Dynamic Multi-Component Nanofibrous Scaffolds for Enhanced Tissue Maturation

Given their ability to dictate initial cell alignment and subsequent matrix organization, aligned electrospun scaffolds are a fitting means for engineering fiber-reinforced, anisotropic tissues such as tendon, ligament, the knee meniscus, and the annulus fibrosus [1]. However, one commonly observed limitation of such scaffolds is the relatively slow infiltration rates of surface-seeded cells, where the central thicknesses of constructs cultured for 10 weeks remain devoid of cells [2]. This limitation arises from the tight packing of fibers which yields small pore sizes, thereby hampering cell migration. Towards accelerating cell ingress, we have designed two-polymer composite scaffolds containing both slow eroding poly(ε-caprolactone) (PCL) fibers as well as water-soluble poly(ethylene oxide) (PEO) fibers that serve as space holders during scaffold formation (Fig1A-D) [3]. To assess the effect of sacrificial PEO fiber content on construct growth, PCL/PEO composites were fabricated with PEO fiber fractions ranging from 0 to 60%. After seeding with human meniscus fibrochondrocytes (hMFCs), the mechanical and biochemical maturation of constructs was assessed over 12 weeks. Nuclear staining of w12 cross-sections confirmed that the inclusion of sacrificial PEO fibers improved cell infiltration, leading to a more continuous collagen distribution (Fig1E-F). Image analysis showed increases in the number of centrally-located cells with higher PEO content (Fig1H). In addition to improved distribution, collagen content was also higher in w12 60% PEO constructs (Fig1G). While higher PEO content reduced the initial modulus of hydrated scaffolds, by 12 weeks of culture, the modulus of 60% PEO constructs group surpassed that of 0% controls (Fig1I). The change in stiffness between weeks 3 and 12 correlated with the PEO content, where 60% PEO constructs demonstrated the most dramatic increase (Fig1J). Expanding beyond two fiber constituents, we have begun to explore new fiber elements with the use of a tri-jet electrospinning device (Fig2A-B). The addition of synthetic fibers that erode over the time course of tissue formation [4], or biomimetic natural polymer fibers such as collagen will allow us to explore the effect of a dynamic nanofibrous microenvironment on *in vitro* tissue development [5].

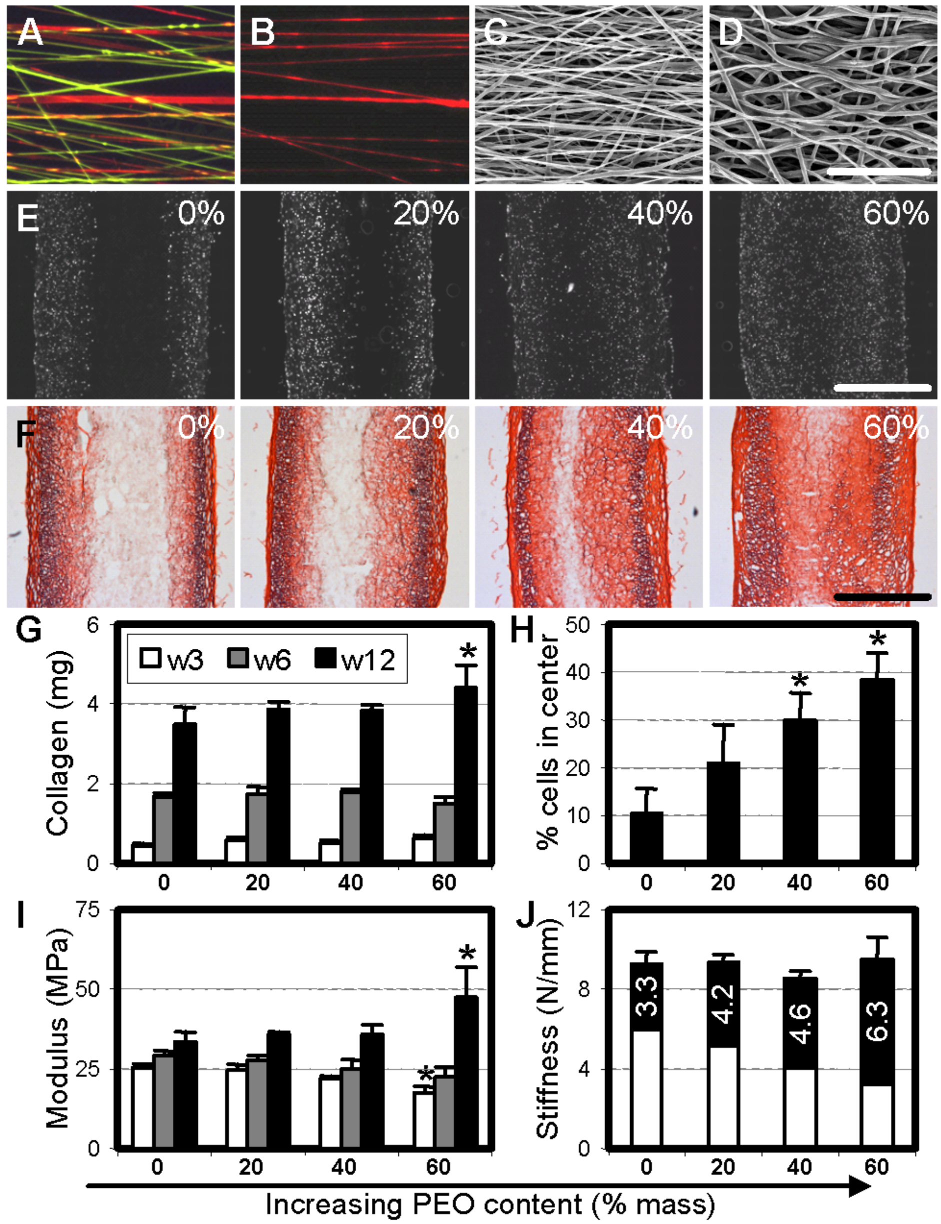

Figure 1. PEO/PCL composites before (A, C) and after (B, D) fiber sacrifice (scale bar: 25µm). Cross-sections stained for nuclei (E) and collagen (F) at w12 (scale bars: 500µm). G) % of cells located in the central 50% thickness at w12. Collagen content (H) and tensile modulus (I) of constructs at all time points. J) Stiffness of w3 and w12 constructs (Δ stiffness within bars). *: $p<0.05$ vs. 0% at same time point, n=6.

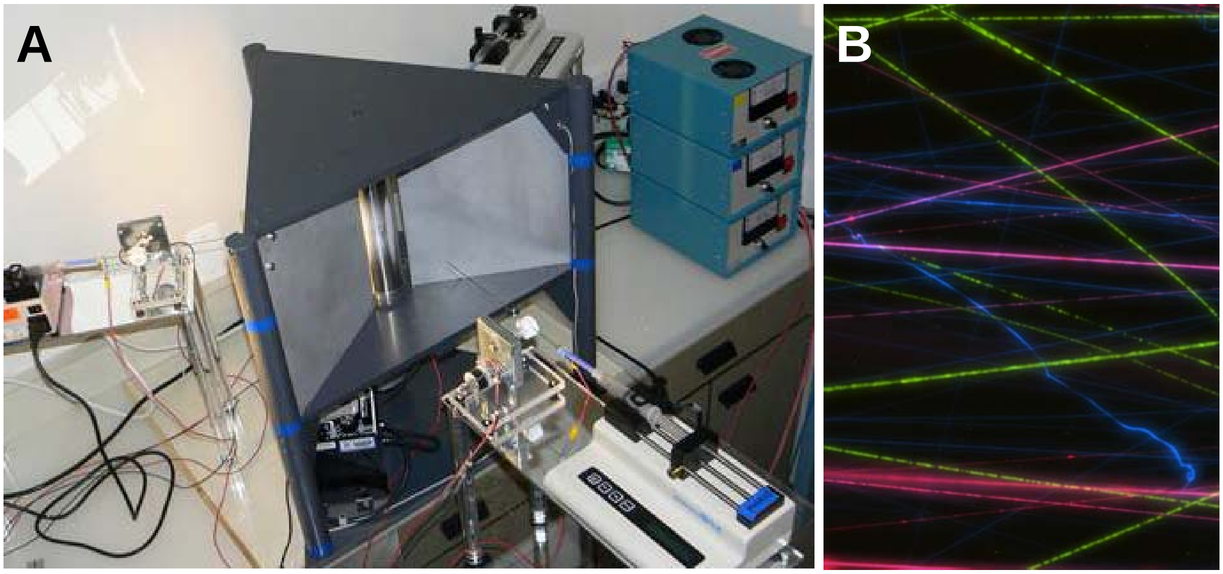

Figure 2. A) Tri-jet electrospinning device for fabricating composite nanofibrous meshes with slow, medium, and fast degrading elements. B) Composites with fluorescently-labeled PCL, PLGA, and PEO nanofibers.

Recent Publications:

1. Mauck RL, Baker BM, Nerurkar NL, Burdick JA, Li WJ, Tuan RS, Elliott DM. "Engineering on the Straight and Narrow: The Mechanics of Nanofibrous Assemblies for Fiber-Reinforced Tissue Regeneration," 2009, Tissue Engineering: Part B, 15(2):171-93.
2. Baker BM, Nathan AS, Huffman GR, Mauck RL. "Tissue Engineering with Meniscus Cells Derived from Surgical Debris," 2009, Osteoarthritis and Cartilage, 17(3):336-45.
3. Baker BM, Gee AO, Metter RB, Nathan AS, Marklein RA, Burdick JA, Mauck RL. "Selective Removal of Sacrificial Fibers Improves Cell Infiltration in Composite Fiber-Aligned Nanofibrous Scaffolds," 2008, Biomaterials, 29(15):2348-58.
4. Baker BM, Nerurkar NL, Burdick JA, Elliott DM, and Mauck RL. "Fabrication and Modeling of Dynamic Multi-Polymer Nanofibrous Scaffolds," 2009, Journal of Biomechanical Engineering, 131(10):101012
5. Gee AO, Baker BM, Montero G, Silverstein AM, Mauck RL. "Fabrication and Evaluation of Biomimetic-Biosynthetic Nanofibrous Composites," Transactions of the 56th Annual Meeting of the Orthopaedic Research Society, New Orleans, LA, March 6-9, 2010.

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