STUDY OF EXTRACELLULAR MATRIX SCAFFOLD REMODELING IN THE PORCINE TEMPOROMANDIBULAR JOINT: UNIAXIAL COMPRESSION AND BIOCHEMICAL ANALYSIS

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Robert Jonathan Mortimer, M.S.

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Regenerative medicine techniques, such as extracellular matrix (ECM) scaffolds, are currently being investigated to address temporomandibular joint (TMJ) disc replacement. Positive results were seen in the canine TMJ model, where ECM scaffolds remodeled to tissue resembling the native TMJ disc biochemically and in compressive properties (Brown et al. 2012, Brown et al. 2011). To further quantify the temporal remodeling of the ECM when implanted in the TMJ, the porcine model was chosen due to similarities with the human TMJ. These pigs underwent bilateral discectomy and a unilateral small intestine submucosa extracellular matrix (SIS-ECM) device implantation and were then euthanized at 1, 3 and 6 months post-op. Unconfined uniaxial compression was performed on the remodeled ECM, and the condylar cartilage from both joints. Biochemical characterization was also performed to measure glycosaminoglycan and DNA content. The results of this study found no statistical (p<0.a05) difference in the mechanical properties between the remodeled tissue and the native tissue at any time point. Additionally, the ipsilateral condylar cartilage was not statistically different from the native condylar cartilage except for peak stress and tangent modulus at the 30% strain rate for the 3 month post implant group. Conversely, the contralateral condylar cartilage at the 6 month time point had a statistically significant difference in the peak stress and tangent modulus at 20% and 30% strain. The mechanical findings are supported by the biochemistry, which shows no statistical difference between native and remodeled tissue. Overall, this study indicates that the SIS-ECMscaffold constructively remodels into a TMJ disc-like structure.

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1.0 INTRODUCTION

The Temporomandibular Joint (TMJ) is a ginglymodiarthroidal synovial joint that is formed by the articulation of the mandibular condyle with the glenoid fossa and articular eminence of the squamous portion of the temporal bone. A biconcave fibrocartilaginous disc known as the TMJ disc separates the two bones (Beek et al., 2001b; Gallo et al., 2000). This disc is essential for the smooth rotation and translation of the joint and is also vital for absorbing shock experienced during mastication (Beek et al., 2001a; Osborn, 1985; Tanaka and van Eijden, 2003). Due to the complex structure and mechanical function of the TMJ, there are numerous problems that can arise leading to Tempromandibular Joint Disorders (TMDs).

It is estimated that TMDs affect nearly one in four Americans, with women being affected 9 times more frequently than men (Solberg et al., 1979). Although no exact etiology has been determined for TMD, it frequently involves a spatial dislocation or defects in the TMJ disc (Brown et al., 2011). A damaged disc often results in problems with mandible articulation, muscle/joint pain, and in extreme cases, crepitus due to bone on bone movement as a result of a perforated disc (Tanaka et al., 2008). Unfortunately, the current standard of care for TMDs has questionable efficacy and has gone relatively unchanged since the 1970s. Treatment for TMD generally begins with non-invasive treatments such as physical therapy or dental appliances. If the non-invasive treatment is not effective and the disease progresses, the next step in treatment involves minimally invasive procedures such as joint arthrocentesis and arthroscopy. Beyond

these minimally invasive procedures, the goal becomes to restore function (Tanaka et al., 2008). Procedures to do this involve discectomy and ultimately a total joint prosthesis. Although both of these do provide a short-term solution, their long-term efficacy is questionable.

To overcome deficiencies in these treatments, the goal over the past several decades has been to create a replacement for a damaged TMJ disc. The first attempts utilized alloplastic devices such as proplast-teflon and silastic implants. However, these devices failed due to particles being produced from the constant articulation, which in turn led to an adverse healing response. This prompted the development of autogenous techniques, such as the use of the temporalis muscle flap. While these procedures do provide positive short-term results, limitations such as donor site morbidity and resorption of the graft, negates their potential for long-term applications. The failures of all of these techniques and approaches have provided insight as to the success criteria for an ideal implant material. This material would provide a substrate for site-appropriate cell ingrowth, protect the condyle and fossa from degenerative changes, and not result in necrosis from the donor site (Brown et al., 2011).

These specifications have made tissue-engineering approaches to joint preservation appealing over the past decade. The goal of tissue engineering is to use natural or synthetic materials to induce the restoration or regeneration of the appropriate tissue in both morphology and function (Brown et al., 2011). There are numerous techniques and methods that can be used as templates for tissue regeneration. However, one that has successfully been used for a variety of applications is allogenic and xenogenic extracellular matrix (ECM). The ECM can be harvested from a variety of tissue types, including the small intestine, urinary bladder, and dermis. The ECM are then processed into 2 or 3-dimensional constructs which are then implanted to form site appropriate tissue. A previous study by Brown et al. (2012) proved that

powdered urinary bladder ECM laminated between sheets of the same material could be formed into a pillow like device that resembles a rudimentary TMJ disc. The results of this canine study showed regeneration of tissue that resembles the native TMJ disc. Although this study was ground breaking, a quantitative temporal analysis of the remodeling was not performed, with measurements such as compressive stiffness or collagen content. Furthermore, it is believed that the porcine model more closely resembles the human TMJ than the canine model, in terms of size and shape of the disc and condyle, the rotation and translation of the condyle during opening and closing, and the size ratio of the temporalis to the masseter.

Therefore, this work will study the potential of a small intestine submucosa (SIS) ECM device to remodel constructively at different time points in the porcine TMJ, as assessed through mechanical and biochemical testing. The characterization of the native articulating tissues of the TMJ is paramount to understanding the efficacy of any device intended to preserve the joint. Previous literature has characterized the porcine TMJ disc under unconfined uniaxial compression at high strain rates (Allen and Athanasiou, 2006). However, the fast 'instantaneous' strain rate of 50% per second is technically difficult to perform due to overshoot of the mechanical testing apparatus. Furthermore, there are no constitutive models for the compressive behavior at those high strain rates. However, compressive behaviors at slow strain rates can be fit to biphasic tissue models (solid and liquid phase) to gain an understanding on the contribution of each phase. For that reason, a slow strain rate will be used for this study as previously described by Hagandora et al. (2011) in the goat model. In addition to determining the properties of the TMJ disc, it is also important to evaluate the properties of the tissue that the disc protects, the mandibular condylar cartilage. The results of testing this tissue will show how well the remodeled ECM protects the condylar head when compared to the native tissue. Previously, the

condylar cartilage had only been tested under dynamic compression, however, for this study the static compression protocol established by Hagandora et al. in 2011 will be followed (Tanaka et al., 2006). Characterizing the properties of both the native condylar cartilage and the TMJ disc are essential in order to establish a baseline by which to compare remodeled ECM.

In addition to the mechanical properties, the biochemical characterization of both the TMJ disc and condylar cartilage is also important in determining the efficacy of the device. This is because in order to prove that this device will be successful long-term, its biochemical composition should be similar to that of the native tissues. Works by Almarza et al. (2005) have highlighted this importance and have provided the suite of biochemical markers that are necessary to accurately characterize TMJ tissues. Almarza et al. (2005) validated previous claims that the TMJ is a relatively acellular tissue, is rich in collagen, and also has small amounts of glycosaminoglycans (GAGs). These metrics will provide a means by which to evaluate the remodeled ECM as well as its effect on the surrounding tissues. The presence of DNA would indicate cellularity, and we would expect a decrease over time as the ECM is remodeling. GAGs in the TMJ are mainly found as highly branched polysaccharides attached to protein cores, proteoglycans, and due to the small amounts (~2% dry weight) found in the TMJ, these proteoglycans are believed to be involved in the control of collagen fiber size and packing (Almarza et al., 2006).

The objective of this study is to evaluate the remodeling of an ECM scaffold in the TMJ through biomechanical and biochemical characterization in the porcine model at various time points, as well as the impact on the condyle cartilage. Uniaxial compression testing will be done on native TMJ discs and mandibular condylar cartilage to determine strength and stiffness. These tests will be done at various strain levels since there is no widely agreed upon level of

strain that the TMJ disc would experience under normal physiological loading. The native TMJ soft tissues will further be characterized through biochemistry, testing for DNA and GAGs. Specimen from 1, 3 and 6 months post implantation of an ECM scaffold will undergo the same compression and biochemical analysis as the native tissue. It is hypothesized that ECM scaffold will remodel into a tissue with no statistical differences in mechanical or biochemical properties between the six month time point and the native disc. Additionally, no statistical difference will be seen between the six month ispilateral condyles and the native condyles in terms of compressive properties.

2.0 MATERIALS AND METHODS

2.1 SPECIMEN PREPARATION

Heads from 9-month old female pigs were obtained from a local abattoir to be used as native samples at n=9. For the experimental group, 3-month old female pigs were implanted with ECM scaffolds and were allowed to heal for 1, 3, and 6 months. These animals underwent bilateral discectomy and then received a SIS-ECM 3D construct that is described in Brown et al. (2012) unilaterally. At this stage of the study, we have remodeled ECM samples and corresponding condyles from 1 month post-op (n=2), 3 month post-op (n=3), and 6 month post-op (n=2). Condyles from the empty contralateral joint were also tested from 1 month post-op (n=5), 3 month post-op (n=5), and 6 month post-op (n=3).

For all specimens, the right TMJ joint was dissected using an anterior approach. Once the joints were dissected and TMJ discs excised, a 4mm dermal biopsy punch was used to remove a punch from the lateral portion of the intermediate zone, as well as from the corresponding location on the mandibular condylar cartilage. Special attention was given to ensure no subchondral bone was attached to the sample. The mandibular condylar cartilage sample, as well as the remaining disc, was immediately wrapped in gauze soaked in 0.1M PBS and frozen at -80°C until testing. The disc punches were immediately embedded in optimal cuttent temperature comound and cryotomed to ~1mm in height to ensure parallel surfaces as

well as an appropriate aspect ratio for mechanical testing. These samples were then wrapped in gauze soaked in PBS and frozen at -80°C until testing.

2.2 BIOMECHANICAL TESTING

2.2.1 Experimental Testing

Before mechanical testing, samples were allowed to thaw and equilibrate in 37°C PBS for a minimum of one hour. After the samples had equilibrated, the diameter was measured using digital calipers. The sample was then adhered to an aluminum stage inside of an empty water bath using cyanoacrylate and preloaded to 0.02N using an MTS insight electromechanical testing system (MTS Systems Corp, Eden Prairie MN). After obtaining the height of the sample, the water bath was filled with 0.1M PBS and heated to 37°C. The platen was then lowered into the water bath down to within 0.1mm of the level previously determined. The load at this point was recorded as buoyancy and the load cell zeroed. The sample was then preloaded to 0.05N and allowed to relax for 30 minutes, and this distance between clamps was used as the specimen height. After the preload, the sample underwent 10 cycles of preconditioning to 10% strain at a strain rate of 9% per minute. Samples were then subjected to stress relaxation testing at 10%, 20%, and 30% strain steps at a rate of 9% per minute and allowed to relax for 30 minutes. Data was collected at 3Hz during ramping and 1Hz during relaxation. After compression testing, the samples were frozen at -80°C. Data was then analyzed using MATLAB® which provided peak stress and the tangent modulus (Hagandora et al. 2011). Stress was calculated by taking the load and dividing it by the cross-sectional area of the sample. Strain was calculated by plate-to-plate

displacement divided by specimen height after pre-load. Peak stress was determined as the highest value of stress at each strain step. The tangent modulus was determined as the slope of the curve at the last 20% of the linear portion of the stress-strain curve.

2.2.2 Protocol Validation

The first step in the mechanical testing procedure included validating the testing equipment as well as establishing a repeatable protocol. Machine validation was first attempted by trying to reproduce known porcine TMJ disc compression data from Allen and Athanasiou (2006). However, as previously mentioned, this proved technically difficult as the strain rate of 50% per second resulted in the machine overshooting the desired strain level, producing inaccurate results. The next attempt at validating the machine was to reproduce data in the goat TMJ model from Hagandora et al. (2010). This study used a slow, reproducible strain rate of 9% per minute. This strain rate was also found to be beneficial as it provides a way to characterize the contributions of the solid and liquid phases. For this validation 6 skeletally mature goats were obtained from a local abattoir and the discs and condylar cartilage were excised. Through this validation the proper sample preparation and testing procedures were determined and are employed in the current study. The results of this validation can be seen in Table 1.

Further validation was performed using the canine model. This was done to ensure the accuracy of the machine and reproducibility of the protocol. For the canine validation, samples were tested and then compared to data from Brown et al. (2011). After this successful validation, the current study commenced.

Table 1-Study Validation- Mechanical testing results as published in Hagandora et al. 2011 and Brown et al. 2012 compared to the data obtained to validate the protocol and mechanical testing equipment for testing of TMJ disc tissues.

		10 % Strain		20 % Strain		30 % Strain	
	Species	Peak Stress (kPa)	Tangent Modulus (kPa)	Peak Stress (kPa)	Tangent Modulus (kPa)	Peak Stress (kPa)	Tangent Modulus (kPa)
Hagandora 2011	Goat	16±7	304±141	61±26	729±267	127±40	1278±385
Mortimer 2014	Goat	13±5	282±105	73±20	757±294	151±49	1560±409
Brown 2012	Canine	2.7±0.6	23.4±6.0	4.8±1.4	68.8±23.8	-	-
Mortimer 2014	Canine	2.0±0.8	18.6±6.3	4.7±1.2	60.1±19.7	-	-

2.3 BIOCHEMICAL ANALYSIS

Once all samples had been mechanically tested, they were placed in 5mL glass tubes and their weight was measured. They were then lyophilized for 72 hours and re-measured so that a dry weight and percent water could be obtained. These samples were then placed in a 4mL papain digest at 60°C for 28 hours. After digest, samples were kept frozen until testing. Glycosaminoglycans were tested using a Blyscan™ dimethylmetheylene blue assay kit (Biocolor, Newtownabbey U.K.). DNA content was measured using PicoGreen dsDNA quantitation assay kit (Molecular Probes, Inc., Eugene, Oregon).

2.4 STATISTICAL ANALYSIS

Both biomechanical and biochemical analyses for all the time points and native tissue were assessed through a one-way ANOVA's with $p<0.05$ being statistically significant. To determine differences among groups, a Tukey's post-hoc test was performed. When only two groups with statistical power were present, a two-tailed t-test was performed. All statistics were calculated using Minitab®. Statistics were only performed when at least 3 samples per group were available to testing.

3.0 RESULTS

3.1 BIOMECHANICAL TESTING

3.1.1 TMJ Disc

The native and experimental TMJ discs were tested as previously described under uniaxial compression. In terms of peak stress (Figure 1), the 1-month group (n=2) seems to have a lower value than native (n=9), and there seems to be a trend of increasing peak stress from 1 month to 3 month, but more samples are needed for statistical comparison. No statistically significant differences ($p<0.05$) in peak stress were seen between the 3-month group (n=3) and the native disc. It also seems that the peak stress for the 6-month group is on par with the native tissue, but again more samples are needed. Similar trends were observed when analyzing the tangent modulus (Figure 2). Complete peak stress and tangent modulus data for the TMJ discs can be seen in Table 2.

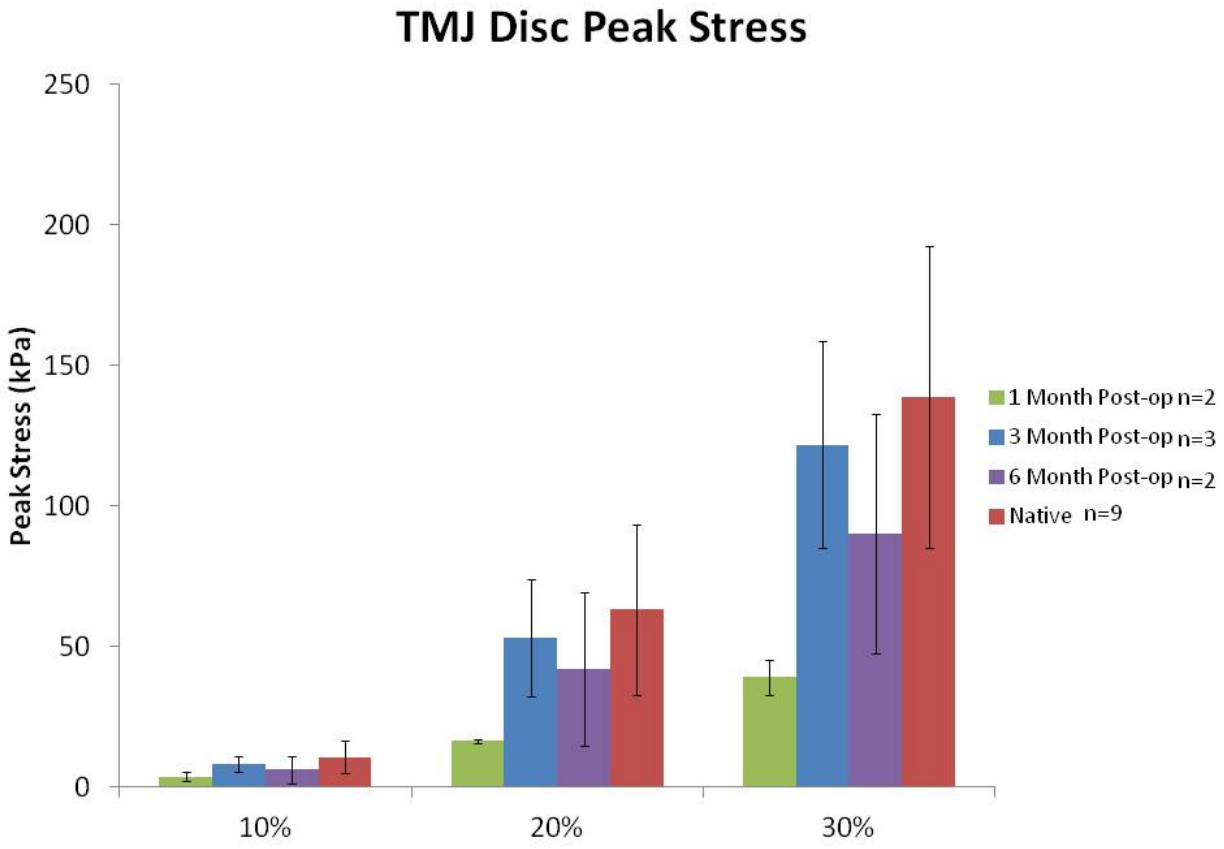

Figure 1- TMJ Disc Peak Stress-Peak stresses are shown for 10%, 20%, and 30% strain rates on the TMJ disc of 1 month post implant pigs (n=2), 3 month post implant pigs (n=3), six month post implant pigs (n=2) and native pigs (n=9). No statistical significant differences were observed among groups.

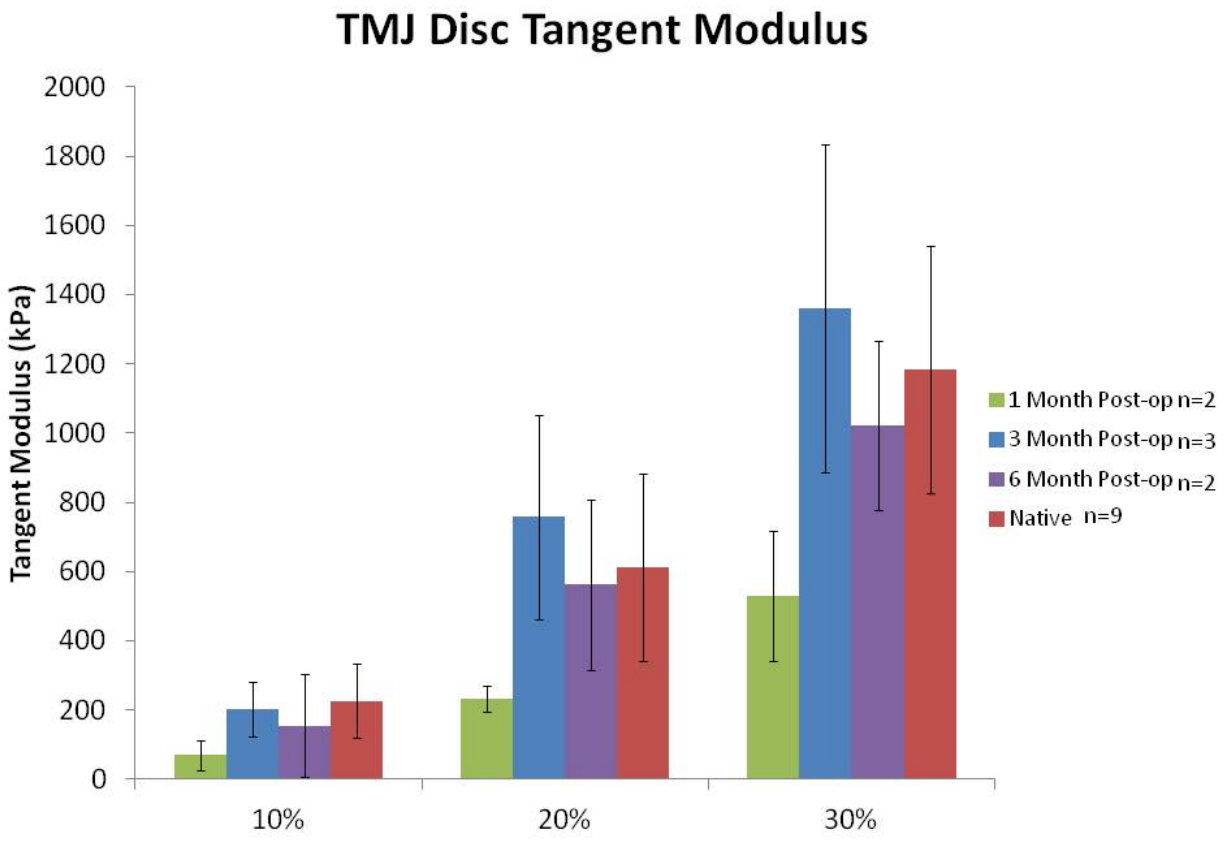

Figure 2- TMJ disc Tangent Modulus- Tangent moduli are shown for 10%, 20%, and 30% strain rates on the TMJ disc of one month post implant pigs (n=2), three month post implant pigs (n=3), six month post implant pigs (n=2), and native pigs (n=9). No statistical significant differences were observed among groups.

Table 2- Mechanical testing data for 1 month, 3 month, and native TMJ discs

	10 % Strain		20 % Strain		30 % Strain	
	Peak Stress (kPa)	**Tangent Modulus**	**Peak Stress (kPa)**	**Tangent Modulus**	**Peak Stress (kPa)**	**Tangent Modulus**
1 Month Pig	6.19±4.35	143.12±130.91	31.36±25.97	427.26±339.80	67.46±49.42	760.91±422.42
3 Month Pig	8.19±2.74	202.75±79.78	53.14±20.63	757.72±294.86	121.89±26.87	1360.38±474.61
Native Pig	10.05±3.92	208.57±96.12	45.04±21.50	604.50±285.66	105.19±38.92	1169.27±376.25

3.1.2 Ipsilateral Condylar Fibrocartilage

The condylar fibrocartilage from the treated side was harvested and mechanically tested as previously described and analyzed for peak stress and tangent modulus. In terms of peak stress (Figure 3), there seems to be a trend of increased peak stress at all time points ($n<3$) when compared to the native specimens at 10%, 20%, and 30% strain levels. The three-month post op group had a statistically significant 100% increase in peak stress at the 30% strain level when compared to the native tissue ($p<0.05$) (Figure 3). Similar trends were seen with the tangent modulus, with an apparent increase stiffness at all time points ($n<3$) when compared to the native specimens at 10%, 20%, and 30% strain levels (Figure 4). The three-month post op group had a significant increase ($p<0.05$) in tangent modulus of 132% when compared to the native tissue at the 30% strain level.

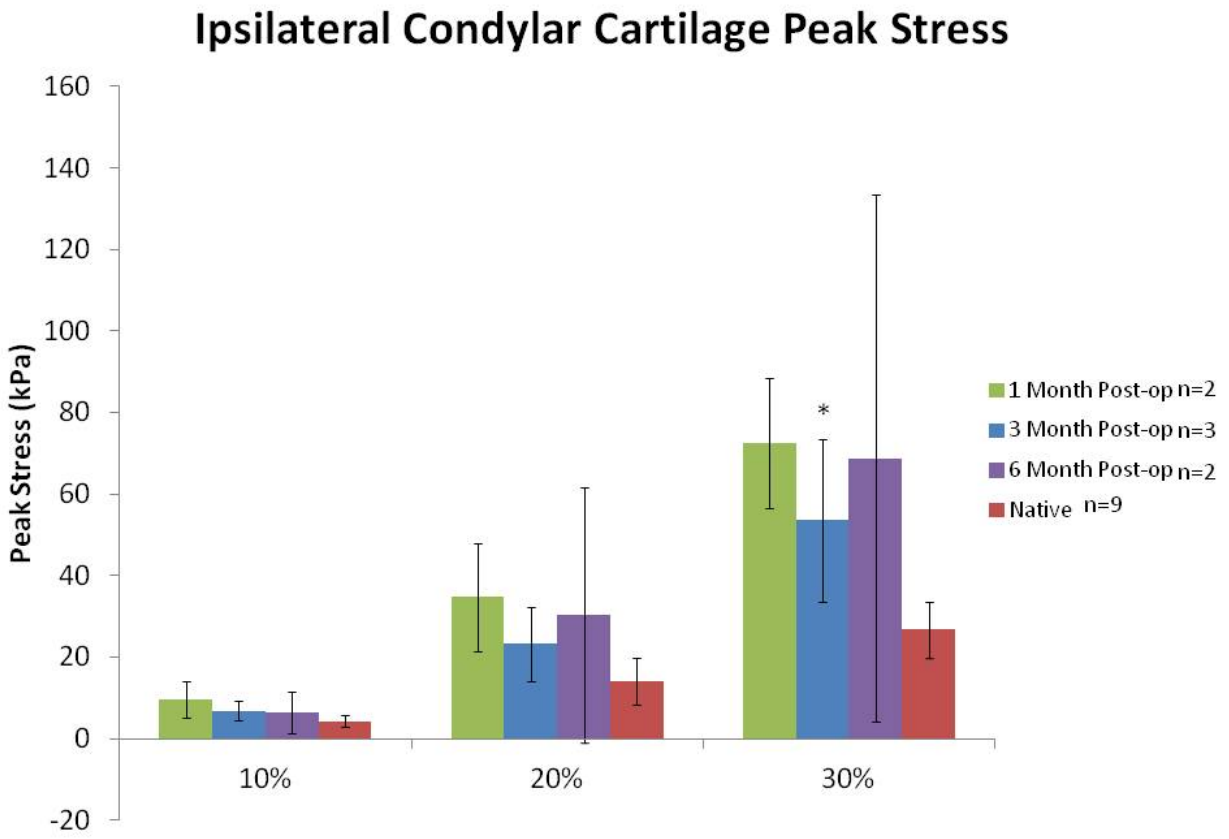

Figure 3-Condylar Cartilage Peak Stress- Peak stresses are shown for 10%, 20%, and 30% strain rates on the ipsilateral condylar cartilage of 1 month post implant pigs (n=2), 3 month post implant pigs (n=3), 6 month post implant pigs (n=2) and a native pigs (n=9). Statistical significance between the 3 month and native pigs can be seen at the 30% strain level.
The * signifies a statistical difference between native and the three-month post op group within the respective strain step.

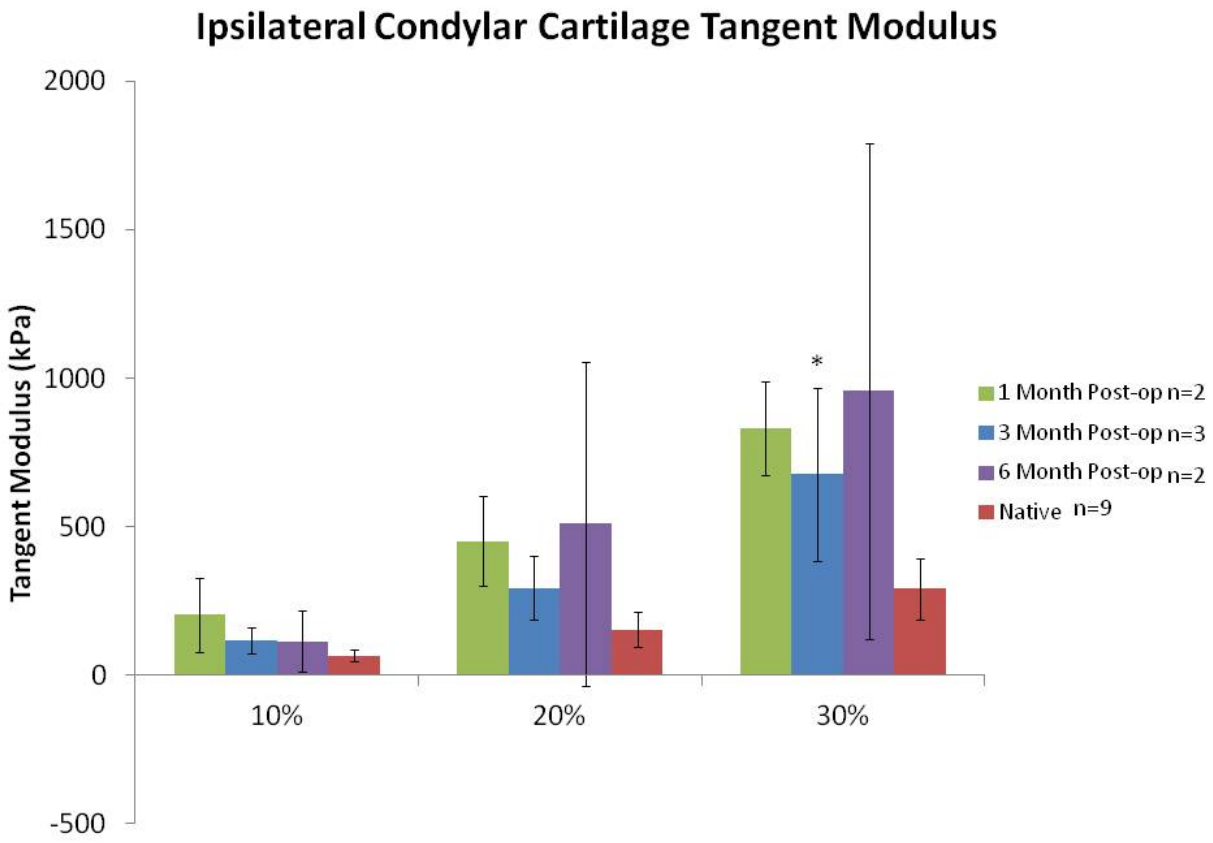

Figure 4-Condylar Cartilage Tangent Modulus- Tangent modulus is shown for 10%, 20%, and 30% strain rates on the ipsilateral condylar fibrocartilage of 1 month post implant pigs (n=2), 3 month post implant pigs (n=3), six month post implant pigs (n=2) and a native pigs (n=9). Statistical significance between the 3 month and native pigs can be seen at the 30% strain level. The * signifies a statistical difference between native and the three-month post op group within the respective strain step.

3.1.3 Contralateral Condylar Fibrocartilage

The contralateral fibrocartilage was mechanically tested with an identical protocol to the aforementioned ipsilateral condylar cartilage. At the one and three-month time points, no statistical significant differences ($p<0.05$) were detected in peak stress when compared to native at all strain levels (Figure 7). At the six-month post implant time point, statistically significant differences were observed when compared to the native specimens at the 20% and 30% strain levels, with increases of 163% and 217%, respectively ($p<0.05$).

When the tangent modulus was analyzed, a trend similar to that seen in the peak stress was observed (Figure 8). At the one and three month post implant times there were no statistically significant ($p<0.05$) differences to native within each strain level. At the six-month post implant time point, however, statistical differences ($p<0.05$) were observed at the 20% and 30% strain levels when compared to the native tissue with increases of 235% and 244%, respectively ($p<0.05$).

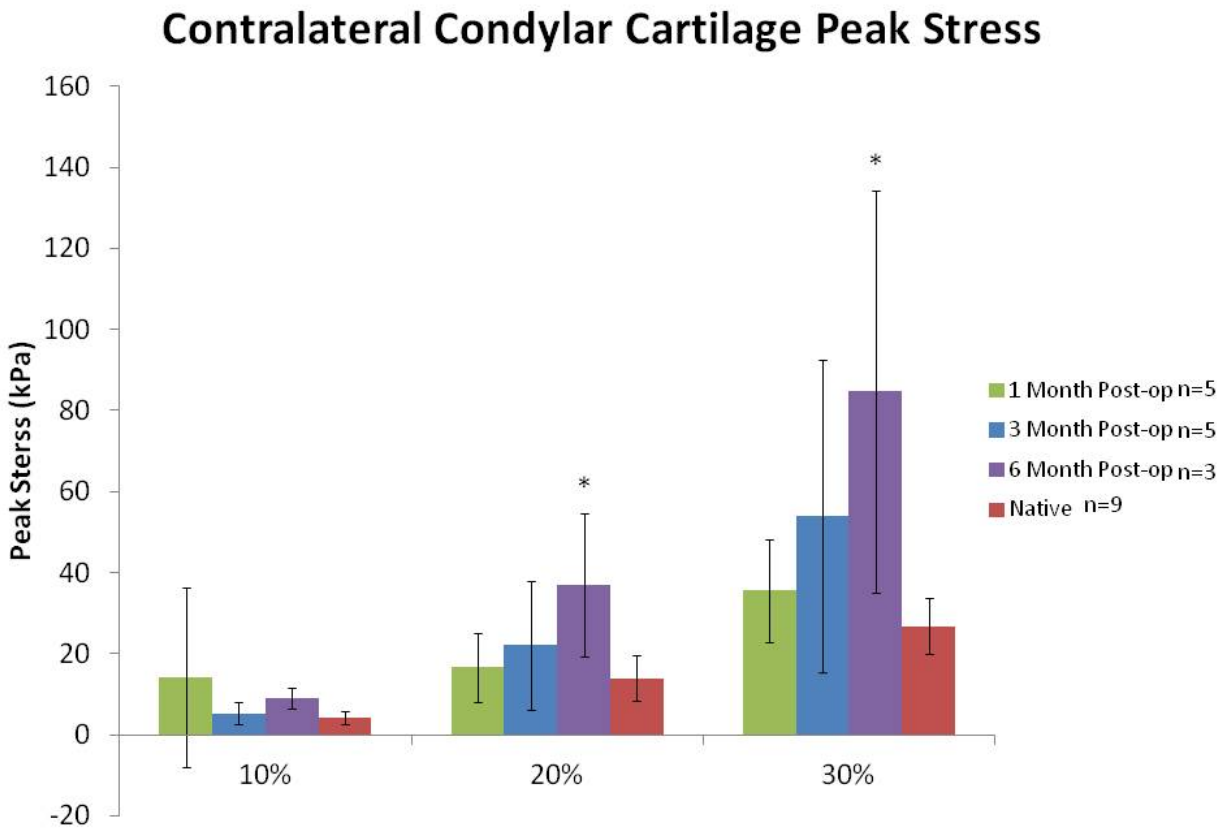

Figure 5- Condylar Cartilage Peak Stress- Peak stresses are shown for 10%, 20%, and 30% strain rates on the contralateral condylar cartilage of 1 month post implant pigs (n=5), 3 month post implant pigs (n=5), 6 month post implant pigs (n=3) and a native pigs (n=9). Statistical significance between the 6 month and native pigs can be seen at the 20% and 30% strain levels. The * signifies a statistical difference between native and the six-month post op group within the respective strain step.

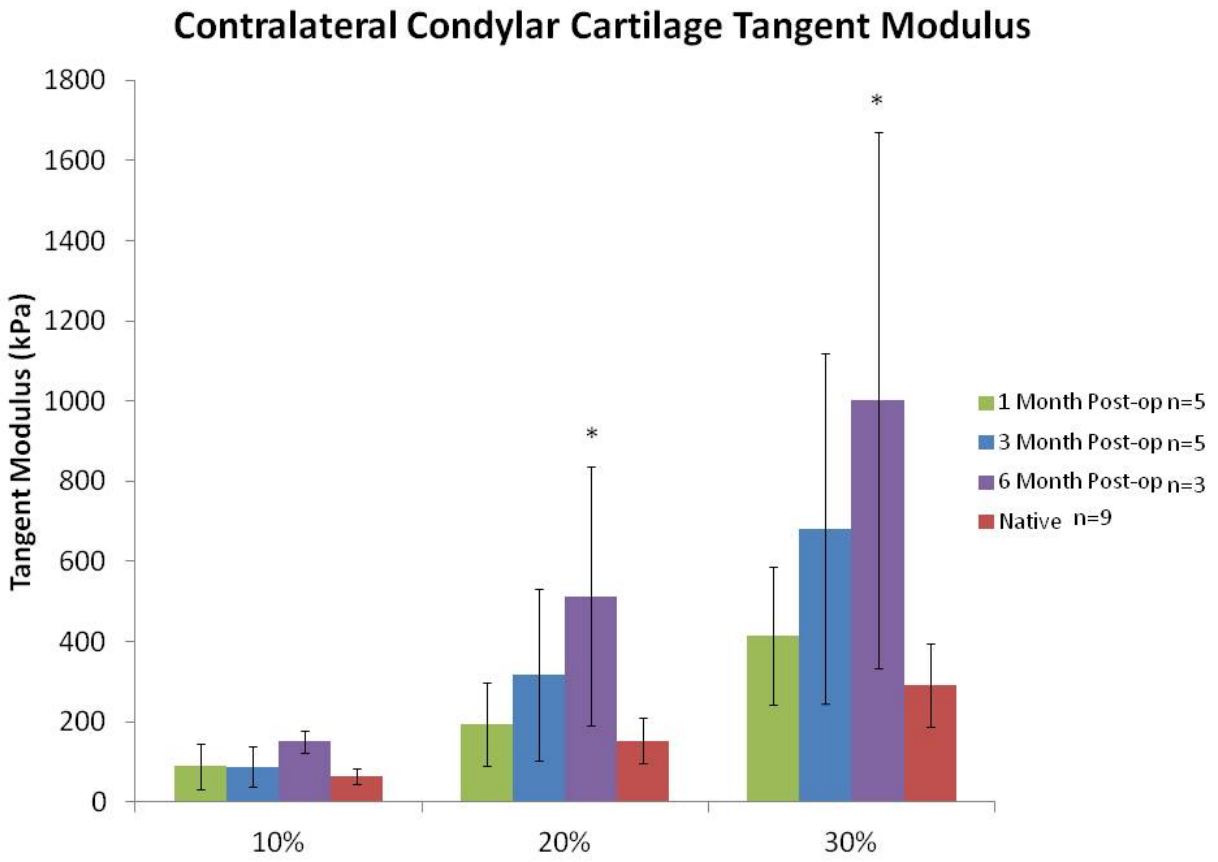

Figure 6- Condylar Cartilage Tangent Modulus- Tangent Modulus is shown for 10%, 20%, and 30% strain rates on the contralateral condylar cartilage of 1 month post implant pigs (n=5), 3 month post implant pigs (n=5), 6 month post implant pigs (n=3) and a native pigs (n=9). Statistical significance between the 6 month and native pigs can be seen at the 20% and 30% strain levels. The * signifies a statistical difference between native and the six-month post op group within the respective strain step.

3.2 BIOCHEMICAL TESTING

3.2.1 Glycosaminoglycans

As previously mentioned, the TMJ disc and both ipsilateral and contralateral condylar cartilage samples underwent biochemical analysis to determine the glycosaminoglycan content at the varying time points (Figure 9). The native TMJ disc had a glycosaminoglycan content of 0.7±0.3% by dry weight. There were no statistical differences ($p<0.05$) observed amongst time points. The native condylar cartilage had a glycosaminoglycan content of 1.8±0.7% by dry weight. Again, there were no statistically significant differences ($p<0.05$) amongst time points. In the contralateral condylar cartilage, there were statistically significant increases ($p<0.05$) in GAG when comparing 1-month to 3-month and native, of about 205%.

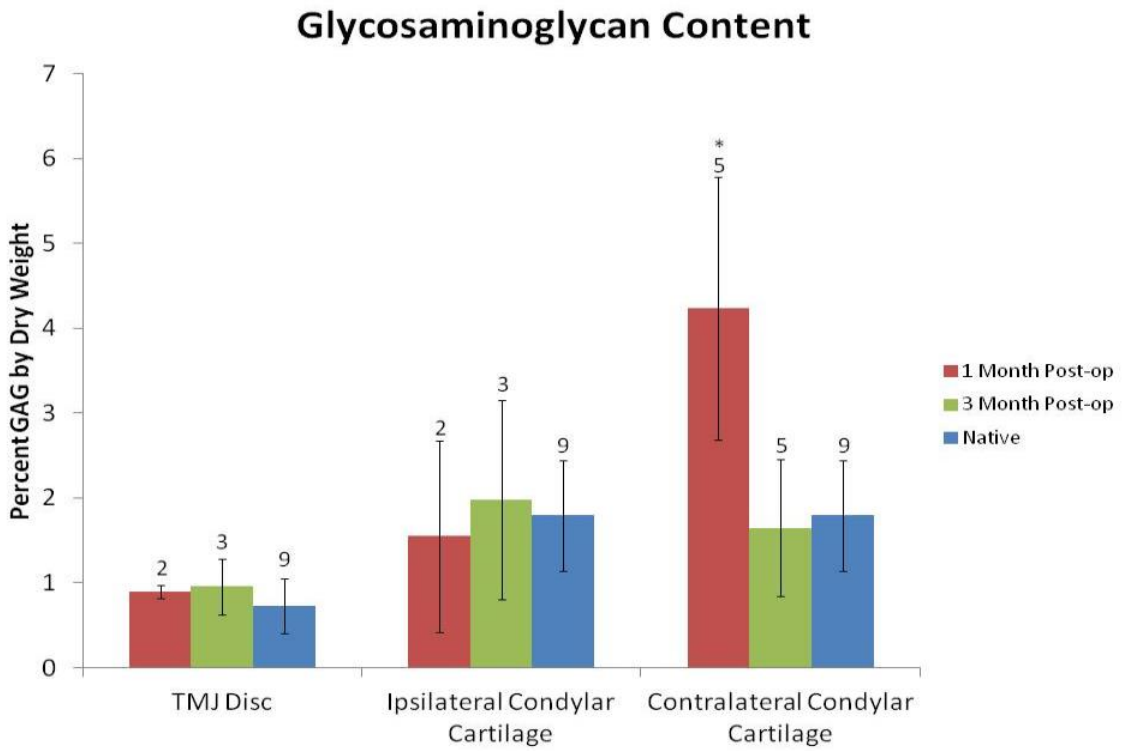

Figure 7- Glycoasaminioglycan Content-There were no statistically significant differences in the amount of GAG seen amongst the groups for the TMJ disc, or ipsilateral condylar cartilage. The one month post-op contralateral cartilage, however, had significantly ($p<0.05$) more GAG by dry weight than the native condylar cartilage. The numbers above each bar signify the number of samples represented. An * indicates a statistically significant difference between the sample and the respective native tissue.

3.2.2 DNA Content

A Picogreen® assay was used to determine the DNA content of both the TMJ discs as well as ipsilateral and contralateral condylar cartilage (Figure 10). The native TMJ disc was found to have a DNA content of 0.01±0.004% by dry weight. At the one-month time point, there was a general trend of increased DNA content, but more samples are needed for statistical comparison. At the three-month time point, there no statistically significant differences found when compared to native. The native condylar cartilage had 0.07±0.02% DNA by dry weight (Figure 10). The one month post implant cartilage seem to have a lower DNA content of 0.04±0.01% by dry weight when compared to native, but more samples are needed for statistical comparison. The three month post implant cartilage had a DNA content of 0.03±0.01% by dry weight, statistically less ($p<0.05$) than the native condylar cartilage. On the contralateral side, DNA content was relatively consistent amongst samples (Figure 10), with no statistical differences ($p<0.05$) observed amongst the groups.

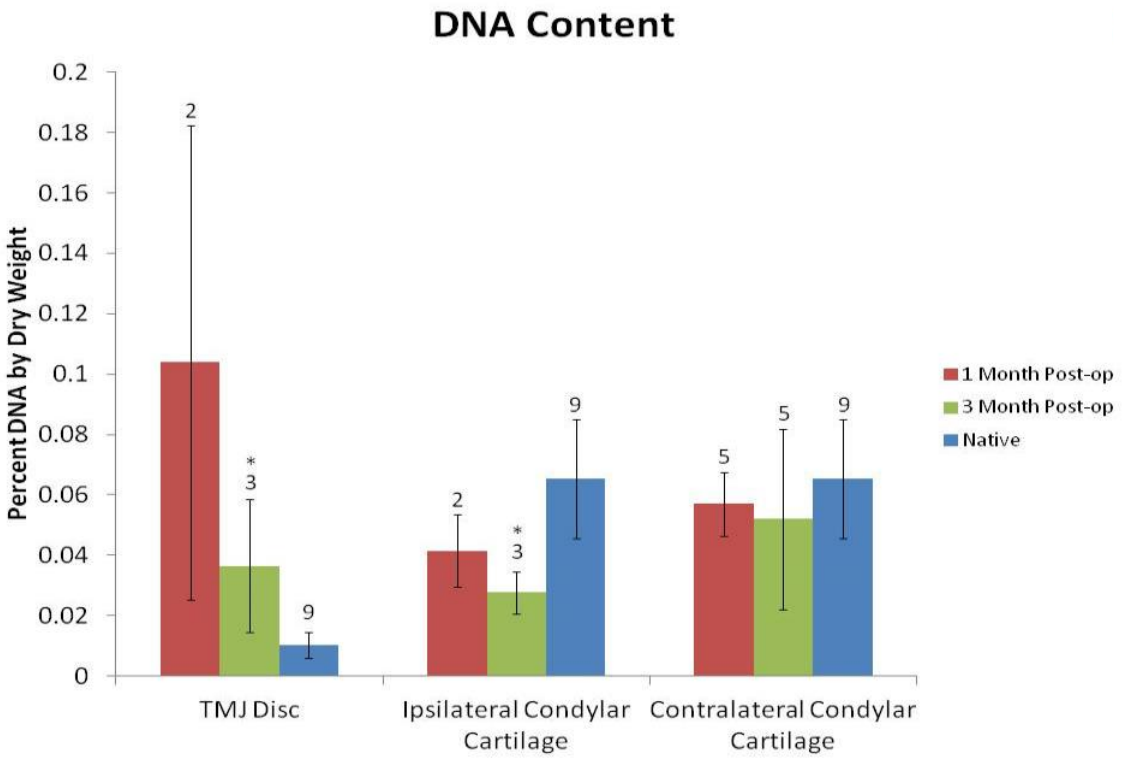

Figure 8-DNA Content- The three month remodeled TMJ disc had a significantly greater ($p<0.05$) percentage of DNA by dry weight than did the native TMJ disc. Conversely, the three month post-op ipsilateral condylar cartilage had a significantly lower percentage of DNA by dry weight than the native condylar cartilage. There were no statistical differences amongst the contralateral condylar cartilage groups. The numbers above each bar signify the number of samples represented. An * indicates a statistically significant difference between the sample and the respective native tissue.

4.0 DISCUSSION

4.1 MECHANICAL TESTING

The results of the biomechanical testing indicate that SIS-ECM has promise as an inductive template for constructive tissue remodeling after discectomy. The current pig model with a xenogenic canine SIS-ECM implant has provided insight that was not available in previous studies (Brown et al. 2011, Brown et al. 2012). This study starts to quantify the temporal remodeling of the ECM, and it effect on the condyle cartilage, from one to three to six months. Of note, this study has shown that in the porcine model, there seems to be little differences in tangent modulus and peak stress when the native tissues are compared to the one and three month post remodeled matrix. This suggest that after only one month, the acellular biologic scaffold remodels into a tissue which may be capable of functioning as a disc analog.

Although the device does seem to remodel in constructive manner, it is important to study the effect the device has on the underlying condylar cartilage. The results suggest that at all time points, the condylar cartilage seems to have a higher peak stress at all strain levels and might be stiffer than the native condylar cartilage. If these findings hold true when more samples are analyzed, it would suggest that the condyle is going through a healing response as well.

In the contralateral joint, where no ECM was placed, the fibrocartilage of the remaining condyle does not appear normal. This joint was left empty to provide a control that demonstrates that the TMJ disc does not regenerate naturally. It also provides a metric by which to measure the damage done by the loss of congruity and new stress distributions. The peak stress and stiffness of the fibrocartilage seems to be higher than the ipsilateral joint. These trends seem to suggest that the remodeled ECM may protect the articulating surfaces, manifested as a less acute of stiffening the condylar fibrocartilage.

4.2 BIOCHEMICAL TESTING

Much like the mechanical data, the biochemical analysis indicates that SIS-ECM shows promise as an inductive scaffold for constructive tissue remodeling after a discectomy. The native data collected was consistent with that of previous literature, validating the methods of the study (Almarza et al. 2006, Hagandora et al. 2012). At the three month post-op time point, the implanted device displayed a glycosaminoglycan content that was not statistically different from the native tissue, indicating a rapid remodeling process. Additionally the condylar cartilage on the ipsilateral side remained largely unaffected, with similar content to the native tissue. This consistency provides evidence that the remodeling SIS-ECM device protects the condylar head and preserves its properties. The contralateral condylar cartilage, however, displayed a noticeable and statistically significant increase in the amount of glycoasaminoglycans at the one month post implant time point when compared to both the native and three month post implant groups.

In addition to the insight provided by glycosaminoglycan content, DNA is also a valuable property in assessing remodeling tissue. This is because DNA presence indicates cellularity, which, in an acellular tissue such as the TMJ disc, indicates tissue remodeling. Therefore, the significantly greater quantity of DNA seen at the three month post-op time point indicates that remodeling is still ongoing in the tissue.

4.3 LIMITATIONS AND FUTURE DIRECTIONS

Although this study did seem to provide support for positive remodeling of the SIS-ECM in the TMJ, there were several limitations to this study. The first major limitation is the lack of tensile testing to mechanically characterize the tissue. The TMJ disc has been proven to function primarily under tension, leaving a large gap in our knowledge of native and constructively remodeled tissues (Detamore and Athanasiou, 2003). Additionally, the lack of collagen biochemistry furthers this lack of understanding of the discs tensile properties and is an important aspect to quantifying any tensile properties.

In the switch from the canine to the porcine model, many new procedures and approaches have been tailored as differences in the models are being found. The porcine model thought to be one of the better large animal models of the human TMJ, however, no previous studies have involved an invasive *in vivo* component (Allen and Athanasiou, 2006; Brown et al. 2012). When performing surgery on the porcine model, Drs. Chung and Brown have noted many differences to the canine model. One such difference is the size of the animals. An ideal animal model for

the implant of the SIS-ECM device would be skeletally mature, much like the majority of human TMD patients (Solberg et al., 1979). However, the experience gained in this study has shown that skeletally mature pigs weight in at over 200kg, making them hard to handle. Furthermore, some of the morphology of the pig is also different, in which the zygomatic arch grows over the joint space throughout ontogeny. With the zygomatic arch covering the joint, accessing the disc for removal and the subsequent device implant is difficult without inflicting excessive damage to the surrounding area.

As a result, the study was performed on juvenile pigs as their smaller size of roughly 40kg is much more manageable for the surgeons. Additionally, the zygomatic arch has not completely covered the joint, making accessing the joint space possible. As such, we are now introducing a new variable where growth of the animal is a factor, as pigs double their weight within the first two months after the operation (Corson et al. 2008). This growth adds an additional layer of complexity to the remodeling of the tissue that would not be seen in skeletally mature dogs.

5.0 CONCLUSION

This study suggests through mechanical and biochemical testing that the SIS-ECM scaffolds do constructively remodel into a disc-like tissue. However, although the results are promising, more samples need to be tested and there is still a need for tensile testing and other measures before the true impact of ECM technology can be elucidated in the TMJ.

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