Mandibular Condyle Shape Changes Following Unilateral Partial Discectomy in Mice

BY

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THESIS

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LIST OF ABBREVIATIONS

CBCT	Cone-beam computed tomography
DICOM	Digital Imaging and Communications in Medicine
GM	Geometric morphometrics
MicroCT	Micro-computed tomography
NSC	Non-surgical control
OA	Osteoarthritis
PC	Principal component
PCA	Principal component analysis
PFA	Paraformaldehyde
RT-PCR	Reverse transcription polymerase chain reaction
TMD	Temporomandibular disorder
TMJ	Temporomandibular joint
UPD	Unilateral partial discectomy

SUMMARY

Temporomandibular joint disorder (TMD) is a relatively common phenomenon, as an estimated 5% to 12% of the population experiences TMD-related symptoms (Matheson et al., 2023). Despite the ubiquitous nature of TMD, there is still much that remains unknown to both researchers and clinicians alike. TMD may be caused by wide variety of factors, and is generally grouped into muscle-related disorders and joint-related disorders. Bony and cartilaginous degeneration as seen in osteoarthritis (OA) of the temporomandibular joint (TMJ) can occur as a result of mechanical overloading of the joint, often in the presence of internal derangements or disc perforations.

Due to the incomplete understanding of TMJ OA, researchers have looked to an animal model of OA induction to be able to better study this phenomenon. Previous research has demonstrated how surgical intervention, including unilateral partial discectomy (UPD), in an animal model can produce regional bony and cartilaginous changes consistent with changes seen in OA. These studies have described bony changes of the condyle on a univariate level. While these measurements are precise, univariate measurements (such as mediodistal width, or anteroposterior length of a condyle) do not capture shape changes occurring in TMJ OA. A multivariate geometric morphometric (GM) approach is necessary to be able to more adequately detect shape changes and give an indication as to where they might be occurring. Additionally, a GM shape analysis lets you distinguish shape from size compared to a standard morphometric approach (using linear and angular measurements) which would not.

The results of this study indicate that performing unilateral partial discectomy produces bony changes in shape, such as condylar flattening that is seen with osteoarthritis. The surgical intervention resulted in an experimental group with condyles that were wider mediolaterally, and flatter superoinferiorly compared to non-surgical controls.

1.0 INTRODUCTION

1.1 **Background**

The temporomandibular joint (TMJ) serves as a pivotal anatomical structure facilitating essential functions such as mastication, speech, and facial expression. Its complex nature, composed of bone, cartilage, ligaments, and muscles underscores its susceptibility to various pathologies. Functional impairment of the joint is referred to as temporomandibular disorder (TMD). TMD encompasses a spectrum of clinical manifestations, ranging from localized discomfort to debilitating pain and restricted jaw movement, with the potential to significantly impact an individual's quality of life. Despite extensive research, the precise etiology of TMD often remains elusive, attributed to a combination of factors including biomechanical, anatomical, psychological, and genetic elements (Chisnoiu et al., 2015).

Osteoarthritis (OA) is defined by the deterioration of joint cartilage and remodeling of the underlying subchondral bone. Important to the understanding and management of TMD is the recognition of OA as a significant contributing factor, particularly in cases involving degenerative changes within the TMJ.. There is an important gap in knowledge in terms of precise descriptions in the change of shape of the mandibular condyle and surrounding structures that occurs as TMJ OA progresses. With a better understanding of change in shape, we can more easily link changes at the cellular level with the anabolic and catabolic processes of the cartilage and subchondral bone.

Animal models serve a valuable role in studying osteoarthritis, as researchers have developed many techniques to induce osteoarthritis in a laboratory animal setting.. A study by

Zhao et. al, 2022 outlines different types of animal models of TMJ OA induction. Among these are intra-articular injection methods, surgical induction models, mechanical loading models, high-fat diet models, sleep deprivation models, naturally occurring models, and genetically modified models.

Each of these methods of OA induction have their advantages and disadvantages. An advantage of surgical models for TMJ OA induction is that they work quickly, and can create large and easily observable lesions. We have developed expertise in performing unilateral partial discectomies in mice, which causes cartilage degeneration, condylar flattening and osteophytic lipping.

Table I shows a literature review of previous studies that have been performed, using an animal model to induce TMJ OA with a surgical technique. The majority of these studies used a mouse as the animal of choice. However, Hinton (1992) used a rat subject, Angelo et al. (2018) used black merino sheep, Saito et al (2021) used rabbits, and Man et al (2009) also used rabbits.

TABLE I

Literature review highlighting the methods of previous studies using surgical models of TMJ OA induction

Author	Year	Study Objectives
Angelo et al.	2018	Black merino sheep were used. There were three experimental groups: discectomy, discopexy, and sham. Histopathologic, imaging and body weight outcomes were examined following bilateral discectomy.
Cohen et al.	2014	Unilateral partial discectomies performed in mice. Histology done to evaluate cartilage on surgical and contralateral side. Tissue was collected at four week intervals (between 4-16 weeks) for histological examination.
Hinton	1992	Unilateral discectomies performed on rats, examining the wet and dry tissue weights of the condylar cartilage, which increased post discectomy
Ishizuka et al.	2021	Partial discectomies performed unilaterally on mice. Histology performed to analyze cartilage changes. This study was interested in looking at muscular changes (the temporalis) in addition to bony changes after OA induction. Univariate measurements performed to analyze volume of condylar head.
Lan et al.	2017	Discectomies performed on mice, then histology was used for evaluation. Immunohistochemistry done to evaluate expression of antibodies such as Notch1, Jagged1, Hes1, and Hes5.
Lei et al.	2020	Discectomies performed on mice. Condyles examined histologically and immunofluorescence performed to examine cartilage and subchondral bone post discectomy.
Liu et al.	2020	TMJ OA was induced through discectomy. MicroCT taken, and gray levels analyzed, trabecular bone also analyzed. Histological analysis completed to evaluate cartilage thickness. Immunohistochemistry performed, as well as immunofluorescence. RT-PCR performed for mRNA evaluation.
Man et al.	2009	Disc perforation performed bilaterally on rabbits. MicroCT taken to evaluate trabecular bone. Condylar thickness evaluated histologically. RT-PCR done to evaluate mRNA expression.

Saito et al.	2021	Discectomies were performed on rabbits. Following discectomies, univariate measurements were taken, such as mandibular ramus height, mandibular length, condylar length, and condylar width. Comparisons were made from a discectomy group, control group, and a discectomy with lower-intensity pulsed ultrasound (LIPUS) group.
Xu et al.	2009	Discectomies, performed on mice. Histology performed, and used to Modified Mankin scoring system to evaluate structural condition of articular cartilage. Immunohistochemistry performed to evaluate for Ddr2 and Mmp-13.

Many of these studies reviewed looked at the cartilaginous changes with histology, immunohistochemistry to assess gene expression, or MicroCT to evaluate trabecular bone. Few studies that have been done that have attempted to describe shape changes. Saito et al (2021) performed a discectomy study which included univariate measurements, such as mandibular ramus height, mandibular length, condylar length, and condylar width. Ishizuka et al (2021) used univariate measurements to determine the volume of the mandibular condyle following discectomy. But to our knowledge, no other study has attempted to use GM to perform a multivariate shape analysis to detect changes in shape of the mandibular condyle that occur after unilateral partial discectomy.

To our knowledge, the only other study that has used GM for analysis of condylar shape in an animal study has been Chen et al. (2022), however this study included extraction of maxillary molars. In their study, they found evidence of degenerative changes, but the mandibular condyle actually become more convex, which is different from the flattening that is typically seen with osteoarthritis.

1.2 **Objectives**

As was postulated in a study by Yotsuya et al. (2020), bony adaptations in the TMJ that occur following destabilization are a mechanism to minimize stress concentrations. The primary hypothesis of this thesis is that after discectomy, mechanical equilibrium is attained through adaptive alterations in the shape of the mandibular condyle and glenoid fossa, thus reducing stress concentrations on the joint. To investigate this hypothesis, we will investigate with two specific aims.

The first aim is to define how the shape of the mandibular condyle changes in mice who had surgical induction of OA through unilateral partial discectomy (UPD) compared to age- and sex-matched non-surgical controls. A second aim of the study was to determine whether the shape of the mandibular condyle of the OA group was different between females and males.

1.3 **Hypotheses**

Based on the aims of this study, we have the following null hypotheses:

H_0: There is no difference between the shape of the mandibular condyle of mice who have undergone unilateral partial discectomy (UPD) and age- and sex-matched non-surgical controls.

H_0: Following UPD, there are no differences between the shape of the mandibular condyle of male mice compared to female mice.

Conversely, we have the following alternative hypotheses:

H_A: There are differences between the shape of the mandibular condyle of mice who have undergone UPD and age- and sex-matched non-surgical controls.

H_A: Following UPD, there are differences between the shape of the mandibular condyle of male mice compared to female mice.

2.0 BACKGROUND

2.1 **Temporomandibular Joint (TMJ) Anatomy and Physiology**

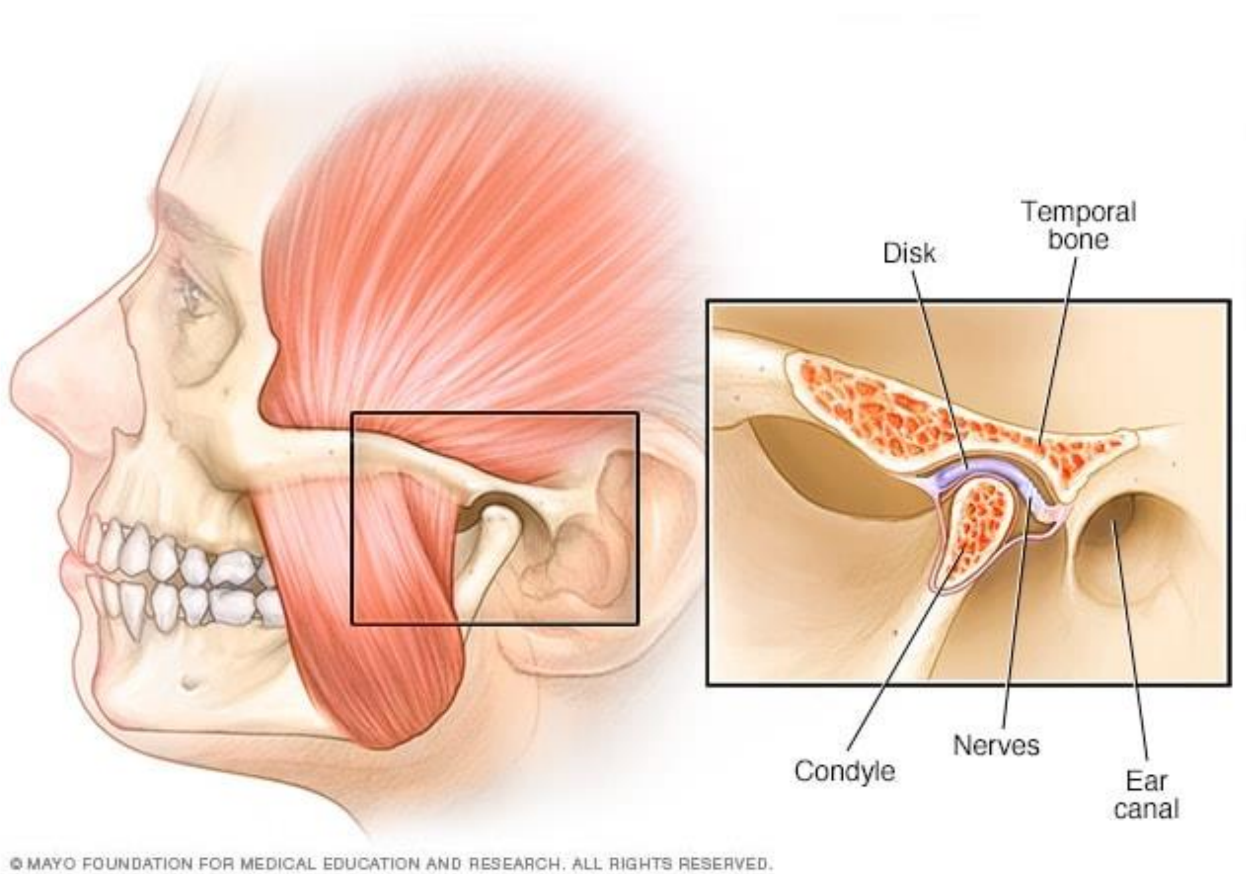

Figure 1: Components of the TMJ, Pruthi (2018)

To better understand shape changes that will occur to the temporomandibular joint (TMJ) with osteoarthritis (OA), it is first important to understand the anatomical components of the TMJ. The TMJ is a sophisticated articulation involving the condylar process of the mandible, the glenoid fossa of the temporal bone, and the articular disc (see Figure 1). This intricate anatomical arrangement enables the TMJ to execute a diverse range of movements crucial for essential functions such as mastication and speech (Okeson, 2019).

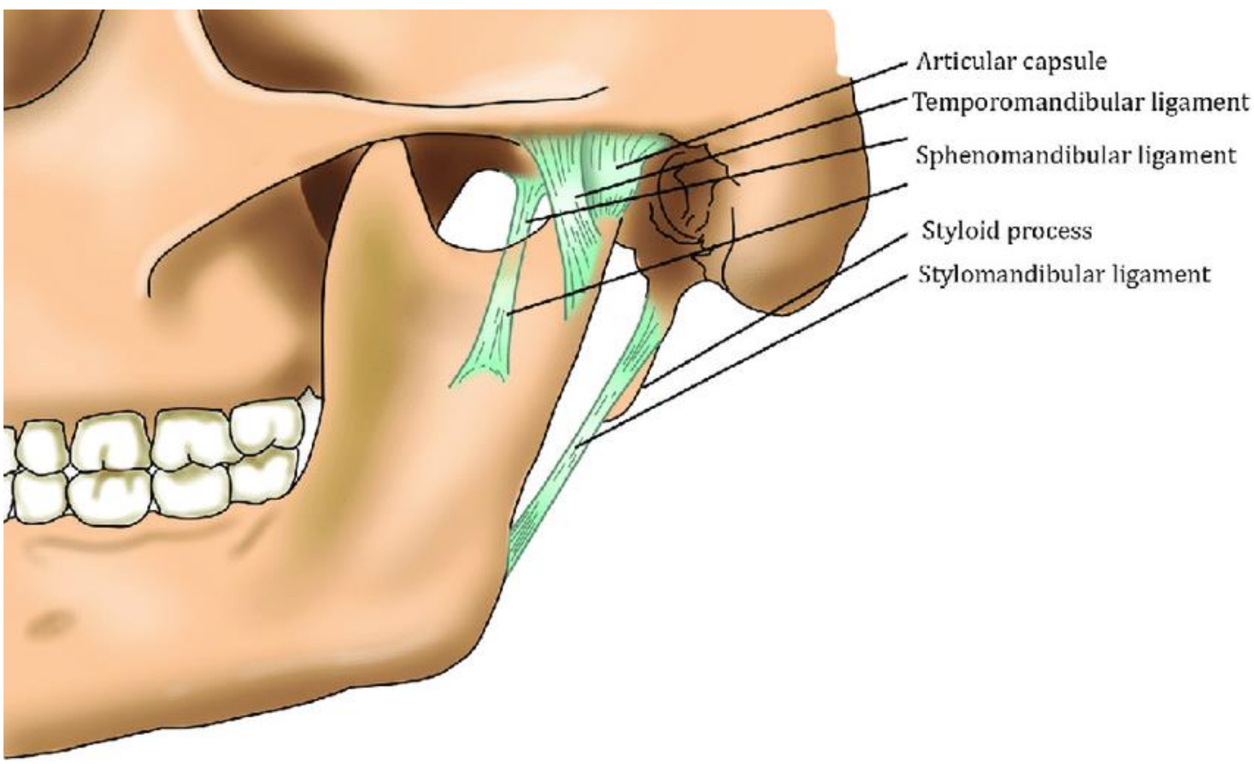

Figure 2: Ligaments of the TMJ, Esmaeelinejad and Sohrabi (2018)

There is an array of ligaments, which provide stability to the joint during movements (see Figure 2). These include the temporomandibular ligament, the sphenomandibular ligament, and the stylomandibular ligament. Furthermore, the TMJ is encapsulated by a fibrous articular capsule housing synovial fluid, ensuring smooth articulation and minimizing friction (Okeson, 2019). This complex anatomical configuration is vital for maintaining the TMJ's functionality and structural integrity.

The muscles governing TMJ movement are the muscles of mastication, infrahyoid, and suprahyoid muscles. The muscles of mastication (masseter, temporalis, lateral pterygoid, medial pterygoid) are primarily responsible for mandible elevation. The suprahyoid muscles (such as the digastric, mylohyoid, and geniohyoid) produce depression of the mandible. The infrahyoid muscles (sternothyroid, thyrohyoid, sternohyoid, omohyoid) are also responsible for depression of the mandible, and help to stabilize the hyoid bone, which creates a firm base for the origin of the suprahyoid muscles to act on for depression of the mandible (Mansfield & Neumann, 2019).

During movements of the mandible, the mandibular condyle rotates as well as translates in the joint apparatus. Rotation takes place as the condyles pivot around a fixed axis located within the condyles. With a pure rotational movement, the mouth can open and close without any movement of the condyles' position. Translation refers to a motion where every point of the object moves in the same direction and at the same velocity simultaneously. This action occurs when the mandible moves forward in protrusion. During this movement, the teeth and entire mandible all shift uniformly in one direction and to an equal degree. During most normal mandibular movements, both rotation and translation occur concurrently (Okeson et al., 2020). In a healthy functioning joint, the articular disc moves with the condyle in translational movements.

The TMJ disc operates as a structure with viscoelastic properties, serving as both a stress absorber and distributor. Consequently, it aids in averting stress concentration and excessive strain on the joint's cartilage and bone elements. These roles likely shield the joint from disc degeneration (such as perforation and thinning) and osteoarthritis (Tanaka & Van Eijden, 2003).

2.2 **Temporomandibular Disorder (TMD)**

Temporomandibular disorder (TMD) may affect the mandibular condyle, associated muscles, and surrounding bony structures. Literature by Kandasamy et al., 2015 has described the etiology of TMD as the following: orthopedic instability coupled with loading, trauma, emotional stress, deep pain input, parafunctional habits, psychosocial stressors, and an acute change in the occlusal condition. TMD manifests through a spectrum of signs and symptoms, including pain, restricted mandibular movement, joint noises, and functional limitations (Maini & Dua, 2024).

The prevalence of TMD varies across populations. In epidemiological studies, the reported prevalence is influenced by demographic factors, diagnostic criteria, and other methodological approaches utilized in these studies. It is estimated that approximately 5% to 12% of the population in the United States experiences TMD-related symptoms (Matheson et al., 2023). Previous research shows TMD has a high predilection for female patients, with studies showing a gender ratio of 2.6:1 to 7.3:1 (Li et al., 2019).

The economic burden associated with TMD encompasses direct healthcare costs, including diagnostic procedures, treatment modalities, and indirect costs related to productivity loss and absenteeism. In the United States alone, TMD imposes a substantial financial burden on both individuals and the healthcare system. The estimated annual direct and indirect costs of TMD management exceed $4 billion, highlighting the significant economic impact of this disorder (Matheson et al., 2023).

2.2.1 Classifications of Temporomandibular Disorder (TMD)

The majority of cases of Temporomandibular Disorder (TMD) can be categorized into either muscle pain or intracapsular disorders. Among these, muscle pain is more prevalent. (Schiffman et al., 1990).

TMD muscle disorders are generally caused by pain of the muscles of mastication. This may be due to overuse and fatigue, although it is often more complicated than that. There is some consensus that muscle pain is influenced by central nervous system mechanisms (Svensson and Graven-Nielsen, 2001). Myofascial pain is a type of muscular pain contributing to TMD that is defined as specific regions of tense and hypersensitive muscle tissue bands (referred to as "trigger points"). Trigger points are painful to palpation, and it is theorized that there may be some underlying localized neurologic sensitization or metabolic changes in the area causing hypersensitivity (Kandasamy et al., 2015).

Joint intracapsular disorders in TMD are alterations to the physical structure of the joint apparatus. The main types of intracapsular disorders are internal derangements, and osteoarthritis (OA). OA, one of the central components of this thesis, will be discussed in the next section (Section 2.3).

Internal derangements are abnormalities in the position of the disc in relation to the mandibular condyle. When the disc is positioned anteriorly, the condyle can load the retrodiscal tissues, which may cause pain (Okeson et al., 2020). Farrar and McCarty (1979) found in their review that nearly 70% of individuals experiencing temporomandibular dysfunction endure disc displacement, underscoring the pivotal role of the articular disc in the series of events contributing to advancing pathology and morbidity. A study by Iwasaki et al. (2009) found that individuals with anterior displacement of the articular disc experience increased loading of the TMJ compared to individuals with a normal disc position. These findings indicate that disc displacement can cause overloading of the joint, and thus lead to TMJ OA.

2.3 **Osteoarthritis of TMJ**

Osteoarthritis (OA) of the temporomandibular joint (TMJ) is a degenerative condition marked by the gradual breakdown of the cartilage of the articulating surfaces of the TMJ, and changes in the structure of the bone beneath the joint cartilage (subchondral bone). This leads to pain, limited jaw movement, and functional impairment. While there are various factors that contribute to the development of TMJ OA, including trauma, and genetic predisposition, one of the key factors that initiates TMJ OA is mechanical overloading of the joint (Laskin et al., 2006). The TMJ, akin to other synovial joints that bear weight, undergoes deterioration due to detrimental molecular processes initiated by excessive strain or systemic illness. When subjected to repetitive or excessive mechanical stress, TMJ experiences pathological changes if its inherent healing or adaptability surpasses its capacity (Laskin et al., 2006).

In TMJ OA, the condyle and fossa undergo significant morphological changes. Progression of the condition affects various structures including cartilage, subchondral bone, synovial membrane, and other surrounding tissues. These changes lead to alterations like bony TMJ remodeling, as well as abrasion and deterioration of the articular cartilage (Al-Ani, 2021). Bony changes are of particular interest to dental professionals and others who routinely view 2D or 3D radiographic images of the skull. The bony changes that can be identified include condylar flattening, condylar erosion, condylar osteophyte formation, condylar sclerosis, and flattening of the articular eminence (Cömert Kiliç et al., 2015). However, these changes may not always reflect clinical symptoms and are only seen in the later stages of osteoarthritis (Stegenga et al., 1991). OA of the TMJ can occur when the joint is overloaded, but it is most commonly takes place in conjunction with disk perforation, or disk displacements (Kandasamy et al., 2015).

2.4 Unilateral Partial Discectomies in Mice

As indicated previously, there are many animal models that have been developed for studying TMJ OA. Discectomies, although technique sensitive, offer rapid induction of TMJ OA. It also induces easily observable lesions of the condyle. However, there is a knowledge gap in the description of these precise mathematical changes of shape that occur that following initiation of OA.

The principle aim of this thesis is to use geometric morphometrics (GM) to analyze the shape changes of mouse TMJ following unilateral partial discectomy. The condylar shape of the surgical group will be compared to age- and sex-matched non-surgical controls to determine if shape changes are statistically significant.

2.5 Geometric Morphometrics (GM)

Geometric Morphometrics (GM) is the field of study that performs statistical analysis of the shape of an object based on landmarks that have associated Cartesian (x,y,z) coordinates (Mitteroecker & Gunz, 2009). Landmarks are defined as "homologous anatomical loci that provide adequate morphology and can be found repeatedly and reliably" (Zelditch et al., 2004). Semilandmarks are points along a curve between landmarks that help capture the shape of the surface of the object. The landmarks and semilandmarks can be thought of as points that can be used to map out the surface of an object. Once the landmarks have been placed, a shape analysis can occur.

Shape is defined as "all the geometric information that remains when location, scale and rotational effects are filtered out from an object" (Kendall,1977). To compare the shape of objects, a superimposition method such as Procrustes Superimposition (PS) performed. PS is used to account for discrepancies in translation, rotation, and scale between different objects (see Figure 4).

To perform a PS, first the objects are centered around a common point, called the centroid. A centroid is calculated by finding mean of each coordinate (x,y,z) separately. Next, the objects are scaled so they have the same centroid size. The centroid size is calculated from the following equation: $\sqrt{\sum_{i=1}^{n}[(x_i - \bar{x})^2 + (y_i - \bar{y})^2 + (z_i - \bar{z})^2 + ...]}$, which refers to the square root of the sum of the squared differences between coordinates and their centroid. Lastly, the objects are corrected for rotation. This is done by rotating the objects about the centroid until the total squared Euclidean distances among corresponding landmarks are minimized (Mitteroecker and Gunz, 2009).

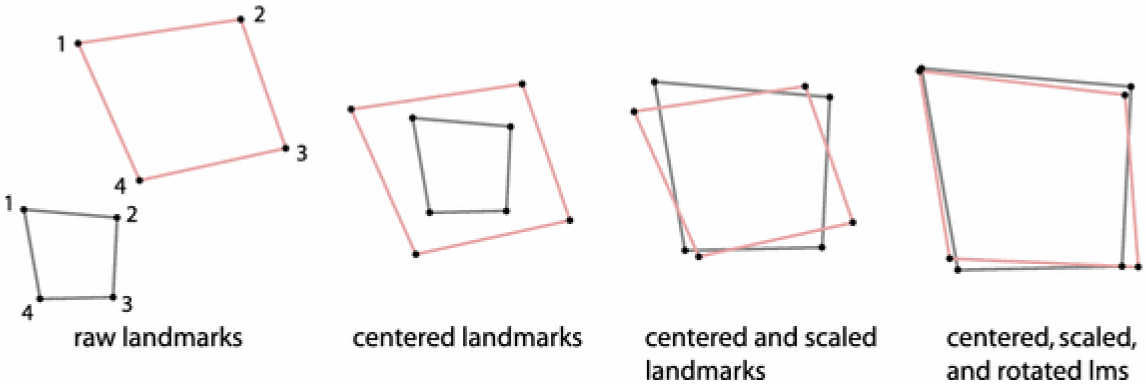

Figure 3: The steps of a Procrustes Superimposition are outlined above. First, the objects must be centered to a common origin. Next, the objects must be scaled to a common size. Lastly, the objects are rotated so that they minimize the distances between the homologous landmarks (Mitterocker and Gunz, 2009).

Following superimposition, all the data from the landmarks and semilandmarks can be simplified with a Principal Component Analysis (PCA). The goal of PCA is to identify the directions (principal components) in which the data varies the most. Principal components capture the maximum amount of variance present in the data. The initial principal component captures the highest amount of variance, followed by the second principal component, and so on. The objective of PCA is to simplify large datasets while preserving as much of the variability within the data as possible.

Using GM to perform shape analysis is way to mathematically and precisely describe the differences in shapes between many specimens of different experimental groups. A univariate shape analysis, such as measuring the mediodistal width or anteroposterior length of a condyle can give valuable information, but it is missing a large portion overall shape of the structure and therefore can miss much of the variation in shape that is occurring. Furthermore, because a GM approach accounts for the differences in sizes between objects, it is therefore able to compare shape.

In summary, a geometric morphometric shape analysis is preferable to a standard morphometric approach (angular and linear measurements) because it is able to capture more information about the variation in shape between different experimental groups and is also able to allow you to separate shape from size.

3.0 MATERIALS AND METHODS

3.1 **Experimental Process and Controls**

Unilateral partial discectomy was performed to induce TMJ OA in accordance with the procedures outlined in Xu et al. (2009) and our prior works (Reed et al., 2019; Yotsuya et al., 2019; Yotsuya et al., 2020). Skeletally mature 16-week-old male and female c57 BL/6 mice given the anesthetics ketamine (100 mg/kg, Henry Schein, Dublin, Ohio) and xylazine (5 mg/kg, Akorn, Lake Forest, IL).

The skin surrounding the TMJ area was shaved, and then cleaned with betadine and 70% ethanol. An incision of 3-5 mm was made above the temporomandibular joint (TMJ) on the right, exposing the lateral capsule. The articular disc was removed, and immediately afterwards irrigation of the joint was performed with sterile 1x Phosphate-Buffered Saline. The surgical incision was stitched closed using 5–0 nylon suture (Ethicon, Bridgewater, NJ).

Throughout the experiment, all mice were maintained on a standard diet. Upon reaching the experimental endpoint, mice were euthanized via CO2 inhalation. The skulls were then collected, fixed overnight in 4% paraformaldehyde, and stored in 70% ethanol before microCT scanning.

As seen in Table II, a total of 14 mice (7 male and 7 female) were used in the experimental group. An equal amount of age- and sex-matched non-surgical controls (NSCs) was used for comparison. Mice were sacrificed four weeks following discectomies, and tissue were collected for scanning. All samples were scanned using either a Scanco microCT 40 or a Scanco 50 microCT scanner at 70 kV and a resolution of 12 μm voxel size.

TABLE III

NUMBER OF CONDYLES OBSERVED BY SEX AND GROUP

	Non-Surgical Control	Discectomy
Males	7	7
Female	7	7
Total	14	14

3.2 **Landmarking**

MicroCT scans were exported as DICOM files, then loaded into 3D Slicer. Mandibular condyles were segmented using 3D Slicer, by isolating the condyle from the surrounding glenoid fossa. STL files of the isolated mandibular condyle were generated. These newly generated three-dimensional STL reconstructions were loaded into 3D slicer and landmarks and semilandmarks were placed.

Descriptions of landmarks and semilandmarks can be seen in Table III and Table IV, respectively. A total of 18 landmarks and semilandmarks were used, see figure 3 for a visual representation of the placement. Landmarks points were placed at medial pole and lateral pole of condyle, as well as the most anteroinferior and posteroinferior surface of the condyle. Seven semilandmarks were placed in the mediodistal center of the condyle, from anterior to posterior on the superior surface of the condyle. Three semilandmarks were placed in the anteroposterior center in the medial to lateral direction. Four semilandmarks were placed around the periphery of the condyle.

TABLE IIIII

CONDYLE LANDMARKS

Landmark	Description
1	Most posterior and inferior point of condyle
5	Most anterior and inferior point of condyle
3	Lateral pole of condyle
7	Medial Pole of condyle

TABLE IVV

CONDYLE SEMILANDMARKS

Semilandmarks	Description
2,4,6,8	Points evenly distributed along the periphery of the condyle
9-15	Points evenly distributed along the midline of the superior surface of the condyle in the anteroposterior dimension.
16-18	Points evenly distributed along the midline of the condyle in the mediolateral direction

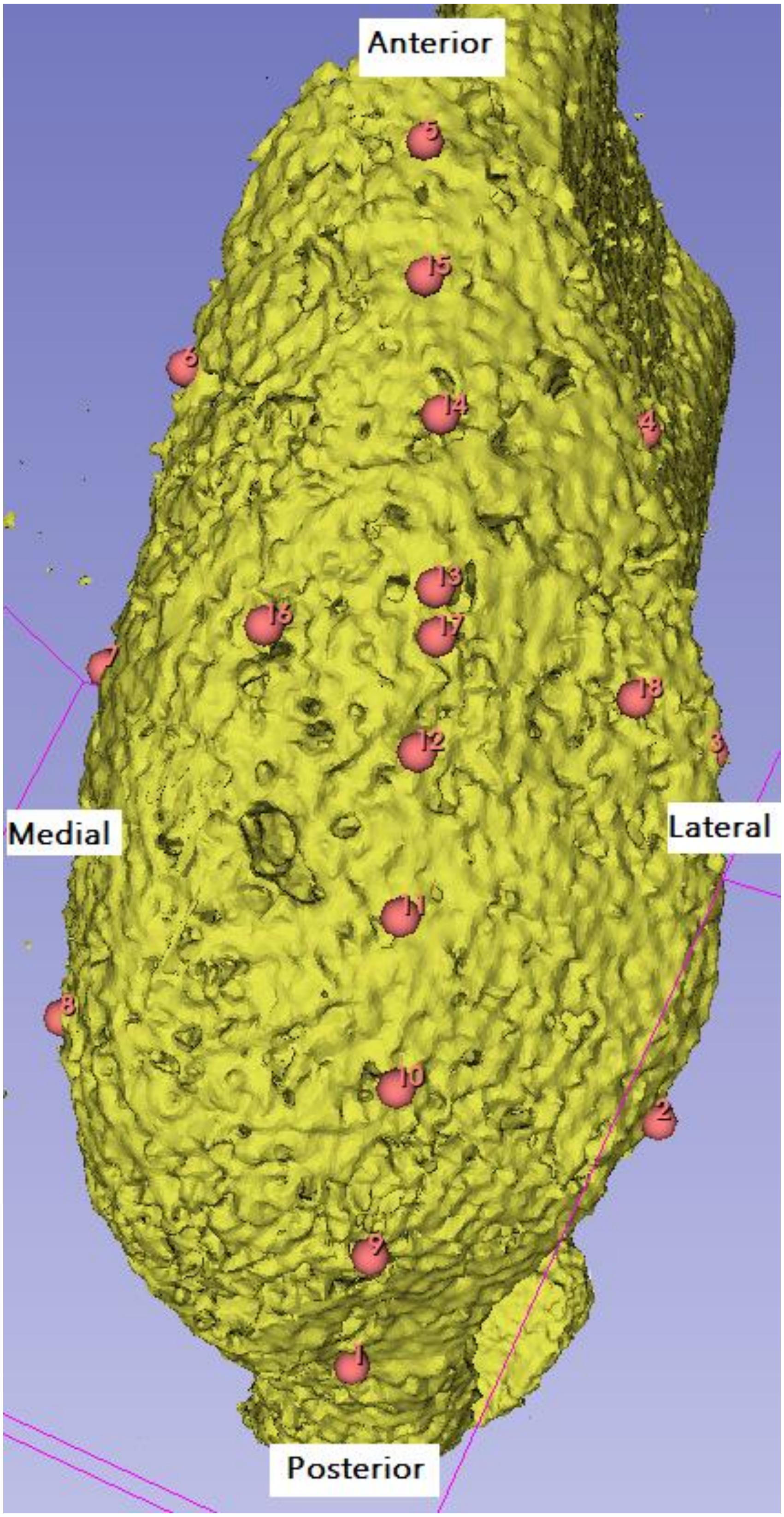

Figure 4: Condyle with landmarks and semilandmarks placed.

3.3 **Geometric Morphometrics**

Following landmark placement, Procrustes superimposition technique was performed. Principal component analysis was then performed. With the principal component analysis, means of experimental group and non-surgical control group were compared to look for statistical differences in shape.

3.4 **Statistical Analysis**

Following principal component analysis (PCA), Shapiro-Wilk normality test was performed on principle components (PCs) to determine if data was normally distributed. Of the PCs selected for further analysis, parametric statistical analysis was conducted on data exhibiting a normal distribution, while non-parametric statistical analyses were used for data that did not adhere to a normal distribution.

For normally distributed data, Welch two sample t-tests were performed to test the difference in means between the control group and osteoarthritis (OA) group. Welch two sample t-tests were also performed to compare means between the OA male and OA female group. Conversely, for non-normally distributed data, Wilcoxon rank sum exact test was used to compare means.

3.5 **Inclusion and Exclusion Criteria**

For inclusion in the study, each specimen needed to have a MicroCT scan with the entire surface of the mandibular condyle present to be able to place all landmarks for proper analysis. Any MicroCT scans where any part of the mandibular condyle was cut off were excluded from the study.

For the surgical group included in the study, it was necessary for the mouse to survive the UPD procedure, and survive another 4 weeks to be able to evaluate the bony OA changes that occur following the surgical intervention.

4.0 RESULTS

4.1 **Results**

Principal component analysis (PCA) identified a total of 27 principal components (PCs). The first five PCs individually accounted for at least 5% of the total variation, and thus were chosen for further analysis. Shapiro-Wilk normality test confirmed all PCs analyzed exhibited normal distribution, except for PC4. Welch two sample t-tests were used to compare means of normally distributed PCs (PC1, PC2, PC3 and PC5). Wilcoxon rank sum exact test used to compare means of experimental groups of PC4.

Our analysis revealed a significant disparity in shape ($p<0.001$) between the control and osteoarthritis (OA) groups along PC1. No statistically differences seen between controls and OA with other PCs. For the OA group, no statically significant differences seen between the sexes.

As can be seen in Figure 5 and Figure 6, the difference in PC1 shape was striking, with no overlap in scores between the two groups. The OA group displayed higher PC1 scores, while the control group was characterized by lower PC1 scores.

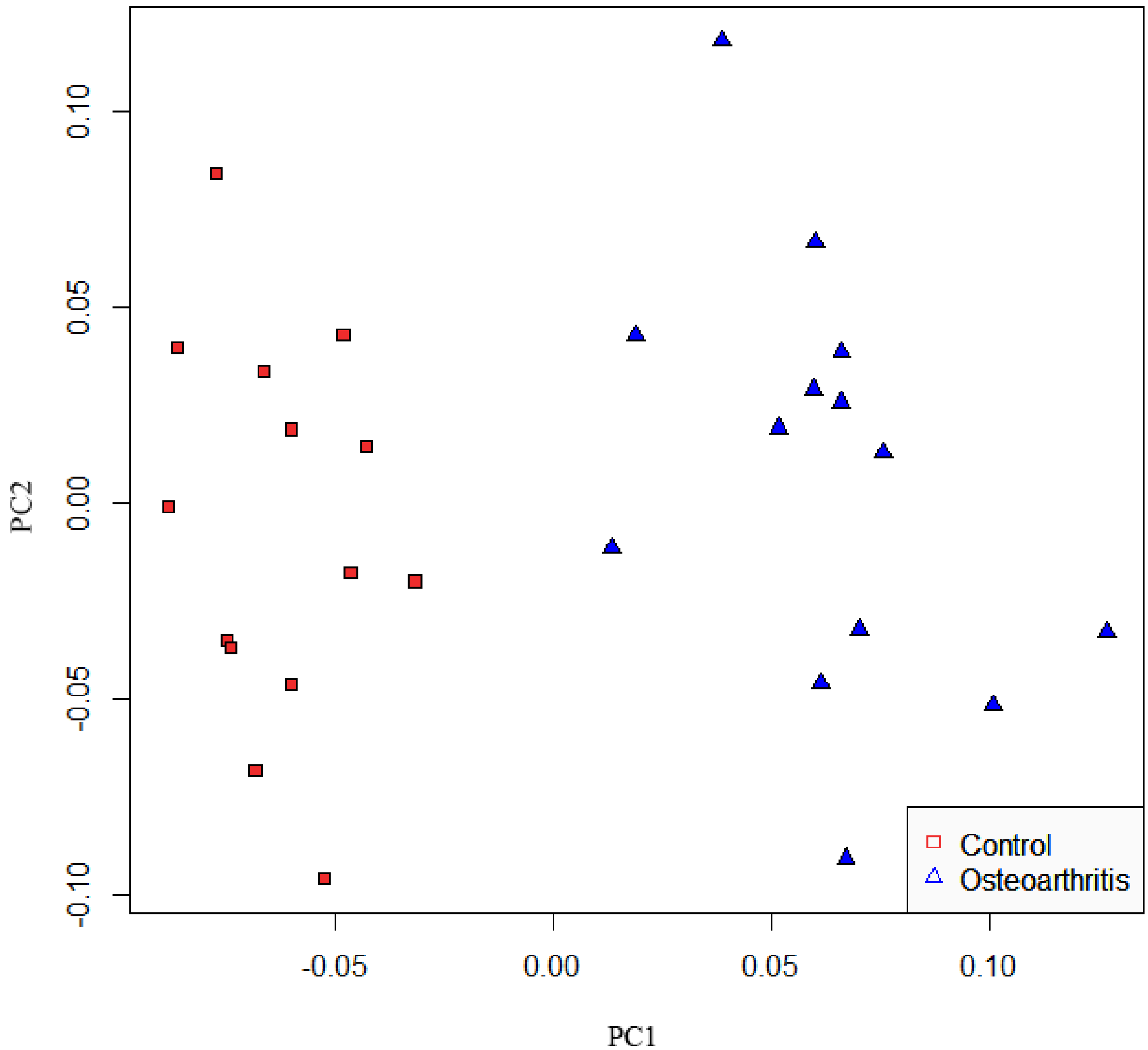

Figure 5: A plot of PC1 on the horizontal axis, and PC2 on the vertical axis. For PC1, there is no overlap between the control group (red boxes on left) and osteoarthritis group (blue triangles on right). For PC2, however, there was no statistically significant difference between either group, as both groups can be seen scattered throughout the plot somewhat evenly in the vertical direction.

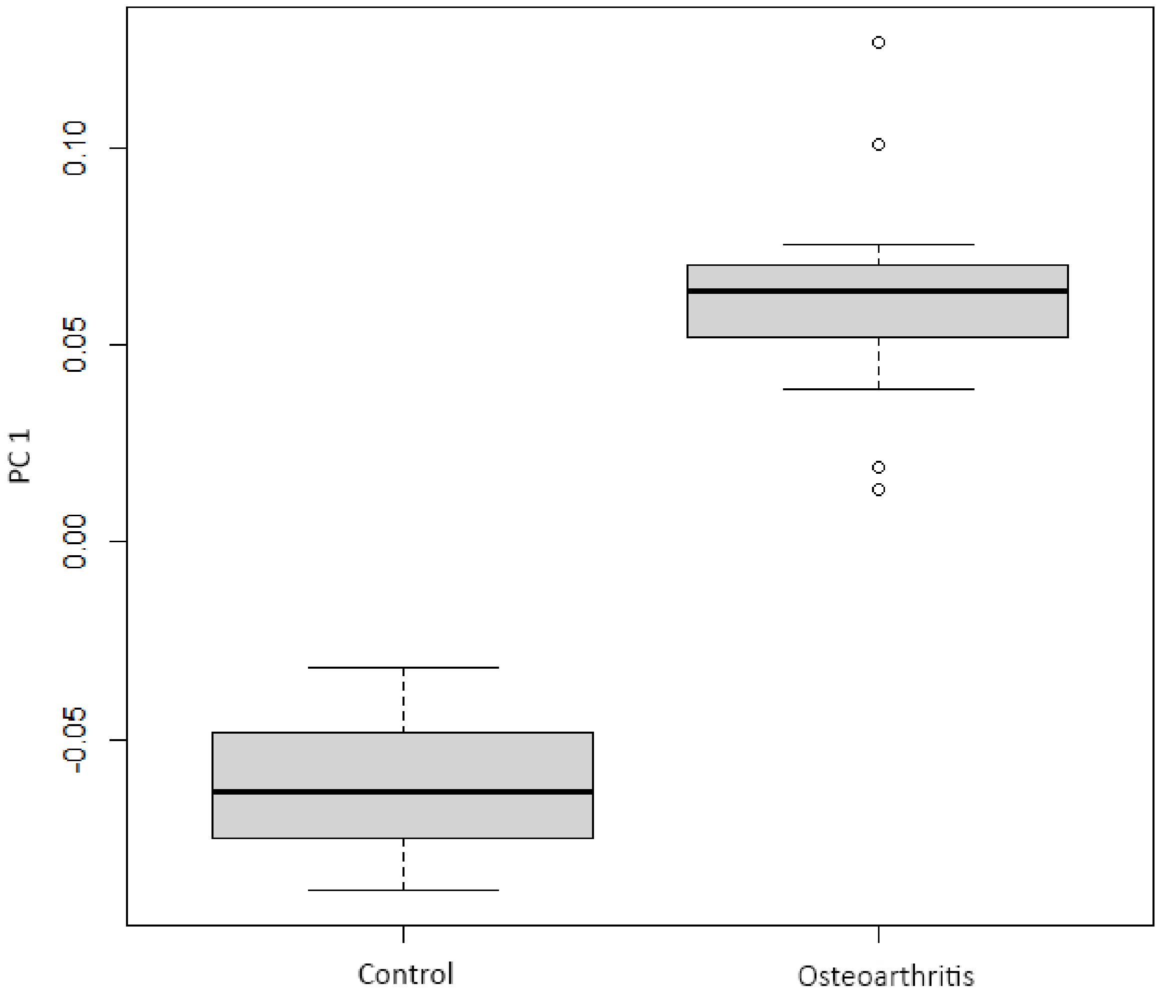

Figure 6: Box plot showing the control group and osteoarthritis (OA) group are statistically significant, with the OA group showing higher PC1 scores.

Figure 7 shows PC1 max, which shows the OA group (darker dots) compared to the average position of each landmark (lighter gray dots). It can be seen that the OA group is wider mediolaterally, and flatter superoinferiorly.

Figure 8 depicts PC1 min, where the darker dots are the control group, compared to the average position of each landmark and semilandmark (lighter gray dots). The control group, represented by PC1 min, is characterized by more rounded condyles, that are taller in the superoinferior direction and thinner in the mediolateral direction.

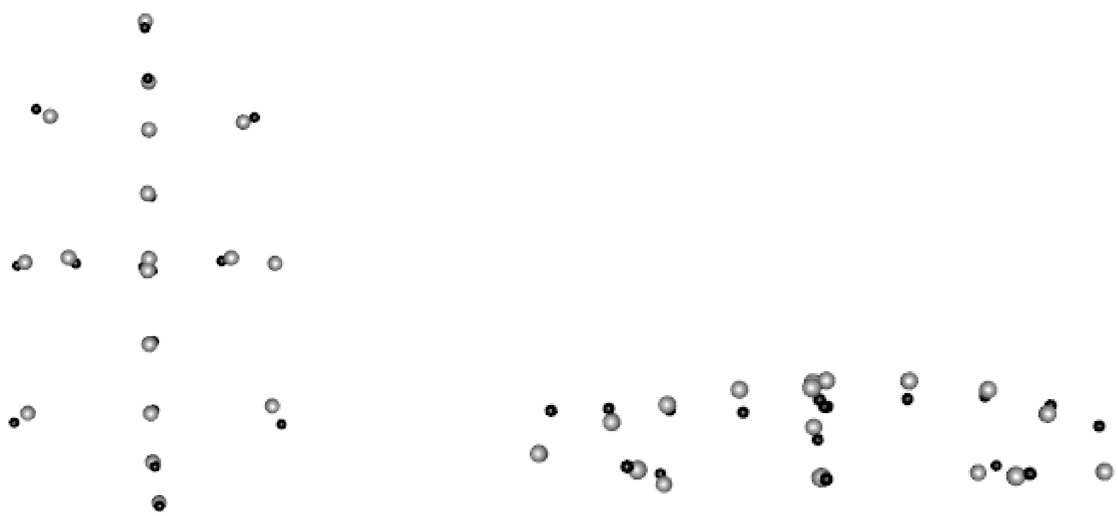

Figure 7: PC1 max from a superior view (left), and a lateral view (right). Darker dots represent the OA group, compared to the overall average (gray). It can be seen from the top-down view that the OA group is wider in the mediolateral direction. From the lateral view, it can be seen that the OA group is flatter in the superoinferior direction.

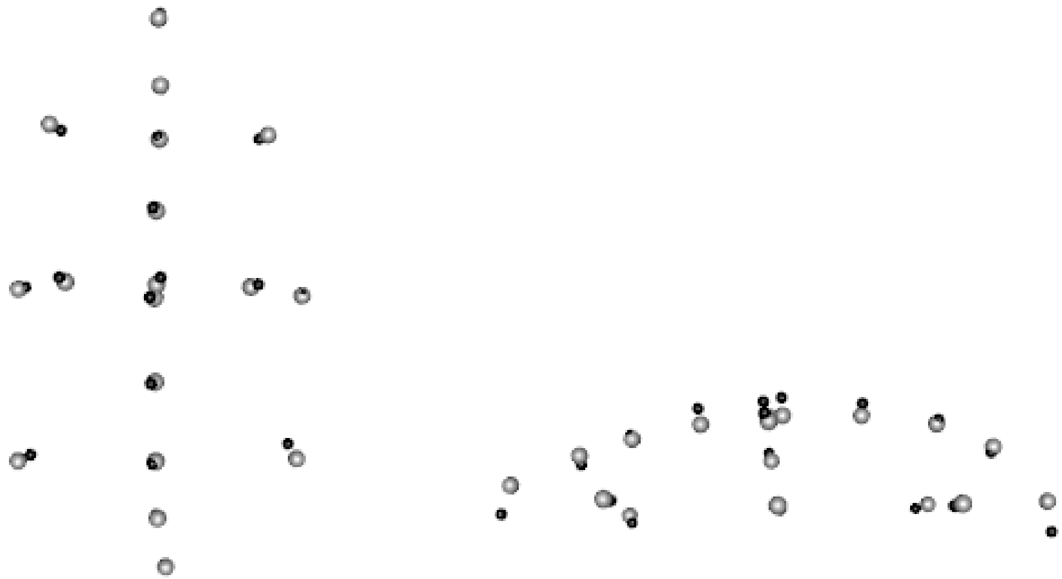

Figure 8: PC1 min from a superior view (left), and a lateral view (right). Darker dots represent PC1 min, compared to the average (gray). It can be seen from the top-down view that the control group (represented by PC1 min) is more narrow in the mediolateral direction. From the lateral view, it can be seen that the control rounded and more tall in the superoinferior direction.

5.0 DISCUSSION

5.1 **Shape Changes Following Osteoarthritis Induction**

Our study found there is a statistically significant difference between the shape of the mandibular condyle of the OA group and control group. This is in line with other studies that have been done, however to our knowledge, this present study was the first to confirm these findings with a multivariate shape analysis.

There were unique challenges to overcome with this research because the use of geometric morphometrics (GM) in this context is still relatively new. For example, there is no standard for where to place landmarks and semilandmarks on the mandibular condyle. The study conducted by Chen et al. (2022) placed landmarks and semilandmarks on the entire mandible, but with far fewer on the mandibular condyle than what we placed. Chen et al. (2022) study also placed landmarks on the condylar neck, which we did not.

The findings of the studies were also different, with Chen et al. reporting the condyles on the experimental group were narrower anteroposteriorly, and the surface of the head was more curved compared to the controls. This differs from our study, where we found condyles that were wider mediolaterally, and actually less convex. This highlights how the bony adaptations of the mouse TMJ may be different when the disc is removed, compared to when teeth are extracted – although it would be important to repeat these studies to confirm these findings.

A study by Derwich et al. (2020) examined the shape of human mandibular condyle with OA compared controls. This study took univariate measurements (condylar width and condylar A-P dimension) on CBCTs. The study concluded that there were no statistically significant differences between the controls and OA group in condylar width or condylar A-P dimensions. A key finding of the study was that condyles observed with more severe OA, nearly all of them (>96%) had condylar flattening. This contrasts with our study slightly, as we found that in mice the condylar width increases. However, similar to humans, we found a pronounced flattening of the mandibular condyle in our OA group. These findings indicated that although there are some differences, mouse TMJ OA induction with unilateral partial discectomy is a good model to study bony shape changes in OA. This model is also highly practical due to the very distinct changes that can be seen in a short time of four weeks.

5.2 **Sex Differences**

Contrary to our expectations, we did not see a sex difference in shape of mandibular condyles of our OA group. This may indicate that in mice, both sexes have similar physical adaptations in response to unilateral partial discectomy. This could also indicate that the duration of the experiment (four weeks) was not long enough to observe potential effect of hormonal differences on the capacity of the condyle to adapt following UPD.

Although we did not observe any difference in shape between sexes, this is not to say there is no difference in bony remodeling. We did not do a trabecular bone analysis, or histological analysis for cartilaginous changes.

5.3 **Limitations**

The limitations of this study can be related to the use of an animal model. Although structurally quite similar, there are differences between the human and mouse TMJ. For example, on an anatomical level mice have no articular eminence or postglenoid processes. Additionally, during mastication mice have no mediolateral movement of the jaw as humans do. Due to these differences, the results may not be fully generalizable to humans.

Because this is a post-traumatic model of osteoarthritis, it may not reflect the same processes that lead to high inflammatory conditions of the TMJ, such as rheumatoid arthritis, or juvenile idiopathic osteoarthritis. In humans, trauma as a causative agent represents a minority of cases of TMJ OA.

An additional limitation of this study is that all landmarks were placed on the condyle, so we are unable to determine changes in shape concerning an unaffected tissue such as the rest of the mandible.

5.4 **Future Directions**

Future research could include an analysis of trabecular bone. Synthesizing trabecular bone with a multivariate analysis of shape, could give us more information about the bony changes that occur in TMJ OA.

Furthermore, a shape analysis could be conducted of the of the glenoid fossa. Shape changes in the mandibular condyle in mice discectomy is likely due to increases in contact stress concentration due to the absence of the shock absorbing properties of the disc. The condyle changes shape to match the shape of the fossa, to equilibrate the stresses on the condyle (Yotsuya et al., 2020). Future research could integrate information from the glenoid fossa to establish mathematical correlations between the shape of the fossa and the condyle.

Future studies could also include a shape analysis of transgenic mice currently being researched at the University of Illinois Chicago, namely the NG2/CSPG4 knockout mice. Utilizing these transgenic mouse lines to investigate the impact of specific gene knockouts on the condyle's capacity to remodel its shape, distinct from alterations in internal geometry or material properties. It can be argued that shape serves as a highly valuable parameter for evaluating the tissue's genetic response to injury.

6.0 CONCLUSIONS

We were able to reject our null hypothesis that there are no differences in the shape of mandibular condyles of our surgical intervention group versus the non-surgical controls. Our results indicate that there is a dramatic shape change of the mandibular condyle that occurs after unilateral partial discectomy (UPD) in mice. We confirmed these findings with our geometric morphometric (GM) multivariate shape analysis. Our findings are in line with other studies, and mimic similar bony osteoarthritic changes that occur in humans with OA. To our knowledge we are the first to use GM to mathematically describe shape changes of the mouse mandibular condyle following induction of OA.

Future studies of TMJ OA would benefit from including a multivariate shape analysis to be able to better describe the bony changes that occur, and to account for size differences of individuals. Synthesizing bony shape changes with other data will allow researchers to understand the anabolic and catabolic processes in subchondral bone in the progression of TMJ OA.

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