Anisotropic Diffusivity Measured with Fluorescence Recovery after Photobleaching (FRAP)

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Diffusive transport of solutes for signaling pathways and metabolic activity plays a key role in maintaining the function of normal tissue. Fibrous tissues often exhibit structural anisotropy that gives rise to anisotropic solute diffusion. A major advantage of tissue engineered fiber-based scaffolds is the control of directionally dependent properties such as diffusivity. However, a standard method for quantifying anisotropic solute diffusion in scaffolds has not yet been established. The goal of this work is to develop a method to quantitatively measure the diffusion tensor in biological tissues and engineered scaffolds. Fluorescence recovery after photobleaching (FRAP) is a technique that measures diffusivity by analyzing the fluorescence recovery of a probe after bleaching a region with a high intensity laser. We have previously developed methods of FRAP that can be used to simultaneously measure the diffusion in two[1,2] or three[3] dimensions with commercially available confocal and multi-photon microscopes. The methods have been validated with computational simulation as well as experimental measurements in isotropic solutions and anisotropic biological tissue. Recently, we have successfully measured anisotropic diffusion in electrospun fiber-based scaffolds with FRAP. In both fibrous biological tissue and electrospun fiber-based scaffolds, the diffusivity in directions parallel to fibers was significantly increased when compared to the diffusivity in directions orthogonal to fibers. In the scaffolds, absolute diffusivity as well as the degree of anisotropy was dependent on fiber density, fiber diameter, tissue porosity, bathing solution, and the molecular weight of the probe. FRAP is a powerful technique for accurate and quantitative measurements of the diffusion tensor in biomaterials. Our validated methods can be used to characterize the diffusion tensor of fiber-based scaffolds.

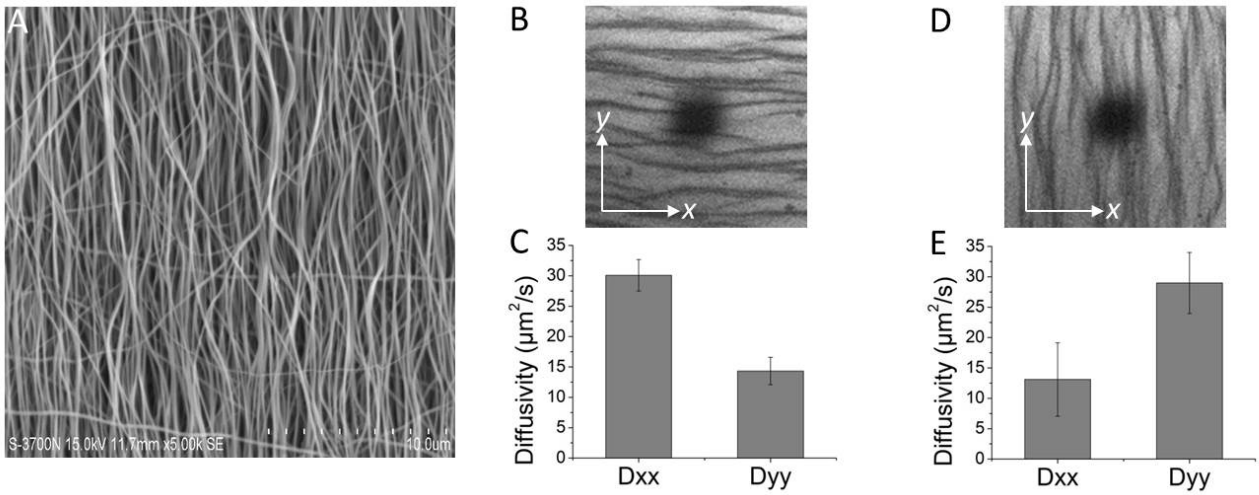

Fig. 1. (A) SEM of electrospun gelatin fibers. FRAP measurements of horizontal (B,C) and vertical (D,E) fibers show increased diffusivity of FITC-dextran 70 kDa in the direction of alignment.

References

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