Non-destructive Determination of Collagen Fibril Width in Extruded Collagen Fibers by Piezoresponse Force Microscopy

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Extruded collagen fibers are a promising platform for tissue engineering applications.[1,2] Ensuring that the functional properties of the engineered tissues are suitable for the intended application requires tools to measure, e.g., the structural properties and the collagen fibril diameter within the fiber. Previous scanning electron microscopy (SEM) and atomic force microscopy (AFM) studies have revealed the structural features of collagen fibrils within such engineered tissues; however, these techniques often require bleaching or staining steps that damage the sample in the process.[3,4] In this study, lateral piezoresponse force microscopy (LPFM), which is sensitive to the polar orientation of piezoelectric collagen,[5–7] is used to determine the width of individual fibrils and moreover map their organization and polar orientation without damaging the sample. The collagen fibrils showed a highly anisotropic arrangement with preferred alignment along the length of the fiber (Figure 1). Fibril widths of 90 ± 14 nm and 96 ± 18 nm in untreated and bleached fibers, respectively, were measured from LPFM amplitude images. These values agreed with SEM (76 ± 11 nm) and AFM (77 ± 17 nm) measurements that could be measured from bleached but not untreated fibers. The presented method can be extended to measure characteristic dimensions and organization of any piezoelectric biopolymer, without the need of destructive chemical treatments.

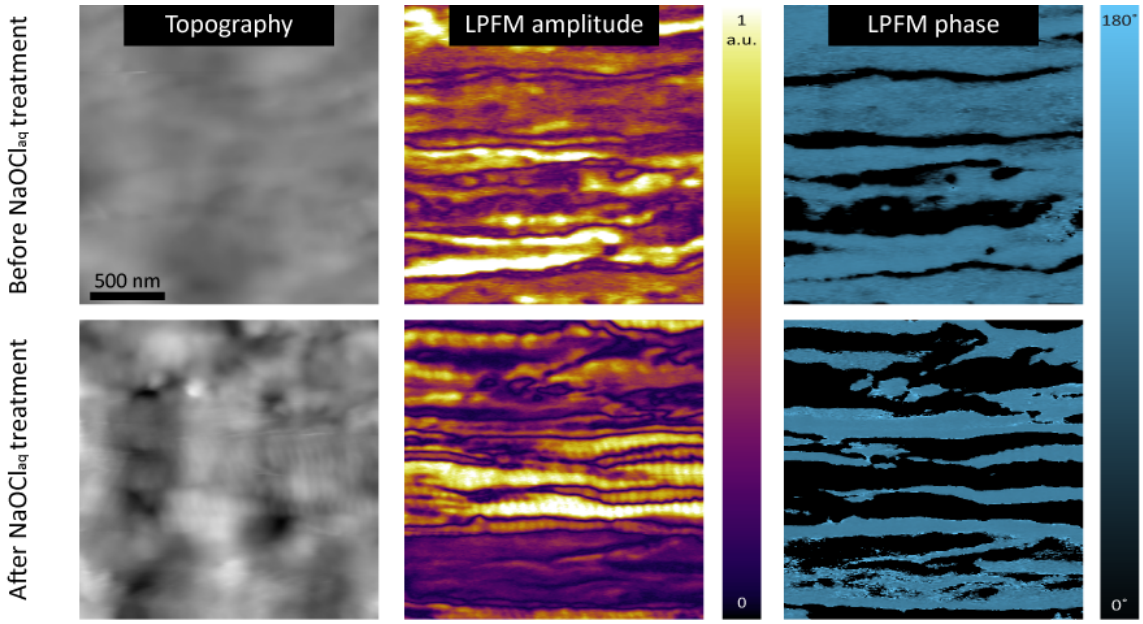

Figure 1. Topography and LPFM amplitude and phase images of untreated and bleached collagen fibers.

References

[1] D.I. Zeugolis, R.G. Paul, and G. Attenburrow, Acta Biomater. **4**, 1646 (2008).
[2] Z. Ahmad, et al., Regen. Biomater. **2**, 77 (2015).
[3] G.D. Pins, D.L. Christiansen, R. Patel, and F.H. Silver, Biophys. J. **73**, 2164 (1997).
[4] A.O. Brightman, et al., Biopolymers **54**, 222 (2000).
[5] M. Minary-Jolandan and M.-F. Yu, Nanotechnology **20**, 085706 (2009).
[6] C. Harnagea, et al., Biophys. J. **98**, 3070 (2010).
[7] D. Denning, S. Alilat, S. Habelitz, A. Fertala, and B.J. Rodriguez, J. Struct. Biol. **180**, 409 (2012).