MICROMECHANICAL BENDING AND STRETCHING OF SINGLE COLLAGEN FIBRILS USING AFM

Martin L. Bennink, Lanti Yang, Kees van der Werf, Pieter J. Dijkstra, Jan Feijen
MESA+ Institute for Nanotechnology and Institute for Biomedical Technology, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands
m.l.bennink@utwente.nl

Collagen is the most abundant protein in the human body. Of the at least 19 types of collagen proteins known, fibril forming collagen is an important component in many structured tissues. The fibril-forming collagens provide the structural framework and are largely responsible for the biomechanical properties of tissues. Collagen based tissues show mechanical properties which are related to their hierarchical structure. In summary, the hierarchical structure can be described as follows (see Fig. 1): Tropocollagen molecules (collagen triple helices from which the propeptides are enzymatically removed) assemble in a staggered arrangement to form collagen fibrils, which leads to a 67-nm banding pattern, that is very characteristic for collagen fibrils. The fibrils further assemble into fibers, which in a next step can form tendon.. It is important to note that there is still a debate about the existence of microfibrils, which are formed by the aggregation of five tropocollagen molecules to form structures of about 4 nm in width. These structures have never been isolated but have been suggested to exist based on data from X-ray analysis AFM imaging.

The mechanical properties of collagen based tissues have been a subject of study for many years. However, the relationship between the mechanical properties and hierarchical structure of collagen based tissues is not fully explored due to the limitations in performing mechanical testing at lower hierarchical structures of collagen, e.g. collagen fibrils.

In this presentation, we present experiments with a slightly adapted atomic force microscope in order to obtain the mechanical behavior of individual collagen fibrils. The first set of experiments are bending experiments, in which individual collagen fibrils are deposited across micrometer wide and deep channels (Fig. 2). Using the atomic force microscope in force-distance mode, measurements have been performed on this sample. By comparing the force distance curves obtained at the collagen fibril supported by the underlying surface with that of the fibril when it is freely suspended, allowed determination of the Young's modulus of the fibril. This method has been further extended using multiple force distance measurements along the suspended fibril. Comparative experiments have been done on collagen fibrils crosslinked with glutaraldehyde and EDC/NHS, and on electrospun fibers to measure the effect of these on the elastic properties.

The second set of experiments are tensile test experiments in which two-component glue is used to attach individual collagen fibrils with one end to the supporting surface and with the other end to the AFM cantilever. Using pre-calibrated cantilevers it was possible to obtain stress-strain curves from which the Young's modulus could be obtained directly. In order to get a better understanding of the viscous properties of the collagen fibrils we have done a number of relaxation experiments. This means we stretch the fiber up till a preset strain and then measure the force relaxation as a function of time. These relaxation data obtained from differently cross-linked collagen fibers indicated the existence of microfibrils, which are thought to be made up from 5 collagen fibrils.

References:

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Figures:

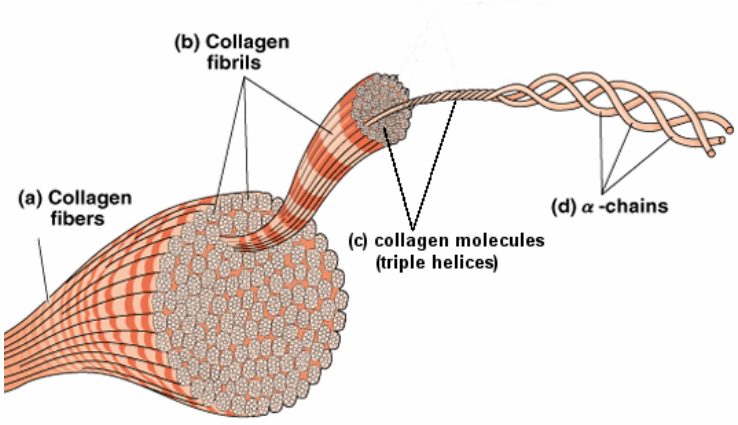
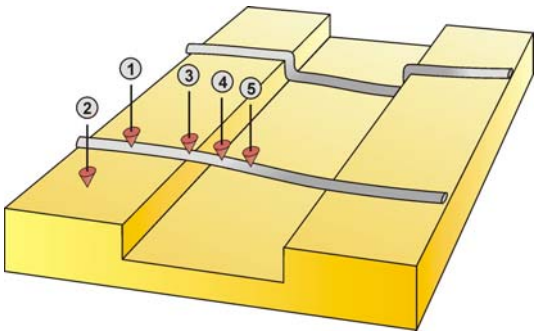


Figure 1
Schematic of the hierarchical structure of collagen. Collagen fibers (a) are large bundles of collagen fibrils (b) (200 nm diameter), which consists of individual collagen molecules (c) (1.5 nm thick and 300 nm long), that again are built up from 3 individual peptide chains (d). The microfibrils that are thought to be consisting of 5 collagen molecules is not depicted here

Figure 2
Schematic representation of how individual collagen fibrils are deposited on a surface that is patterned with microchannels. Atomic force microscopy in force-distance mode is now applied to measure the apparent elasticity, from which the Young's modulus can be calculated.



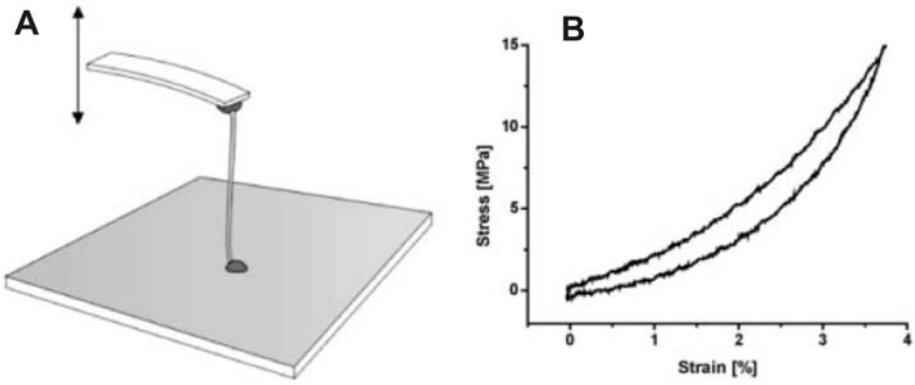


Figure 3
(A) Schematic representation of a tensile test experiment in which an individual collagen molecule is glued between the surface and the AFM cantilever. (B) Typical stress strain curve obtained from stretching and relaxing a collagen fibril in liquid conditions.