Roles of Collagen V in the Structure and Mechanics of TMJ Condyle Cartilage: A Fibro-Hyaline Hybrid

Prashant Chandrasekaran, PhD[1]
Qing Li[1]
Chao Wang[1]
Mei Sun[2]
Louis Soslowsky, PhD[3]
David Birk, PhD[2]
Lin Han, PhD[1]

[1]Drexel University
Philadelphia, PA

[2]University of South Florida
Tampa, FL

[3]McKay Orthopaedic Research Laboratory
University of Pennsylvania

Introduction

The mandibular condyle cartilage in the temporomandibular joint (TMJ) has a unique bi-layered layout of a collagen I-dominated fibrocartilage layer covering a collagen II and aggrecan-dominated hyaline cartilage layer[1]. This distinctive hybrid structure endows the mandibular cartilage with its specialized biomechanical functions for the high frequency loading of the TMJ during daily speaking and chewing activities[2]. Similar to knee osteoarthritis (OA), degeneration of mandibular cartilage is a hallmark of TMJ OA[3], affecting 10-16% of the US population[4]. Currently, there is very limited understanding of the molecular mechanisms governing the formation of this hybrid tissue extracellular matrix (ECM)[5]. Such knowledge is critical for documenting TMJ OA progression and for designing tissue repair strategies. The initial ECM fibrillogenesis of collagens I and II are regulated by collagens V and XI, respectively[6], and the importance of collagen XI in TMJ function has been highlighted by the phenotype of *Col11a1*$^{+/-}$ (*Cho/+*) murine TMJ[7]. Thus, this study aims to reveal the roles of collagen V in TMJ condyle cartilage structure and function. We also will provide new insights into the high prevalence of TMJ disorder[8] in classical Ehlers-Danlos Syndrome (EDS), a human genetic disorder due to collagen V deficiency[9].

Methods

TMJs were harvested from 3-month old wild-type (WT) and *Col5a1*$^{+/-}$ C57BL/6J mice. The null mice (*Col5a1*$^{-/-}$) were not included as they are embryonic lethal[10]. We applied histology and immunofluorescence (IF) imaging to quantify the TMJ morphology and sulfated glycosaminoglycans (sGAG) staining and the presence of collagen V. We performed SEM imaging on the mandibular condyle surface[11] to quantify collagen fibril diameter. To quantify the modulus of the surface fibrocartilage layer, AFM-based nanoindentation was performed with a microspherical tip ($R \approx 5$ μm, $k \approx 2$ N/m, μMasch) on the central region of a freshly dissected condyle following our established procedure[12].To quantify the mechanical properties of the hyaline layer, we performed nanoindentation on 5-μm thick, unfixed cryosections of the TMJ condyle using Kawamoto's tape method[13]. Hyaline cartilage has two disctinct domains of pericellular and territorial/interterritorial matrices (PCM versus ECM).To delineate these two regions, we applied perlecan IF- image-guided AFM-nanomechanical mapping in PBS. In brief,AFM was performed on each 20 × 20 μm^2 region with ring-shaped PCM terrains using a microspherical tip ($R \approx 2.25$ μm, $k \approx 1$ N/m, μMasch) and a MFP3D (Asylum Research). Effective indentation modulus, E_{ind}, was calculated via finite thickness-corrected Hertz model[14]. The Mann-Whitney U test was used to detect the significance between WT and *Col5a1*$^{+/-}$ cartilage at $\alpha = 0.05$.

Results

In comparison to the WT control, the *Col5a1*$^{+/-}$ mandibular condyle exhibits altered gross-level morphology,and substantial reduction in sGAG staining in histology (Fig. 1). Meanwhile, IF-imaging of collagen V confirms the presence of collagen V in the WT condyle cartilage, and its reduction in *Col5a1*$^{+/-}$ mice (Fig 1). On the mandibular surface, *Col5a1*$^{+/-}$ cartilage exhibits significantly larger fibril diameters (30 ± 6 nm versus 25 ± 7 nm, mean ± std, ≥

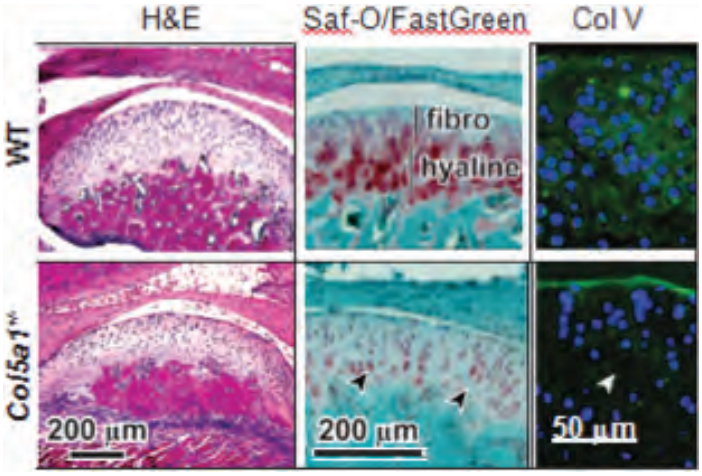

Figure 1 Histological and immunofluorescence imaging of wild-type (WT) and *Col5a1*$^{+/-}$ murine TMJ condyle. H&E shows overall condyle morphology, and Saf-O staining shows reduced sGAGs (black arrowhead) in *Col5a1*$^{+/-}$ condyle cartilage. IF imaging of col V confirms the reduction of collagen V in *Col5a1*$^{+/-}$ cartilage (while arrowhead).

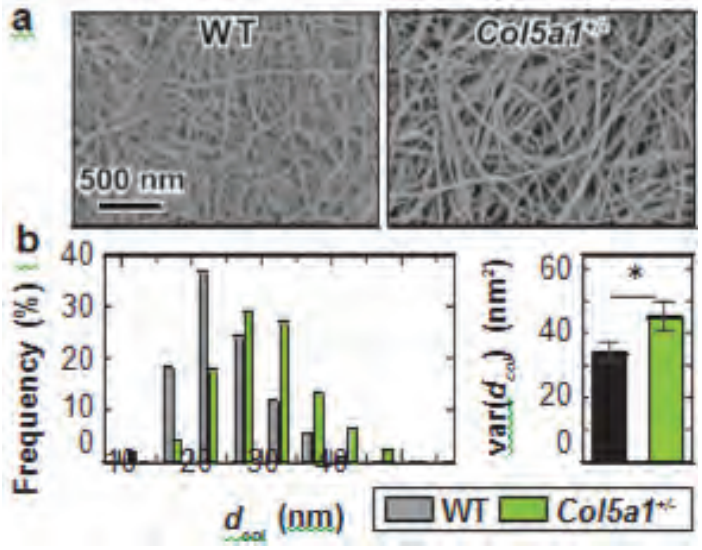

Figure 2 (A) Representative SEM images of TMJ condyle cartilage surfaces. **(B)** Quantitative analysis shows increased collagen fibril diameter and increased heterogeneity (variance) on *Col5a1*$^{+/-}$ condyle surface (mean ± 95% CI, *: $p < 0.05$ from $n > 300$ fibrils on 3 animals).

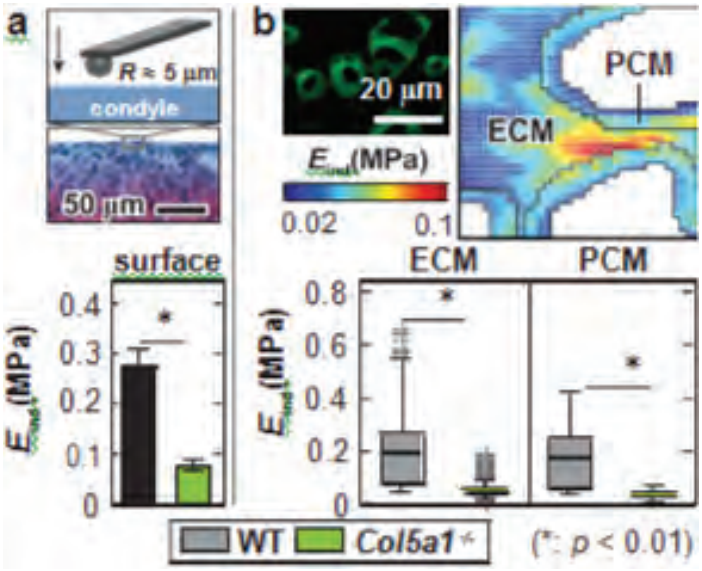

Figure 3 (A) The superficial fibrous layer of *Col5a1*$^{+/-}$ cartilage shows reduced modulus than the WT (mean mean ± 95% CI, ≥ 45 locations of 3 animals). **(B)** Perlecan IF-guided AFM on the cryo-section of secondary hyaline layer shows reduced modulus in both ECM and PCM in *Col5a1*$^{+/-}$ cartilage ($n = 3$).

700 fibrils from $n = 3$ animals) and increased heterogeneity (variance) (Fig 2a, b). Under AFM, both the fibrous and hyaline layers show significant reduction of E_{ind} in *Col5a1*$^{+/-}$ mice (Fig. 3a,b). Notably, in the hyaline layer, the reduction of E_{ind} was significant in both the PCM and ECM (Fig. 3b).

Discussion

This study illustrates the importance of collagen V in regulating the formation of both the fibrocartilage layer, and unexpectedly, the secondary hyaline layer of the mandibular condyle cartilage. In the fibrocartilage layer, the fibril thickening in *Col5a1*$^{+/-}$ mice is in agreement with the known roles of collagen V. During collagen I fibrillogenesis, collagen V co-assembles with collagen I to initiate fibril nucleation[10]. The reduction of collagen V thus leads to increased fibril diameter and heterogeneity (Fig. 2). These structural defects contribute to the loss of tissue integrity, as manifested by the reduction of surface modulus (Fig. 3a).

The reduction of sGAG staining (Fig. 1) and modulus (Fig. 3b) of the hyaline layer, which is unexpected, suggests that collagen V is also critical to the assembly of hyaline cartilage. The mandibular cartilage is an integrated unit of fibrous and hyaline layers (Fig. 1). Therefore, collagen V can influence the hyaline layer possibly through governing the growth of the fibrous layer, or through directly regulating the assembly of hyaline layer. It is possible that the lateral over-growth of collagen I fibrils could influence collagen II fibril assembly in the hyaline layer, resulting in reduced inter-fibril spacing and aggrecan. Meanwhile, it is also possible for collagen V to directly regulate collagen II fibril structure, since we observed salient defects in the hyaline cartilage PCM, the region where cell-mediated initial fibril assembly takes place[15]. This possibility is also supported by the fact that collagen V is more abundant in mature articular cartilage in the form of collagen V/XI heterotypic chains[16]. Therefore, our ongoing studies are developing collagen V/XI compound inducible knockout mice to elucidate their coordinated activities in regulating the formation of this uniquely structured, fibro-hyaline hybrid cartilage.

Significance

The newly discovered role of collagen V in regulating hyaline cartilage has the potential to provide new paths for understanding disease progression and regeneration of TMJ tissues.

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