

Multi-Scale Tissue Engineering of the Intervertebral Disc

The intervertebral disc permits flexibility and motion of the spine. However, nutrient and waste exchange between the body and this dense, avascular tissue may result in the progressive degeneration of the disc, resulting in low back pain and herniation. Successful replacement of the disc requires replication of its mechanical properties. Because function and form are intimately related, our approach for disc tissue engineering is motivated by anatomic form, and the hierarchical organization of the disc (**Fig. 1**). Specifically, we have employed electrospinning to fabricate aligned nanofibrous scaffolds. These scaffolds mimic the scale and organization of native extracellular matrix and nanofiber alignment presages cell alignment. In fact, scaffold alignment directs collagen synthesis and deposition, instructing the formation of collagen rich tissues that possess anisotropy common to many fiber-reinforced soft tissues such as tendon, ligament, meniscus and the annulus fibrosus of the intervertebral disc [1]. We have employed this technology to engineer single-lamellar constructs that possess sub-lamellar collagen alignment [2, 3]. More recently, we have expanded this approach to engineer nanofibrous biologic laminates that replicate the form *and* function of the native annulus fibrosus (**Fig. 2**) [4]. These biologic laminates have also enhanced our understanding of how structure and function are related in such tissues, illustrating how biomimetic materials and engineered tissues can serve as simple analogues for complex tissues, providing key insights into the function of their complex native counterparts. Finally, we have incorporated nanofibrous laminates into nanofiber-hydrogel composites to form whole-disc constructs, possessing both a central gelatinous nucleus pulposus and a multi-lamellar angle-ply annulus fibrosus (**Fig 3**).

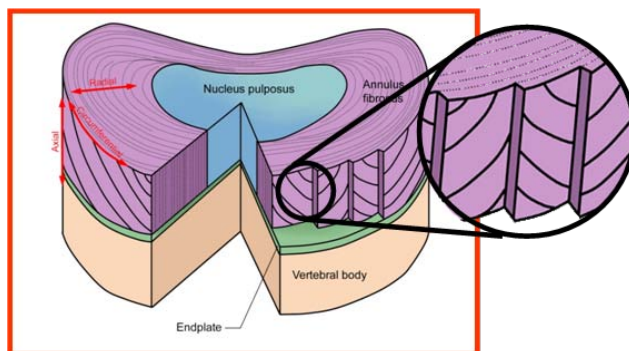


Figure 1. Anatomy of the intervertebral disc. The disc is composed of a central gelatinous nucleus pulposus surrounded circumferentially by the annulus fibrosus. Each lamella of the annulus consists of unidirectionally aligned collagen fibers, and these lamellae are arranged such that from one to the next the direction of alignment alternates by $+30^\circ$ and -30° with respect to the circumferential direction of the disc. This angle-ply organization is key to the disc's ability to withstand loads encountered during axial loading and torsion of the spine [5].

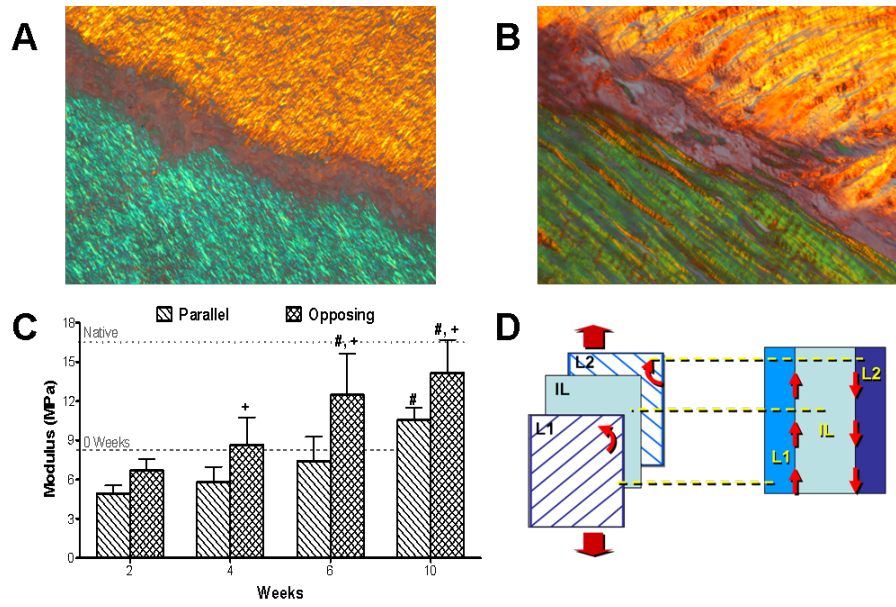


Figure 2. Nanofibrous biologic laminates. Engineered biologic laminates (A) replicate the angle-ply collagen organization of the native annulus fibrosus (B). Comparison of laminates with $\pm 30^\circ$ opposing fiber orientations with $+/+30^\circ$ parallel orientation indicates that, while both develop improved tensile properties with *in vitro* culture, only the opposing bilayers reach comparable values to native annulus fibrosus by 10 weeks (C). We have determined that this disparity in tensile properties between parallel and opposing laminates is the result of inter-lamellar as fibers within opposing laminates reorient in opposing directions (D) [4].

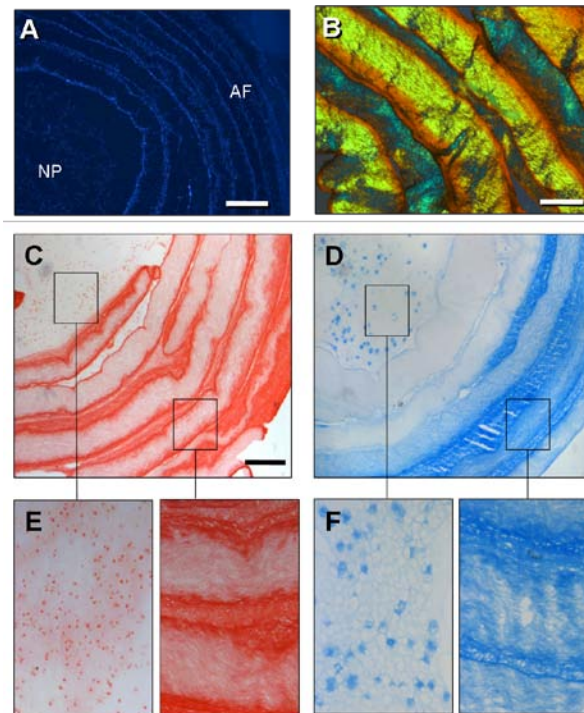


Figure 3. Tissue engineering of a whole disc composite. An intervertebral disc was formed with stem cells encapsulated in agarose to form the nucleus pulposus (NP, A) and stem cells seeded onto aligned nanofibrous scaffolds to form the annulus (AF, B). In the AF region, collagen was deposited along opposing and alternating orientations of $\pm 30^\circ$, replicating the architecture of the intervertebral disc (C). Collagen (C, E) and GAG (D, F) were deposited within the AF and NP regions. The observed heterogeneity in matrix deposition points to a challenge in the future of disc tissue engineering to ensure nutrient transport through the thickness of large, dense tissues [6].

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, in press.
2. Nerurkar NL, Elliott DM, Mauck RL. "Mechanics of oriented electrospun nanofibrous scaffolds for annulus fibrosus tissue engineering," 2007, Journal of Orthopaedic Research, 25(8):1018-28.
3. Nerurkar NL, Mauck RL, Elliott DM. "ISSLS prize winner: integrating theoretical and experimental methods for functional tissue engineering of the annulus fibrosus," 2008, Spine, 33(25):2691-701.
4. Nerurkar NL, Baker BM, Sen S, Wible EW, Elliott DM, Mauck RL. "Nanofibrous biologic laminates replicate the form and function of the annulus fibrosus," 2009, Nature Materials, 8(12): 986-92.
5. Nerurkar NL, Elliott DM, Mauck RL. Mechanical design criteria for intervertebral disc tissue engineering. Journal of Biomechanics (submitted).
6. Nerurkar NL, Sen S, Huang AH, Elliott DM, Mauck RL. "Engineered disc-like angle-ply structures for intervertebral disc replacement," 2010 Spine Special Issue (submitted).

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