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Short Communication

Regionally variant collagen alignment correlates with viscoelastic properties of the disc of the human temporomandibular joint

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ABSTRACT

Objective: To determine the regionally variant quality of collagen alignment in human TMJ discs and its statistical correlation with viscoelastic properties.

Design: For quantitative analysis of the quality of collagen alignment, horizontal sections of human TMJ discs with Pricrosirius Red staining were imaged under circularly polarized microscopy. Mean angle and angular deviation of collagen fibers in each region were analyzed using a well-established automated image-processing for angular gradient. Instantaneous and relaxation moduli of each disc region were measured under stress-relaxation test both in tensile and compression. Then Spearman correlation analysis was performed between the angular deviation and the moduli. To understand the effect of glycosaminoglycans on the correlation, TMJ disc samples were treated by chondroitinase ABC (C-ABC).

Results: Our imaging processing analysis showed the region-variant direction of collagen alignment, consistently with previous findings. Interestingly, the quality of collagen alignment, not only the directions, was significantly different in between the regions. The angular deviation of fiber alignment in the anterior and intermediate regions were significantly smaller than the posterior region. Medial and lateral regions showed significantly bigger angular deviation than all the other regions. The regionally variant angular deviation values showed statistically significant correlation with the tensile instantaneous modulus and the relaxation modulus, partially dependent on C-ABC treatment.

Conclusion: Our findings suggest the region-variant degree of collagen fiber alignment is likely attributed to the heterogeneous viscoelastic properties of TMJ disc that may have significant implications in development of regenerative therapy for TMJ disc.

1. Introduction

Temporomandibular joint disorders (TMJDs) affect over 10 million Americans with an annual cost for treatment at approximately $4 billion as per National Institute of Dental and Craniofacial Research (NIDCR). Damage or displacement of TMJ discs are highly associated with TMJDs, frequently leading to surgical procedure such as discectomy (Allen & Athanasiou, 2006a; Dimitroulis, 2011). Given the controversial clinical outcome of discectomy (Dimitroulis, 2011; Hagandora & Almarza, 2012), synthetic or alloplastic TMJ disc replacements have been applied to relieve the symptoms but failed to result in satisfactory outcome (Estabrooks, Fairbanks, Collett, & MillerEstabrooks, 1990; Henry & Wolford, 1993; Kalpakci, Willard, Wong, &Athanasiou, 2011). More recently, TMJ disc regeneration has been attempted using stem cells, biomaterials, and biochemical signals to overcome limitations of the previous disc grafts (Ahtiainen et al., 2013; Allen & Athanasiou, 2006a; Hagandora, Gao, Wang, & Almarza, 2013; Legemate, Tarafder, Jun, & Lee, 2016; MacBarb, Chen, Hu, & Athanasiou, 2013; Tarafder et al., 2016).

The complex biochemical structure and composition of the disc represent a challenging feature for TMJ disc regeneration. Collagen is the predominant extracellular matrix component in TMJ disc that is densely aligned dependent on region and direction. Histological and scanning electron microscopic analyses revealed that collagen fibers have circumferential orientation in the peripheral bands and anteroposterior alignment in the intermediate zone (Allen & Athanasiou, 2006a; Kalpakci et al., 2011; Scapino, Canham, Finlay, & Mills, 1996; Willard, Kalpakci, Reimer, & Athanasiou, 2012). The anisotropic collagen fibers orientation is largely attributed to the tensile properties of TMJ discs varied by region and direction (Allen & Athanasiou, 2006a;

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Kalpakci et al., 2011; Scapino et al., 1996). In the intermediate zone, tensile modulus in the anteroposterior direction is a higher order in the mediolateral direction (Kalpakci et al., 2011). Similarly, tensile modulus in the peripheral band is significantly higher in the circumferential direction in parallel to the collagen alignment than in the perpendicular direction of collagen alignment (Kalpakci et al., 2011).

The anisotropic collagen structure has been applied in few recent studies for TMJ disc tissue engineering (Legemate et al., 2016; MacBarb et al., 2013; Tarafder et al., 2016). In a biconcave and TMJ-shaped molds, meniscus cells and articular chondrocytes were co-cultured to engineer anisotropic collagen structure in support with passive axial compressive loads and bioactive agents (MacBarb et al., 2013). The engineered anisotropic fibrocartilage exhibited higher tensile properties in the direction of collagen alignment, reminiscent of native TMJ discs (MacBarb et al., 2013). More recently, TMJ disc regeneration has been attempted using 3D-printed bioscaffolds that were constructed with repeats of biodegradable microfibers oriented in the anteroposterior and circumferential directions in the intermediate zone and the peripheral bands, respectively (Legemate et al., 2016; Tarafder et al., 2016). The region/direction-dependent mechanical properties of TMJ discs were successfully reconstructed in the 3D-printed scaffolds (Legemate et al., 2016) that consequently led to TMJ disc regeneration in rabbits (Tarafder et al., 2016).

Despite the meritorious findings, the previous analysis of collagen fibers alignment in TMJ disc has been limited in qualitative measurements. Histological and scanning electronic microscope evaluation used in the previous studies were only sufficient to provide the major orientation direction of collagen fibers not degree and/or quality of the fibers' alignment (Scapino et al., 1996; Shi, Wright, Ex-Lubeskie, Bradshaw, & Yao, 2013; Stankovic et al., 2013). However, previous mechanical characterizations of TMJ discs consistently demonstrated that tensile properties in parallel to collagen orientation are different in between different regions despite the relatively homogeneous collagen contents (Allen & Athanasiou, 2006a; Kalpakci et al., 2011). Accordingly, we performed a quantitative analysis of collagen fibers alignment in different regions to test a hypothesis that qualify or degree of fibers alignment is varied in different regions of TMJ discs. Then we tested a statistical correlation between the degree of fiber alignment and the regional-variant mechanical properties of TMJ disc. Our data below suggest that the quality of collagen fiber alignment are varied in different regions and statistically correlated with viscoelastic properties of TMJ disc.

2. Materials and methods

2.1. Quantitative analysis of collagen fibers alignment in TMJ discs

Total 12 tissue samples were prepared from human TMJ discs obtained from National Disease Research Interchange (NDRI) (age 47–65; 55.3 ± 9 years old; male 67%; female 33%). The fresh-harvested samples were delivered overnight kept at 4 °C without fixation or freezing. Due to the nature of this study, it was granted an exemption in writing by the Columbia University IRB. For histological analysis, harvested TMJ disc samples were fixed, embedded in paraffin, and horizontally sectioned in 5-μm thickness. The tissue sections were then stained with Picrosirius Red and imaged under circularly polarized microscope (Lee et al., 2015). Randomly selected sections from anterior, posterior, medial, and lateral bands, and intermediate zones throughout different depths were imaged (n = 10 per section and region), and then collagen fiber alignment was analyzed using a digital image processing technique as established in our previous works (Lee et al., 2005; Lee et al., 2015). Sections from the superficial zone was excluded given the purpose of our study. The automated image-processing method has been to estimate local directionality and angular deviation in images of oriented textiles, as well as in biological tissues and cultured cells (Lee et al., 2005). In this method, the local orientation in images is determined by forming a pixel-by-pixel gradient vector and the spatial intensity gradient was calculated in the horizontal and vertical directions (Lee et al., 2005). The analysis of each image resulted in quantitative data of distribution of fiber orientations, ranging from −90° to 90°, where 0° was defined as the mean angle. The degree of collagen fiber alignment was quantified as the angular deviation value that represent a statistical deviation of alignment angle of a selected set of fiber segments (Lee et al., 2005). The angular deviation values was calculated using circular statistics (Lee et al., 2005) and the algorithm was implemented using MATLAB (Mathworks Inc., Natick, MA, USA).

2.2. Mechanical tests

TMJ disc samples from different regions were prepared for mechanical tests, separately from the samples for imaging. For tensile tests, tissue samples in parallel with the regional collagen orientation were prepared in a dog shape with length of 25 mm and average thickness of 1 mm. For compression tests, disc-shaped samples ($5 \times 2\ mm^2$) were prepared from different regions of the TMJ disc samples (Supplementary Fig. 1). After preconditioning of 15 cycles of 0–5% strain, a 20% step strain was applied and held up to 15 mins allowing all the samples to reach their relaxation plateau, while maintaining the humidity of tissue samples. The time vs. stress curves were then fitted to a Prony series of stress relaxation model (Kim et al., 2005) as follow:

$$E(t) = E_\infty + \sum_{j=1}^{3} E_j \cdot \exp\left(-\frac{t}{\tau_j}\right)$$

where E(t) is the time function of modulus, E(∞) is relaxation modulus (E_r), τ_j is relaxation time. E(t) when t = 0 represents instantaneous modulus (E_i). Then E_i, E_r and τ_j were calculated from each data curve using MATLAB curve fitting tool as per our prior method (Lee et al., 2014; Legemate et al., 2016). A Prony series for stress relaxation function is consisted of a series of constants and Maxwell elements, which has widely been utilized to evaluate viscoelastic properties of polymers and biological tissues showing better fitting to data from quasi-linear tests (e.g. creep and stress relaxation) (Chen, 2000; Palacio-Torralba et al., 2015). All the mechanical tests were performed using BioDynamics 5100 testing system (TA instruments, New Castle, DE), equipped with tensile jigs and compression plateaus designed for soft tissue mechanics. To determine effects of glycosaminoglycans (GAGs) on the mechanical properties of TMJ disc, separate tissue samples were treated by c-ABC (1 U/mL) for 3 h (n = 5 each region). GAGs contents before and after the c-ABC treatment were measured by Blyscan™ GAG Assay kit (Biocolor Ltd, UK) following our previous methods (Lee et al., 2010a; Lee, Shah, Moioli, & Mao, 2010b).

2.3. Statistical analysis

Upon confirmation of normal data distribution, One-way ANOVA with a post-hoc Tukey test were used to compare between the groups with p value < 0.05 considered significant. Spearman correlation analysis was performed to test statistical correlation between the regional angular deviation and the instantaneous and relaxation moduli using SPSS (IBM Corporation, Armonk, NY). Following previously described methods (Andarawis-Puri, Sereysky, Sun, Jepsen, & Flatow, 2012), the Spearman correlation coefficient (R) was calculated with a significance level at $p < 0.01$.

3. Results

3.1. Regionally variant collagen alignment in human TMJ discs

Our automated imaging processing demonstrated the regionally