ENZYMATIC DIGESTION OF TENDONOUS COLLAGEN SHOWS HIGH SPECIFICITY AT THE LEVEL OF THE INDIVIDUAL FIBRIL

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Introduction

Collagen fibrils (CFs) are the structural foundation of many tissues in the human body. Nevertheless, CFs are continually remodeled, through breakdown by enzymes, such as Matrix Metalloproteinases (MMP), and formation of new CFs. Supposed mechanisms by which MMPs break down CFs have up until now mostly been explored from the perspective of molecular simulations or whole tissue level experiments. Variability in CFs is consequently often ignored or assumed to be negligible. This study demonstrates how the breakdown of tendonous CFs can be observed at the level of the individual fibril via atomic force microscope (AFM). Our findings reveal significant variations in the behavior of individual CFs during digestion that cannot be explained through existing molecular models of collagen structure [1] or MMP digestion [1,2].

Methods

Mouse tail tendon CFs (WT, female, 6 months) were prepared on rectangular coverslips that were equipped with dental silicon fluid cells. For digestion, CFs were incubated in a buffer solution (25 mM HEPES, 2mM CaCl2, 50 mM NaCl) containing 20 nM of activated MMP-1 at 37°C for 5 hours. Fibril topography data were collected with an AFM (Nanowizard 3, Bruker-JPK). Images were taken in dry state (tapping mode) in 20 µm x 20 µm regions of interest (ROI) with a resolution of 1024x1024 pixels using a rectangular cantilever (Nanosensors PPP-NCHR, f=330 kHz, k=42 N/m) before and after incubation in the digestion solution. For analysis, a custom Matlab script was used. First, AFM images were flattened followed by semi-automatic segmentation and classification of individual CFs (N = 414). Metrics like the CFs' height at the apex, their aspect ratio and cross-sectional area were then retrieved from the segments and individually compared between the before and after images.

Results

Analyzing the digestion behavior of over 400 individual CFs reveals that neither damage, nor fibril size are reliable predictors of partial or full digestion, as can be seen in Figure 1. Though CFs that were classified as partially damaged had an overall higher chance of being digested, there were many that remained unchanged, despite visible damage. The only type of fibril that was consistently digested was the one described as fully ruptured, exposing a typical zig-zag pattern (see [3]).

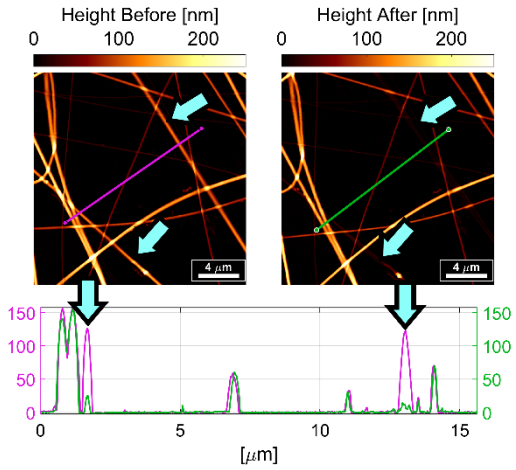

Figure 1: AFM image of a region of interest of CFs on a glass slide before and after digestion through MMP-1. Azure arrows indicate fully digested CFs. The dark green and pink lines correspond to the height profiles at the bottom. None of the CFs in the before image show apparent damage and all of them are very similar in appearance. Yet, two CFs have been digested fully while others remained almost unphased.

Discussion

Previous research on collagen digestion has primarily focused on either large-scale examination of bulk materials or molecular-level analysis of low concentrations. This study, however, reveals that there are intriguing mechanisms to be uncovered by studying individual CFs at the scale of full digestion.

A core limitation of our approach is the fact that our ROIs do not cover the full lengths of individual CFs. While this will obscure the demarcation between the classes of intact/partially damaged CFs, the fact that we also observed many partially damaged CFs that were not digested despite offering many possible MMP-1 binding sites suggests that there must be a different mechanism at play.

References

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