintenance issues, albeit at an increased cost to the study.

In conclusion, the differences found between the 3, 6, and 9 month properties of the condyle cartilage maybe enough to prompt for future studies to perform age match controls. However, there did not seem to be age differences for the TMJ disk, but the error in the tests may mask some of those comparisons. It should be noted that changes in the skeletal anatomy due to rapid growth may necessitate the use of animals within a specific age range for surgical studies. The potential for the proposed tensile method, and the reduction of the variability in tensile modulus, suggest that this method is useful for future biomechanical characterization with TMJ disks.

Funding Data

- National Institute of Biomedical Imaging and Bioengineering (Grant No. T32 EB003392).
- National Institute of Dental and Craniofacial Research (Grant Nos. 1F31 DE025810 and R01 DE022055).

References

- [1] Beek, M., Koolstra, J. H., van Ruijven, L. J., and van Eijden, T. M., 2000, "Three-Dimensional Finite Element Analysis of the Human Temporomandibular Joint Disc," *J. Biomech.*, **33**(3), pp. 307–316.
- [2] Donzelli, P. S., Gallo, L. M., Spilker, R. L., and Palla, S., 2004, "Biphasic Finite Element Simulation of the TMJ Disc From In Vivo Kinematic and Geometric Measurements," *J. Biomech.*, **37**(11), pp. 1787–1791.
- [3] Tanaka, E., del Pozo, R., Tanaka, M., Asai, D., Hirose, M., Iwabe, T., and Tanne, K., 2004, "Three-Dimensional Finite Element Analysis of Human Temporomandibular Joint With and Without Disc Displacement During Jaw Opening," *Med. Eng. Phys.*, **26**(6), pp. 503–511.
- [4] Tanaka, E., and van Eijden, T., 2003, "Biomechanical Behavior of the Temporomandibular Joint Disc," *Crit. Rev. Oral Biol. Med.*, **14**(2), pp. 138–150.
- [5] Estabrooks, L. N., Fairbanks, C. E., Collett, R. J., and Miller, L., 1990, "A Retrospective Evaluation of 301 TMJ Proplast-Teflon Implants," *Oral Surg. Oral Med. Oral Pathol.*, **70**(3), pp. 381–386.
- [6] Henry, C. H., and Wolford, L. M., 1993, "Treatment Outcomes for Temporomandibular Joint Reconstruction After Proplast-Teflon Implant Failure," *J. Oral Maxillofac. Surg.*, **51**(4), pp. 352–360.
- [7] Brown, B. N., Chung, W. L., Almarza, A. J., Pavlick, M. D., Reppas, S. N., Ochs, M. W., Russell, A. J., and Badylak, S. F., 2012, "Inductive, Scaffold-Based, Regenerative Medicine Approach to Reconstruction of the Temporomandibular Joint Disc," *J. Oral Maxillofac. Surg.*, **70**(11), pp. 2656–2668.
- [8] Brown, B. N., Chung, W. L., Pavlick, M., Reppas, S., Ochs, M. W., Russell, A. J., and Badylak, S. F., 2011, "Extracellular Matrix as an Inductive Template for Temporomandibular Joint Meniscus Reconstruction: A Pilot Study," *J. Oral Maxillofac. Surg.*, **69**(12), pp. e488–e505.
- [9] Puelacher, W. C., Visser, J., Vacanti, C. A., Ferraro, N. F., Jaramillo, D., and Vacanti, J. P., 1994, "Temporomandibular Joint Disc Replacement Made by Tissue-Engineered Growth of Cartilage," *J. Oral Maxillofac. Surg.*, **52**(11), pp. 1172–1178.
- [10] Tarafder, S., Koch, A., Jun, Y., Chou, C., Awadallah, M. R., and Lee, C. H., 2016, "Micro-Precise Spatiotemporal Delivery System Embedded in 3D Printing for Complex Tissue Regeneration," *Biofabrication*, **8**(2), p. 025003.
- [11] Wu, Y., Gong, Z., Li, J., Meng, Q., Fang, W., and Long, X., 2014, "The Pilot Study of Fibrin With Temporomandibular Joint Derived Synovial Stem Cells in Repairing TMJ Disc Perforation," *Biomed. Res. Int.*, **2014**, p. 454021.
- [12] Hagandora, C. K., Tudares, M. A., and Almarza, A. J., 2012, "The Effect of Magnesium Ion Concentration on the Fibrocartilage Regeneration Potential of Goat Costal Chondrocytes," *Ann. Biomed. Eng.*, **40**(3), pp. 688–696.
- [13] Almarza, A. J., and Athanasiou, K. A., 2005, "Effects of Initial Cell Seeding Density for the Tissue Engineering of the Temporomandibular Joint Disc," *Ann. Biomed. Eng.*, **33**(7), pp. 943–950.
- [14] Almarza, A. J., and Athanasiou, K. A., 2006, "Evaluation of Three Growth Factors in Combinations of Two for Temporomandibular Joint Disc Tissue Engineering," *Arch. Oral Biol.*, **51**(3), pp. 215–221.
- [15] Hagandora, C. K., Gao, J., Wang, Y., and Almarza, A. J., 2013, "Poly (Glycerol Sebacate): A Novel Scaffold Material for Temporomandibular Joint Disc Engineering," *Tissue Eng., Part A*, **19**(5–6), pp. 729–737.
- [16] Almarza, A. J., and Athanasiou, K. A., 2004, "Seeding Techniques and Scaffolding Choice for Tissue Engineering of the Temporomandibular Joint Disc," *Tissue Eng.*, **10**(11–12), pp. 1787–1795.
- [17] Reiland, S., 1978, "Growth and Skeletal Development of the Pig," *Acta Radiol. Suppl.*, **358**, pp. 15–22.
- [18] Almarza, A. J., Bean, A. C., Baggett, L. S., and Athanasiou, K. A., 2006, "Biochemical Analysis of the Porcine Temporomandibular Joint Disc," *Br. J. Oral Maxillofac. Surg.*, **44**(2), pp. 124–128.
- [19] Beatty, M. W., Bruno, M. J., Iwasaki, L. R., and Nickel, J. C., 2001, "Strain Rate Dependent Orthotropic Properties of Pristine and Impulsively Loaded Porcine Temporomandibular Joint Disc," *J. Biomed. Mater. Res.*, **57**(1), pp. 25–34.
- [20] Chladek, W., and Czerwik, I., 2008, "Mechanical Properties of Temporomandibular Joint Disc on the Basis of Porcine Preparation Investigations," *Acta Bioeng. Biomech.*, **10**(4), pp. 15–20.
- [21] Detamore, M. S., and Athanasiou, K. A., 2003, "Tensile Properties of the Porcine Temporomandibular Joint Disc," *ASME J. Biomech. Eng.*, **125**(4), pp. 558–565.
- [22] Detamore, M. S., Orfanos, J. G., Almarza, A. J., French, M. M., Wong, M. E., and Athanasiou, K. A., 2005, "Quantitative Analysis and Comparative Regional Investigation of the Extracellular Matrix of the Porcine Temporomandibular Joint Disc," *Matrix Biol.*, **24**(1), pp. 45–57.
- [23] Kalpakci, K. N., Willard, V. P., Wong, M. E., and Athanasiou, K. A., 2011, "An Interspecies Comparison of the Temporomandibular Joint Disc," *J. Dent. Res.*, **90**(2), pp. 193–198.
- [24] Kang, H., Bao, G., Dong, Y., Yi, X., Chao, Y., and Chen, M., 2000, "Tensile Mechanics of Mandibular Condylar Cartilage," *Hua Xi Kou Qiang Yi Xue Za Zhi*, **18**(2), pp. 85–87.
- [25] Kim, K. W., Wong, M. E., Helfrick, J. F., Thomas, J. B., and Athanasiou, K. A., 2003, "Biomechanical Tissue Characterization of the Superior Joint Space of the Porcine Temporomandibular Joint," *Ann. Biomed. Eng.*, **31**(8), pp. 924–930.
- [26] Koolstra, J. H., Tanaka, E., and Van Eijden, T. M., 2007, "Viscoelastic Material Model for the Temporomandibular Joint Disc Derived From Dynamic Shear Tests or Strain-Relaxation Tests," *J. Biomech.*, **40**(10), pp. 2330–2334.
- [27] Kuboki, T., Shinoda, M., Orsini, M. G., and Yamashita, A., 1997, "Viscoelastic Properties of the Pig Temporomandibular Joint Articular Soft Tissues of the Condyle and Disc," *J. Dent. Res.*, **76**(11), pp. 1760–1769.
- [28] Lamela, M. J., Fernandez, P., Ramos, A., Fernandez-Canteli, A., and Tanaka, E., 2013, "Dynamic Compressive Properties of Articular Cartilages in the

- Porcine Temporomandibular Joint,” *J. Mech. Behav. Biomed. Mater.*, **23**, pp. 62–70.
- [29] Lumpkins, S. B., and McFetridge, P. S., 2009, “Regional Variations in the Viscoelastic Compressive Properties of the Temporomandibular Joint Disc and Implications Toward Tissue Engineering,” *J. Biomed. Mater. Res. A*, **90**(3), pp. 784–791.
- [30] Matuska, A. M., Muller, S., Dolwick, M. F., and McFetridge, P. S., 2016, “Biomechanical and Biochemical Outcomes of Porcine Temporomandibular Joint Disc Deformation,” *Arch. Oral Biol.*, **64**, pp. 72–79.
- [31] Murphy, M. K., Arzi, B., Hu, J. C., and Athanasiou, K. A., 2013, “Tensile Characterization of Porcine Temporomandibular Joint Disc Attachments,” *J. Dent. Res.*, **92**(8), pp. 753–758.
- [32] Ruggiero, L., Zimmerman, B. K., Park, M., Han, L., Wang, L., Burris, D. L., and Lu, X. L., 2015, “Roles of the Fibrous Superficial Zone in the Mechanical Behavior of TMJ Condylar Cartilage,” *Ann. Biomed. Eng.*, **43**(11), pp. 2652–2662.
- [33] Singh, M., and Detamore, M. S., 2008, “Tensile Properties of the Mandibular Condylar Cartilage,” *ASME J. Biomech. Eng.*, **130**(1), p. 011009.
- [34] Singh, M., and Detamore, M. S., 2009, “Stress Relaxation Behavior of Mandibular Condylar Cartilage Under High-Strain Compression,” *ASME J. Biomech. Eng.*, **131**(6), p. 061008.
- [35] Snider, G. R., Lomakin, J., Singh, M., Gehrke, S. H., and Detamore, M. S., 2008, “Regional Dynamic Tensile Properties of the TMJ Disc,” *J. Dent. Res.*, **87**(11), pp. 1053–1057.
- [36] Tanaka, E., Hanaoka, K., van Eijden, T., Tanaka, M., Watanabe, M., Nishi, M., Kawai, N., Murata, H., Hamada, T., and Tanne, K., 2003, “Dynamic Shear Properties of the Temporomandibular Joint Disc,” *J. Dent. Res.*, **82**(3), pp. 228–231.
- [37] Tanaka, E., Yamano, E., Dalla-Bona, D. A., Watanabe, M., Inubushi, T., Shirakura, M., Sano, R., Takahashi, K., van Eijden, T., and Tanne, K., 2006, “Dynamic Compressive Properties of the Mandibular Condylar Cartilage,” *J. Dent. Res.*, **85**(6), pp. 571–575.
- [38] Willard, V. P., Kalpakci, K. N., Reimer, A. J., and Athanasiou, K. A., 2012, “The Regional Contribution of Glycosaminoglycans to Temporomandibular Joint Disc Compressive Properties,” *ASME J. Biomech. Eng.*, **134**(1), p. 011011.
- [39] Hagandora, C. K., Chase, T. W., and Almaraz, A. J., 2011, “A Comparison of the Mechanical Properties of the Goat Temporomandibular Joint Disc to the Mandibular Condylar Cartilage in Unconfined Compression,” *J. Dent. Biomech.*, **2011**(1), p. 212385.
- [40] Fazaeli, S., Ghazanfari, S., Everts, V., Smit, T. H., and Koolstra, J. H., 2016, “The Contribution of Collagen Fibers to the Mechanical Compressive Properties of the Temporomandibular Joint Disc,” *Osteoarthritis Cartilage*, **24**(7), pp. 1292–1301.