Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602102984

Surface Organization and Nanopatterning of Collagen by Dip Pen Nanolithography

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Collagens, as a group of fibrous proteins, are programmed at the level of primary sequence to self-assemble into a varied array of molecular materials on the nano and microscale. These materials are characterized by the ability to self-assemble into liquid crystalline mesophases leading to a complex structural hierarchy that influences cellular responses and material properties. The basic helical repeat (glycine-proline-hydroxyproline, and variants on this theme) drives this hierarchical assembly and can be studied with synthetic peptide models of the triple helix. The spontaneous organization of these rod-like molecules occurs in highly concentrated solutions at pH 3, where nematic and cholesteric mesophases have been observed. The mechanism by which fibrillar collagen structures form from liquid crystalline mesophases is not well characterized. Therefore, self-assembly and regular organization of collagens as structural models provides a useful system to develop a better understanding of the assembly process through patterning and surface assembly studies. Patterning a monolayer of precursor "director" molecules on length scales ranging from the nanometer to micrometer, should provide predictable and controllable assemblies. [1]

As one option to study collagen self-assembly, positive printing of collagen and a collagen-like peptide down to 30-50 nm line widths was accomplished, using the atomic force microscopy technique of dip pen nanolithography (DPN). [2] The method preserved the triple helical structure and biological activity of collagen and even fostered the formation of characteristic higher-levels of structural organization. A thiolation modification of the molecules was necessary to ensure attachment to the gold substrate. It was also possible to control the spacing between the lines as well as patterning on larger scales, such as drawing 2μm x 2μm squares and line widths varying for 0.2-0.8 μm. The 'direct-write' capability of biologically-relevant molecules while preserving their structure and functionality provides tremendous flexibility in future biological device applications, in proteomics arrays as well as a new strategy to study the important hierarchical assembly processes of biological systems.

We have also studied the spontaneous supramolecular organizations of different synthetic collagen-like peptides. Our efforts were aimed at developing a systematic understanding of the relationship between primary sequence, concentration and pH on the nature of the liquid crystalline patterns formed and the extent to which the periodicity of the patterns can be manipulated. Four peptides were studied: $(Glu)_5(Gly\text{-}Xaa\text{-}Hyp\text{-}Gly\text{-}Pro\text{-}Hyp)_6(Glu)_5$ where the residue at position Xaa was systematically altered (proline, alanine, valine or serine) to probe the influence of hydrophobicity and potential hydrogen bonding on the structure. Supramolecular organization was determined by polarizing microscopy. The results showed that, depending on the amino acid sequence, the peptides assembled into nematic phases or into spherulites resembling cholesteric liquid crystals.

The degree of fluidity or crystallinity in these spherulites varies with the amino acid sequence of the collagen-like polypeptide. [3]

References

[1] S. Hong, J. Zhu, and C. A. Mirkin, *Science* 286 (1999) 523.
[2] D. L. Wilson, R. Martin, M. Cronin-Golomb, Y. Hong, C. Mirkin, and D. L. Kaplan, *Proc. Natl. Acad. Sci.*, 98 (2001) 13660.
[3] We thank the National Aeronautics and Space Administration and the Air Force Multidisciplinary University Research Initiative program for support of this effort. We also thank Peggy Cebe of Tufts University for help with preparation of the gold substrates.

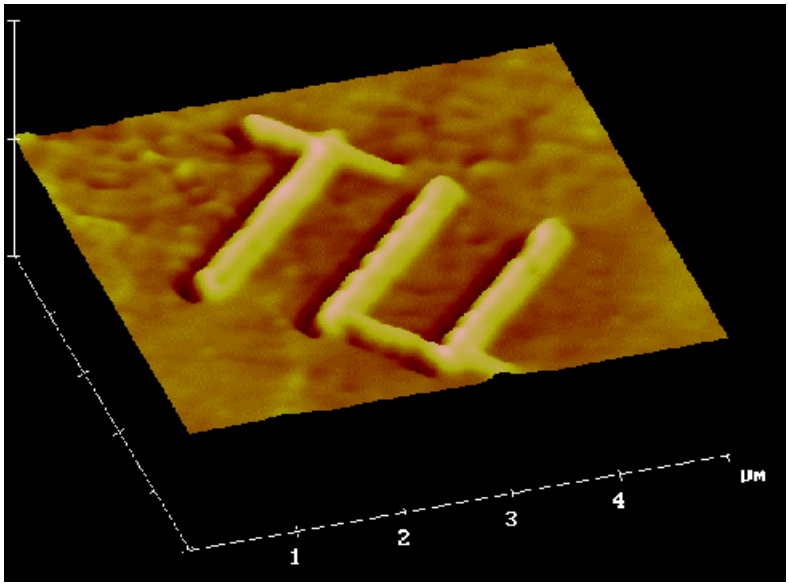

Fig. 1. Phase image of thiolated collagen on Au substrate. The uniform deposition and 120 ± 1.6 nm line widthscan be easily visualized.

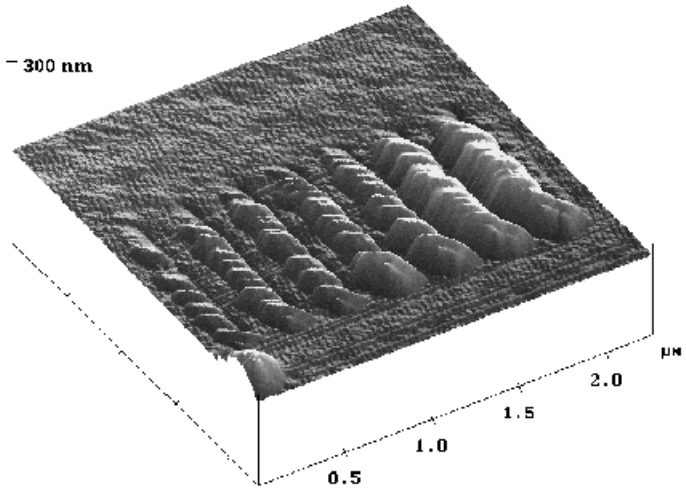

Fig. 2. Top and surface plot views of a topography image of modified collagen molecules deposited on Au substrates

https://doi.org/10.1017/S1431927602102984 Published online by Cambridge University Press