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Biomechanical and biochemical outcomes of porcine temporomandibular joint disc deformation

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ABSTRACT

Objective: The structure–function relationship in the healthy temporomandibular joint (TMJ) disc has been well established, however the changes in dysfunctional joints has yet to be systematically evaluated. Due to the poor understanding of the etiology of temporomandibular disorders (TMDs) this study evaluated naturally occurring degenerative remodeling in aged female porcine temporomandibular joint (TMJ) discs in order to gain insight into the progression and effects on possible treatment strategies of TMDs.

Design: Surface and regional biomechanical and biochemical properties of discal tissues were determined in grossly deformed (≥Wilkes Stage 3) and morphologically normal (≤Wilkes Stage 2) TMJ discs.

Results: Compared to normal disc structure the deformed discs lacked a smooth biconcave shape and characteristic ECM organization. Reduction in tensile biomechanical integrity and increased compressive stiffness and cellularity was found in deformed discs. Regionally, the posterior and intermediate zones of the disc were most frequently affected along with the inferior surface.

Conclusions: The frequency of degeneration observed on the inferior surface of the disc (predominantly posterior), suggests that a disruption in the disc-condyle relationship likely contributes to the progression of joint dysfunction more than the temporodiscal relationship. As such, the inferior joint space may be an important consideration in early clinical diagnosis and treatment of TMDs, as it is overlooked in techniques performed in the upper joint space, including arthroscopy and arthrocentesis. Furthermore, permanent damage to the disc mechanical properties would limit the ability to successfully reposition deformed discs, highlighting the importance of emerging therapies such as tissue engineering.

1. Introduction

The temporomandibular joint (TMJ) is a mechanically and biologically complex joint comprising of a thick fibrocartilage disc that minimizes the incongruity between the articulation of the fossa-eminence of the temporal bone and mandibular condyle. Normally, the biconcave disc dynamically adapts to distribute compressive, tensile and shear stress throughout a sequence of movements (Detamore & Athanasiou, 2003a; Allen & Athanasiou, 2006; Juran, Dolwick, & McFetridge, 2013). The inferior surface of the disc primarily experiences rotation through its close association to the condyle while the superior surface experiences mainly translation along the temporal bone (Griffin, Hawthorn, & Harris, 1975; Piette, 1993).

Temporomandibular disorders (TMDs) are a wide range of disorders that affect both the joint and/or the associated structures; however the TMJ disc is affected in as many as 70% of all cases (Farrar & McCarty, 1979a; Schiffman et al., 2010; Ahmad et al., 2009). Patients are usually affected by unidirectional or multidirectional displacement of the intra-articular disc either anteriorly, medially, laterally, or (very rarely) posteriorly (Molinari et al., 2007; Farrar & McCarty, 1979b). In disc displacement, the disc can either be recaptured into a normal position during mouth opening termed disc displacement with reduction (DDWR), or can remain displaced termed disc displacement without reduction (DDWOR). An MRI study by Hirata et al. showed disc morphology to remain primarily biconcave (79%) in DDWR, while disc morphology is altered in DDWOR. Disc shape transitions to a predominantly folded configuration (43%) in addition to hemi-convex (21%) and biconvex (21%) shapes DDWOR (Hirata et al., 2007). The frequently used Wilkes scale groups TMDs into five stages of increasing dysfunction, with Stage III indicative of DDWOR, resulting in obvious disc deformation, which disrupts the

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discs ability to adapt to the condyle and fossa (Molinari et al., 2007; Wilkes, 1989).

The complex loading normally distributed by the disc leads to a very distinct regional variations and structure–function relationships in a healthy joint (Detamore & Athanasiou, 2003b). It would be expected that the disruption of normal function in dysfunctional joints would results in adaptive remodeling and degenerative changes to the disc structure that could be systematically evaluated. Similarity between porcine and human TMJ anatomy has led to many bioengineering studies utilizing the porcine TMJ disc as a model. Similarities include size, extracellular matrix alignment, collagen content, region specific biomechanical and biochemical properties, and an omnivorous diet leading to similar mechanical loading conditions (Kalpakci, Willard, Wong, & Athanasiou, 2011; Herring, Decker, Liu, & Ma, 2002; Herring, 2003; Allen & Athanasiou, 2005; Detamore, Orfanos, Almarza, French, Wong, & Athanasiou, 2005).

Using naturally deformed female porcine discs as a model structure, this study sought to determine how surface and regional biomechanical and biochemical properties of discal tissues differ between grossly deformed and morphologically normal TMJ discs from the same population. The hypothesis was that by studying how the structure of the disc is altered in deformation, understanding of the functional changes of the diseased joint can be elucidated which may provide insight into the identification, progression, and influence of treatment strategies of TMDs.

2. Materials and methods

2.1. Tissue collection

Unmatched *en bloc* porcine temporomandibular joints were obtained from Yorkshire sows (IACUC # 201207534, Animal Technologies, Tyler, TX, $n = 12$; 7 left/5 right). Exact age was unknown, however they were known to be retired breeding sows aged 2+ years. Discs were dissected from the joints, with peripheral attachments left intact, by disarticulating the joint after severing the masseter and lateral pterygoid. The criterion for disc deformation was the lack of smooth biconcavity after removal from the joint correlating with progression to Wilkes Stage 3. Following dissection of the disc and washing in phosphate-buffered saline (PBS), they were stored frozen at −20 °C in gauze soaked with saline containing protease inhibitors (2.3 mM EDTA (Acros Organics, Morris Plains, NJ), 10 mM *N*-ethylmaleimide, and 1 mM phenylmethanesulfonyl fluoride (Sigma, St. Louis, MO, USA (Allen & Athanasiou, 2005)).

2.2. Sample preparation

On the day of testing, discs were thawed and equilibrated in PBS at 37 °C for 1 h. Before regional specimen isolation, disc measurements were obtained in triplicate using calipers in the anterior–posterior (AP) and medial–lateral (ML) direction as well as the thickness of the posterior band (PB), intermediate zone (IZ), and anterior band (AB). Duplicate tensile specimens in the lateral, central, and medial portions of the disc were prepared using a custom parallel blade apparatus to produce 1 mm wide section in the anterior–posterior direction and then cut to 10 mm in length. Lastly, superior and inferior surface samples were obtained by sectioning 1 mm sections from the superior and inferior surfaces after the desired surface was pressed flat against another surface. Posterior and anterior tensile specimens were prepared partly into the attachment tissues to give a measure of disc/attachment strength. Compressive specimens were prepared using 3 mm biopsy punches (Miltex, York, PA) in the lateral, medial, anterior, posterior and intermediate portions of the disc. The top and bottom 1 mm of the punches were obtained for superior and inferior testing samples as were obtained in tensile testing (Fig. 1).

2.3. Biomechanical evaluation

The ends of the rectangular tensile specimens were mounted vertically between two hemostats, leaving a 5 mm gauge length. An Instron 5542 mechanical testing instrument (Instron Corporation; Norwood, MA, USA) was used to apply an extension rate of 1% strain/sec (Murphy, Arzi, Hu, & Athanasiou, 2013) within a PBS hydrated chamber at room temperature following cyclic preconditioning in the toe region at 5% strain (Detamore & Athanasiou, 2003a). Young's modulus (E), ultimate tensile strength (UTS), and strain at failure were calculated from stress–strain curves. Samples that broke at the grips were discarded from analysis and where duplicate measures were obtained they were averaged together before further analysis.

Compressive disc specimens were placed in a hydrated testing chamber in a Biomomentum Mach-1 micromechanical system (Biomomentum Inc., Laval, Quebec, Canada) and subject to cyclic

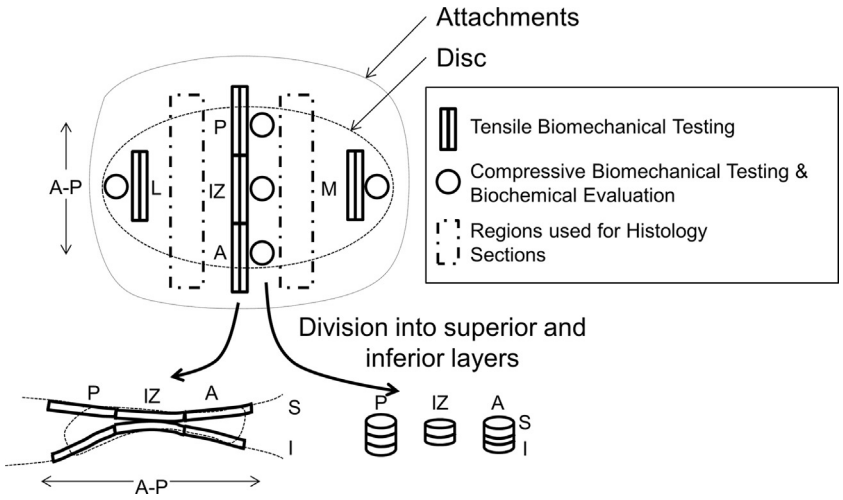

Fig. 1. Disc testing regions. Lateral (L), posterior (P), intermediate zone (IZ), anterior (A), and medial (M) regions were evaluated during testing. Sections were further divided into superior (S) and inferior (I) samples. Rectangular sections were used for tensile biomechanical evaluation, while circular regions were used compressive biomechanical and biochemical analysis. Histology was performed on remaining untested tissue regions.