In Vitro Mechanical Testing of Collagen Nanofibrils

Zhilei Liu Shen[1], Mohammad Reza Dodge[1], Harold Kahn[1], Roberto Ballarini[2], Steven J. Eppell[1].
[1]Case Western Reserve University, Cleveland, OH;
[2]University of Minnesota, Minneapolis, MN.

Statement of Purpose: To fully understand the mechanical behavior of collagenous tissues, to predict their behavior using biomimetic multiscale models, and to provide design constraints for future biomaterials requires mechanical tests at different length scales. Studies have been done at tissue, fiber and molecular levels. However, experimental data on collagen fibrils with diameters around a few hundred nanometers are lacking. To fill this gap, we developed a Microelectromechanical Systems (MEMS) based technology allowing *in vitro* uniaxial tensile tests on type I collagen fibrils.

Methods: MEMS devices designed and fabricated to test collagen fibrils *in vitro* were similar to those used previously for in-air studies [1, 2]. Type I collagen fibrils isolated from sea cucumber dermis were fixed between stationary and movable pads using micron size epoxy droplets. MEMS devices with fibrils attached were mounted on a piezo stage (Physik Instruments L.P., Auburn, MA), used to drive displacement at a rate of ~34 nm/sec until the fibrils fractured. A 60X water immersion objective lens (Nikon, Japan) was used to visualize the devices in PBS buffer. A digital camera (QImaging, Surrey, BC, Canada) was used to capture images at a frame rates up to 0.5 fps while the fibrils were being stretched *in vitro* at room temperature. Displacements of the fibril and the force gauge were determined by comparing the images collected during the fracture test with the zero load image using digital image correlation (DIC). Deformation of the force gauge was converted to force applied to the fibril using the force-displacement response of polysilicon obtained via FEA. Thus force-displacement curves of fibrils were obtained. Scanning electron microscopy (SEM, Hitachi S4500, Japan) was used to record diameters and gauge lengths of the fibrils. Diameters were corrected for shrinkage due to drying by calibrating against in-vitro atomic force microscope images. The force-displacement curve was then converted to the nominal stress-engineering strain curve.

Results: *In vitro* fracture tests were performed on thirteen collagen fibril specimens having hydrated diameters of 330 ± 150 nm. The mean gauge length, measured from SEM images, was 10.1 ± 3.6 μm. Each specimen was monotonically stretched to fracture at a strain rate of 0.4 ± 0.1 %/sec. As expected for a multiphase biological structure, we found substantial variability in mechanical behavior among the specimens. This variability can be categorized using three groups: (I) linear response to fracture (6 specimens, Fig. 1a), (II) multiple linear regions assigned discrete slopes prior to fracture (5 specimens, Fig. 1b-1c), and (III) multiple linear regions followed by a step-wise post-yield region prior to fracture (2 specimens, Fig. 1d). Mechanical properties of collagen fibrils such as elastic modulus, yield strength/strain, and fracture strength/strain were measured from stress-strain curves and compared with a previous in-air study [2] (table 1).

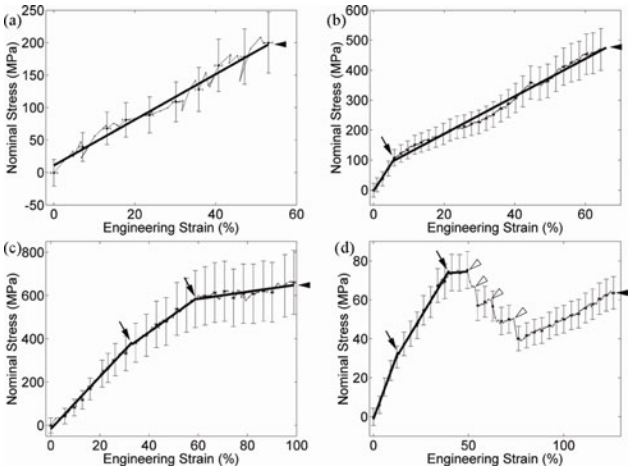

Figure 1. Mechanical behavior of four representative fibrils.

	Elastic Modulus (MPa)	Yield Strength (MPa)	Yield Strain (%)	Fracture Strength (MPa)	Fracture Strain (%)
In vitro	580 ± 640 (n = 13)	160 ± 150 (n = 7)	20 ± 17 (n = 7)	280± 310 (n = 13)	75 ± 43 (n = 13)
In air [2]	860 ± 450 (n = 13)	220 ± 140 (n = 7)	21 ± 13 (n = 7)	200 ± 10 (n = 3)	42 ± 21 (n = 3)

Table 1. Comparison of the mechanical properties of collagen fibrils obtained *in vitro* and in air.

Though the mean values of elastic modulus, yield strength, fracture strength, and fracture strain obtained *in vitro* appeared to be different from those obtained in air, student-t tests showed that there were no statistically significant differences. Elastic modulus, yield strength, and fracture strength decreased with increasing fibril diameter, indicating structural size-dependent mechanical behavior. However, we do not have enough data at this point to make statistically significant statements for each fibril diameter.

Conclusions: We have obtained stress-strain curves of collagen fibrils *in vitro*, which is closer to physiological conditions than previous in-air studies [1, 2]. To our knowledge, these are the first measurements of fracture properties of such specimens. Collagen fibrils displayed structural size-dependent mechanical behavior *in vitro*, indicating that they behave like complex structures rather than homogenous materials, just like the ones tested in air [2]. The mechanical properties obtained from the stress-strain curves, such as the elastic modulus, yield/fracture strength/strain, will serve as constitutive input parameters for multiscale models and design constraints for future biomaterials.

References:

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