A Visual and Quantitative Analysis of Collagen Self-Assembly Under Microfluidic Hydrodynamic Flow

Jennie B. Leach[1,*], Sarah Koester[1,2], Thomas Pfohl[2], Joyce Wong[1]
[1] Dept of Biomedical Engineering, Boston University, Boston, MA
[2] Max Planck Institute for Dynamics and Self-Organization, Bunsenstraße 10, 37073 Göttingen, Germany
[*] Current affiliation: Dept of Chem & Biochem Engineering, University of Maryland, Baltimore County, Baltimore, MD

Statement of Purpose: Collagen is a major component of the extracellular matrix that exhibits unique hierarchical organization at multiple length scales ranging from nano- to macroscale. This multiscale structure of collagen fibers impacts tissue mechanical properties as well as cell function (e.g., morphology, migration, proliferation and gene expression). To date, however, there are few methods available for precisely controlling collagen self-assembly on the nano- to micro-size scale. *Our objective* was to create highly aligned collagen substrata to systematically determine the effects of nano- and micro-scale collagen alignment on cellular behavior. We implemented a microfluidic diffusive mixing device to create a defined pH gradient in a microchannel, which in turn initiates the self-assembly and alignment of soluble collagen into fibrils under flow. The device is designed for in situ visualization and characterization of collagen self-assembly using polarization microscopy and x-ray diffraction. We further demonstrate that finite element method (FEM) simulations provide a good description of our experimental results regarding the diffusive phenomena, flow profile and pH distribution. This approach has broader impact and provides a powerful means of investigating the hierarchical self-assembly process of biomolecules.

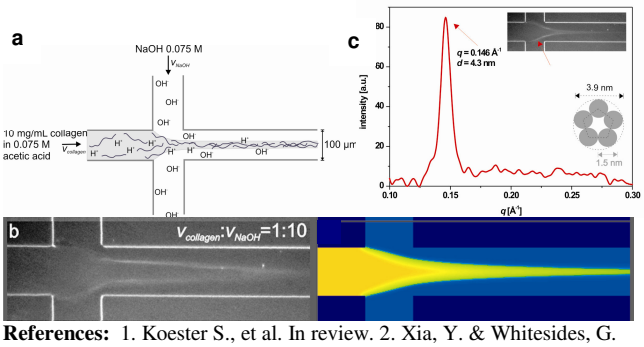
Methods: *Hydrodynamic focusing of collagen:* A 'cross' configuration of polydimethylsiloxane microchannels (35 µm deep, 100 µm wide) was fabricated (Fig. a) using standard soft lithography techniques [1-3]. The fluids were pumped through the microfluidic devices with custom-made syringe pumps controlled by LabView (National Instruments, Austin, TX). A 10 mg/mL solution of collagen-I (calf skin, USB Corporation, Cleveland, OH) in 0.075 M acetic acid (pH 3.7) was injected into the main channel. Into the side channels, 0.075 M NaOH solutions (pH 13) were pumped. The fluid velocity in the main channel (1-8 mm/s) was slower than the side channels (41 mm/s), leading to a hydrodynamically focused collagen stream [4]. *Visualization of collagen self-assembly:* Polarized light microscopy (BX61 Olympus microscope, Germany; SensiCam CCD camera, PCO, Germany) was used to obtain birefringence images in the microfluidic device. *FEM simulations:* To obtain predictions of the pH profile within the microchannels, FEM simulations were carried out with Femlab (Comsol, Burlington, MA) and Matlab (Mathworks, Natick, MA). *X-ray diffraction:* The small angle X-ray microdiffraction experiments were conducted at the beam-line ID10B of the European Synchrotron Radiation Facility (ESRF, Grenoble, France).

Results / Discussion: The collagen self-assembly process was monitored using polarization microscopy to visualize the birefringence signal. A brighter signal was caused by collagen monomer alignment and extension [5]. Collagen self-assembly was initiated as the pH increased from 3.7 towards neutral. One advantage of hydrodynamic focusing is that diffusive mixing of the collagen and NaOH solutions gradually and controllably increases the collagen solution pH. Therefore by observing an increase in birefringence intensity, we are able to locate regions of collagen self-assembly and correlate this information to simulated flow and pH profiles (Fig. b). The FEM simulations predict a stream with a shape that corresponds well with the birefringence signal. We found that the shape of the collagen flow and the diffusion rate are strongly dependent on the flow rate ratio, $v_{collagen}$:v_{NaOH}. Preliminary x-ray diffraction experiments (Fig. c) show a correlation peak at d=4.3 nm, which is suggestive of the formation of critical subunits that further assemble into larger aggregates [6]. If we assume an arrangement of close-packed, regular pentagons made up of cylindrical molecules with a diameter of 1.5 nm (the diameter of the collagen-I triple helices), this gives a diameter of 3.9 nm for the subunits (inset in Fig. c). The formation of these subunits is likely to be the step following triple helix formation in the hierarchical process of collagen self-assembly.

Conclusions: We have described a system that can align collagen molecules at the nanoscale through the generation of diffusion-controlled pH gradients. This technique could be used to elucidate dynamic processes of collagen fiber assembly, and more importantly, has the potential to generate defined structures of collagen for the investigation of cellular response. Such experiments will bring new insights into collagen structure formation and self-assembly and provide fundamental knowledge crucial for the design of new engineered tissues.

Acknowledgments: D. Steinhauser, B. Struth for x-ray experiments. U. Krafft for technical assistance. ESRF synchrotron radiation facilities (ID10b). *Support:* AHA Postdoctoral Fellowship and Clare Boothe Luce Professorship (Henry Luce Foundation) to J.B.L.; DFG E. Noether Program to T.P.; NIH, NASA, and Dupont to J.Y.W.

Figures: (a) Microfluidic device, (b) *left:* polarization microscopy image of a birefringent collagen jet, *right:* FEM simulation of pH (yellow, pH ~4; green, pH ~7; blue, pH ~ 12), (c) x-ray diffraction signal of flow-aligned collagen. [1]



References: 1. Koester S., et al. In review. 2. Xia, Y. & Whitesides, G. (1998) *Ann. Rev. Mat. Sci* 28, 153-184. 3. Pfohl, T., et al. (2003) *ChemPhysChem* 4, 1291-1298. 4. Knight, J.B., et al. (1998) *Phys. Rev. Lett.* 80, 3863-3866. 5. Junqueira, L.C. & Carneiro, J. (1996) *Histologie* (Springer, Heidelberg). 6. Christiansen, D. L., et al. (2000) *Matrix Biol.* 19, 409-420.