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The contribution of collagen fibers to the mechanical compressive properties of the temporomandibular joint disc

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S U M M A R Y

Objective: The Temporomandibular Joint (TMJ) disc is a fibrocartilaginous structure located between the mandibular condyle and the temporal bone, facilitating smooth movements of the jaw. The load-bearing properties of its anisotropic collagenous network have been well characterized under tensile loading conditions. However, recently it has also been speculated that the collagen fibers may contribute dominantly in reinforcing the disc under compression. Therefore, in this study, the structural-functional role of collagen fibers in mechanical compressive properties of TMJ disc was investigated.
Design: Intact porcine TMJ discs were enzymatically digested with collagenase to disrupt the collagenous network of the cartilage. The digested and non-digested articular discs were analyzed mechanically, biochemically and histologically in five various regions. These tests included: (1) cyclic compression tests, (2) biochemical quantification of collagen and glycosaminoglycan (GAG) content and (3) visualization of collagen fibers' alignment by polarized light microscopy (PLM).
Results: The instantaneous compressive moduli of the articular discs were reduced by as much as 50–90% depending on the region after the collagenase treatment. The energy dissipation properties of the digested discs showed a similar tendency. Biochemical analysis of the digested samples demonstrated an average of 14% and 35% loss in collagen and GAG, respectively. Despite the low reduction of collagen content the PLM images showed considerable perturbation of the collagenous network of the TMJ disc.
Conclusions: The results indicated that even mild disruption of collagen fibers can lead to substantial mechanical softening of TMJ disc undermining its reinforcement and mechanical stability under compression.

Introduction

The disc of the temporomandibular joint (TMJ) is a robust fibrocartilaginous structure located between the articulating surfaces of the mandibular condyle and temporal bone (Fig. 1). It facilitates smooth jaw movement by increasing congruity, mediating forces, and absorbing impact loads exerted by the articular bones during everyday activities such as mastication, talking and yawing[1,2]. Most epidemiological studies have reported that 20–25% of the population exhibits one or more symptoms of TMJ disorder (TMD)[3]. Of the three TMJ components, the disc is of particular interest since approximately 70% of patients with TMD suffer from malpositioning of the disc, known as internal derangement[4]. Despite the elusive mechanism underlying disease progression, internal derangement appears to be highly correlated with TMJ osteoarthritis (OA) as an accompanying sign or a subsequent factor in a later stage[5]. Due to a poor understanding of TMD etiology, options for treatment of severely damaged disc are restricted to its resection[6,7]. However, since the disc is a crucial functional component of TMJ, its removal can lead to further pain and dysfunction, and eventually degeneration of the whole joint[7]. The presence of a functionally reliable replacement could be helpful for

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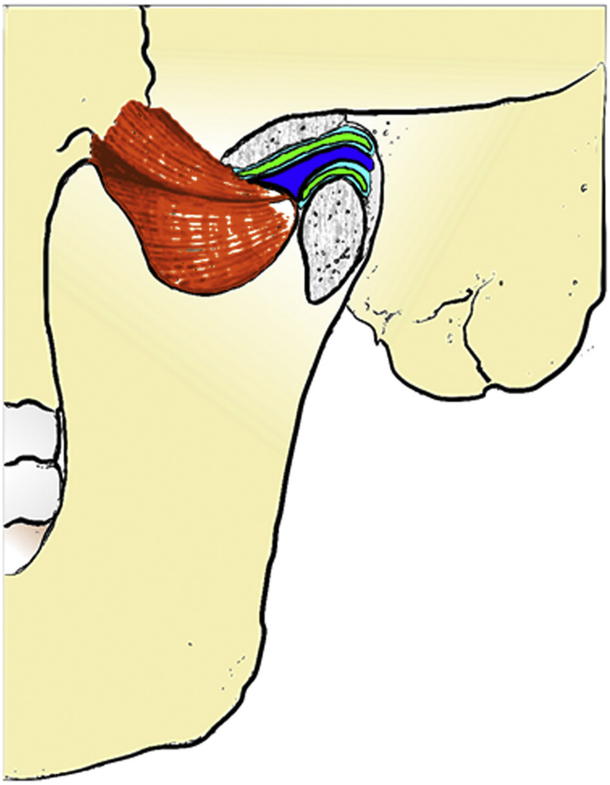

Fig. 1. Schematic view of the TMJ. The TMJ disc (blue), and the articular cartilage of the condyle and mandibular fossa (green) are shown from sagittal view.

treatment, but such has not been engineered yet. To reach that aim the structural-functional relationship of the native TMJ disc needs to be established.

The human TMJ disc is composed of two principal components: (1) a solid extracellular matrix, occupied predominantly by a highly organized collagenous network, and a sparse amount of GAG; and (2) a movable interstitial fluid containing water[8,9]. In contrast to other joints 70–80% of the dry weight of the TMJ disc consists of collagen type I, while GAG constitute only 0.6–10% of it[10]. In the intermediate zone (IZ), the collagen fibers are aligned anteroposteriorly, while in the periphery they show a ring-like orientation, merging with mediolaterally aligned fibers in the posterior and anterior bands[11]. It is commonly assumed that the highly negatively charged GAG and other proteoglycans (PGs) are considered to create an intra-tissue osmotic swelling pressure. Herewith, they respond to compression-induced hydrostatic pressures to spread the force away from directly loaded areas[12,13]. This is performed by interaction with the highly organized region-specific fibrillar collagen of the extracellular matrix of the TMJ disc[11,14]. These features provide the disc with anisotropic and heterogeneous properties that reinforce its structure and maintain its integrity under various types of loading. In a TMJ disc, which is pathologically degenerated by extrinsic factors (e.g., abnormal/repetitive mechanical stress) or/and intrinsic factors (e.g., enzymatic-induced degradation), collagenous network loses its dense integrity. This eventually leads to a disruption of the solid-fluid load-bearing continuum in the cartilage[4,5,15].

Generally, the mechanical compressive properties have been attributed to the GAG content[16,17], while tensile mechanical properties have been related to the amount and organization of collagen fibers[18,19]. However, Willard *et al.*[20] recently indicated that the regional mechanical compressive properties of the disc have a stronger correlation with the associated collagen density and structure than with the GAG content. Despite few studies[8,17,21,22] describing region-dependent mechanical compressive properties of the excised TMJ disc samples, the question still remains how collagen content and structure correspond to regional variations in compressive stiffness of the disc.

Therefore, we measured the regional mechanical compressive properties of TMJ discs before and after enzymatic digestion of their collagen fibers. This approach helps us to evaluate the contribution of collagen fibers in providing reinforcement and stability to the extracellular matrix of TMJ disc as a whole and in relation to their region-specific morphology.

Methods

Sample preparation

Five young porcine heads were obtained from a local slaughterhouse and processed immediately after sacrifice. First, the TMJ discs and condylar head were dissected *en bloc* and then the discs were carefully isolated by removing all bony parts and peripheral soft tissue. All discs were inspected visually and no gross abnormalities were observed. After isolation, the TMJ discs were washed in phosphate buffered saline (PBS), wrapped in gauze soaked with solution of PBS and a mixture of protease inhibitors (Roche Diagnostics, Germany), and then stored at −20°C at which temperature the biomechanical properties of the disc are not affected[23].

Experimental apparatus

The mechanical loading experiment was performed by a custom-made instrument (Fig. 2) capable of generating sinusoidal displacement with a resolution of 0.1 μm at a rate of maximally 30 Hz as described previously by Berendsen *et al.*[24]. The instrument consists of a chamber and two cylindrical stainless steel indenters with diameter of 4 mm. The bottom indenter was fixed to the surface of the chamber and it was aligned with the top indenter whose displacement was controlled by a custom-made software (implemented in LabVIEW 8.2, National Instruments, Austin TX). The samples were placed on the lower indenter, inside the PBS-filled chamber, and the rigid upper indenter was used to apply cyclic compressive displacement. The normal reaction force exerted to the top indenter was measured by a 25 N load cell (Honeywell Model 11, Honeywell, Golden Valley MN). The signal of the load cell was amplified by a bridge amplifier (HBM K10, HBM, Darmstadt,

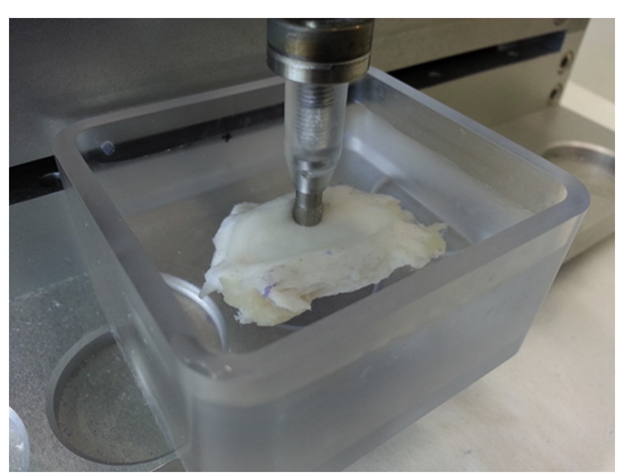

Fig. 2. Indentation test on TMJ disc of porcine sample by the custom-made indentation device.

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