CROSS-LINK DENSITY AND TYPE ARE STRONG DETERMINANTS OF COLLAGEN FIBRIL NANOELASTICITY

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Introduction

At the tissue level, nonenzymatic glycation of rat tail tendon leads to stiffening of the collagen matrix [Reddy 2004]. While the effect of non-enzymatic glycation on mechanics at the tissue level has been previously experimentally documented, a nanoscale mechanical investigation is lacking to date. Yet, the density of collagen cross-links is predicted to determine fracture mechanisms of collagen fibrils [Buehler 2008]. Here we show the direct effect of both cross-link density and type on the nanoelasticity of collagen fibrils.

Methods

Collagen fibrils were obtained from bronchial biopsies from three volunteers by fibreoptic bronchoscopy after ethical approval and informed consent and from mouse tail tendon. The type of cross-links in bronchial human collagen was determined via LC-MS-MS. Non-enzymatic glycation was mimicked in murine collagen by treatment with 0.6 M Ribose in Hank's balanced salt solution (HBSS). Samples were harvested from Ribose and Ribose-free HBSS after 3 and 5 days of treatment. Collagen fibrils where deposited on microscope glass slides, washed with distilled water and air dried. Atomic Force Microscopy (AFM) in cantilever-based nanoindentation mode produces loading and unloading curves of force vs. indentation depth. To reveal the elastic component of modulus (nanoelasticity) the unloading part of the curves was analyzed similarly to Loparic *et al.* AFM experiments were performed by means of an MFP-3D AFM (Asylum Research) using NSC15 cantilevers (MicroMasch), with spring constant k ~ 40 nN/nm and 10 nm tip radius.

Results

After 3 and 5 days of ribose treatment, the modulus of collagen fibrils was significantly increased compared to control (Figure 1C). A strong negative correlation (Pearson's r=–093) between the modulus of human collagen fibrils and the immature to mature cross-links ratio was found (Figure 2B).

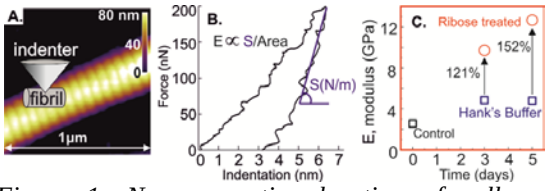

Figure 1: Non-enzymatic glycation of collagen fibrils leads to increase in nanoelasticity. (A.) AFM image of a collagen fibril from mouse tail tendon. (B.) Force curve recorded over the crest of the fibril. (C.) Nanoelasticity increases due to accumulation of non-enzymatic glycation end products.

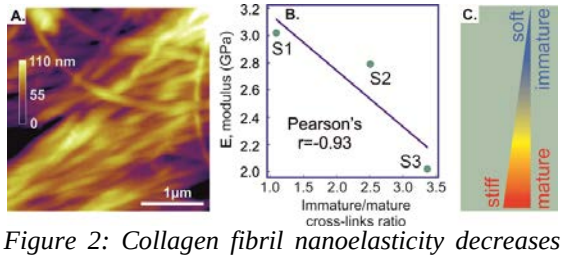

Figure 2: Collagen fibril nanoelasticity decreases when immature cross-links predominate. (A.) AFM image of collagen fibrils from bronchial biopsy. (B.) Decrease in modulus with increase in immature cross-links. (C.) Behaviour of collagen nanoelasticity with type of cross-links.

Discussion

Here we show that the nanoelasticity of collagen fibrils increases with accumulation of nonenzymatic glycation end products (cross-link density). Further, collagen fibrils are softer when immature cross-links predominate. Such alterations could be responsible for changes in tissue physiology leading to impaired functionality; e.g. lung function in severe asthma drops dramatically due to a number of changes, including increased collagen deposition in the airways and likely also the type of collagen cross-linking. Understanding of mechanical implications of collagen cross-link chemistry will undoubtedly provide insights into pathological mechanisms in severe asthma and other collagen-related diseases.

References

Buehler, M.J., J Mech Behav Biomed 1(1):59-67,2008; Reddy, G.K., Exp Diab Res 5:143-153,2004; 2003; Loparic, M. et al, Biophys J 98(11):2731-2740,2010.