

Spatially-Resolved ECM Nanotopology via Gold Nanorod Diffusion Mapping Using Polarization-Sensitive OCT

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Abstract: We demonstrate using PS-OCT to sense cross-sectional ECM nanotopology by mapping spatially resolved GNR diffusion. This novel approach will enable new applications in studying ECM remodeling such as tumorigenesis.

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1. Introduction

The viscoelastic connective tissue comprising cells is primarily made of collagen, a polymeric protein, to form the extracellular matrix (ECM). Mechanical properties of the extracellular matrix (ECM), maintained by stromal fibroblasts, have been shown to impact cellular response that may regulate tumorigenesis [1]. Monitoring ECM stiffness may provide information relevant to cancer progression. However, current minimally invasive methods of assessing ECM *in vitro* are lacking.

We previously describe a method of inferring the nanotopology of the ECM surrounding reduction mammary fibroblasts (RMFs) using diffusing gold nanorods (GNRs) [2]. We hypothesized that as RMFs grow and remodel the ECM, spacing between biopolymers (i.e., the effective pore size) would decrease, resulting in stiffening ECM. By diffusing GNRs throughout ECM of 3D RMF cultures, we could monitor GNR diffusion, increasingly hindered by decreasing pore size, to infer the degree to which RMFs were remodeling the ECM for increasing cell seed densities and incubation periods. Using polarization-sensitive optical coherence tomography (PS-OCT), we were able apply dynamic light scattering theory to quantify diffusion rates from light scattered by GNRs throughout the ECM [3].

However, these studies revealed that for the most populated cell cultures at longer incubation periods (>48 hours), the decreasing trend in GNR diffusion unexpectedly reversed, but with high standard deviations. We suspect that as cells grow and multiply, the ECM microenvironment becomes more heterogeneous than can be described by a spatially-averaged GNR diffusion measurement. Here we report a method of spatially resolving ECM pore size via GNR diffusion mapping in 2D cross-sections, which has the potential to disentangle heterogeneous pore size distributions in ECM such as those developed via remodeling processes in tumorigenesis [4].

2. Methods

High concentration rat tail collagen (Corning), was used to prepared collagen gels with densities of 2, 5, and 8 mg/ml using manufacturer dilution specifications. Each gel was premixed with PEGylated GNRs (83±7 x 22±3) to ensure an even distribution throughout. Samples were gelled by heating at 40°C for >2 hours prior to imaging.

PS-OCT was used to image GNR diffusion throughout each sample. The system is comprised of a Ti:Sapphire laser ($\lambda=800$ nm, $\Delta\lambda=125$ nm, Griffin, KMLabs, Inc.) with 3×12 μm (axial × transverse) resolution in air. Linearly polarized laser light was directed into a Michelson interferometer configuration with outputs separated into co- and cross-polarization states (HH and HV) imaged by a 4096 pixel line-scan camera (Piranha, Dalsa, Inc.) at 25 kHz.

M-mode images (i.e., $z-t$) were collected by repeatedly capturing 4000 A-scans. The images were used to quantify depth resolved pixel intensity fluctuation through time. M-mode image stacks were collected by scanning the OCT beam 2 mm in the x-direction in increments of 20 μm , for a total of n=100 stacks.

The normalized, first-order autocorrelation, g , was computed on the pixel intensity fluctuation at each depth and averaged over a depth of 3 pixels. GNR diffusion was then calculated using the value at which the averaged autocorrelation decayed to 1/e ($\tau_{1/e}$) according to:

$$g_{avg_{x,z}} = e^{-q^2 D T_{map_{x,z}} \tau_{1/e}}, \quad (1)$$

where $D_{Tmap\ x,z}$ is the translational diffusion coefficient at each pixel location, $q=4\pi n/\lambda_0$, and n is the refractive index of the medium. $D_{Tmap\ x,z}$ thus represents a map of the diffusion rate within the cross-section (x and z).

3. Results

Fig. 1. A. shows the bulk GNR diffusion decreasing due to decreased collagen pore size throughout homogenous collagen samples for densities equal to 2, 5, and 8 mg/ml. Spatially resolved GNR diffusion maps show consistent measurements throughout homogenous collagen as expected. Fig. 1. B. shows a cross-sectional OCT image, with overlaid co- and cross-polarization states, of 2 mg/ml collagen diagonally layered onto 8 mg/ml collagen and the corresponding GNR diffusion map. While the GNR concentration is evenly distributed, the diffusion map clearly shows a transition from high to low diffusion rates across the boundary between the collagen concentrations.

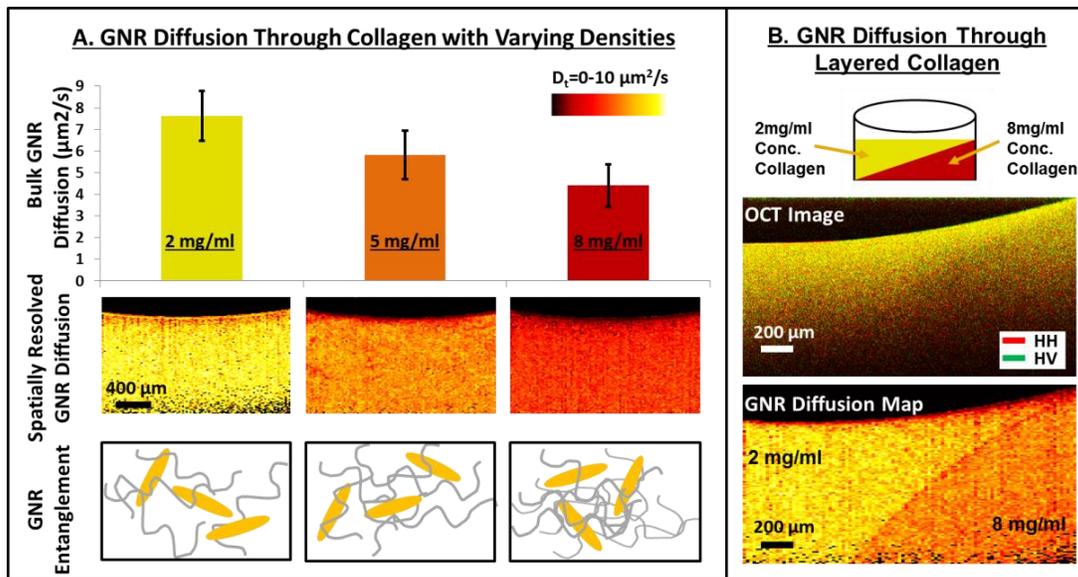


Figure 1. **A.** Top – Bar graph showing GNR diffusion rate through collagen at different densities ($n > 200$, $p < 0.0001$ for each). Middle – GNR diffusion maps through collagen at different densities. Bottom – GNR entanglement by collagen fibers for increasing densities. **B.** Top – Diagram of collagen sample with 2 mg/ml density collagen layered onto 8 mg/ml density collagen. Middle – Cross-sectional image of layered collagen. Bottom – GNR diffusion map showing discrimination between the two collagen layers.

4. Conclusion

The ECM is a complex network of biopolymers that play a role in cellular communication. We present a method of using GNRs imaged with PS-OCT to spatially resolve the ECM pore size and demonstrate this method in collagen at varying densities. Successful spatial resolution of varying collagen density throughout our samples demonstrates the potential of GNR diffusion mapping as a novel tool, which will enable new studies into the role of mechanical signaling in tumorigenesis.

5. Acknowledgements

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6. References

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