INTRODUCTION: Synthetic nanofibrous scaffolds generated via electrospinning have demonstrated marked potential for a wide range of tissue engineering applications as they recapitulate the micro-and nano-scale architecture of the native extracellular matrix (ECM). Aligning fibers in 3D scaffolds engenders both structural and mechanical anisotropy, making them well-suited for the engineering of fiber-reinforced tissues such as meniscus, tendon and ligament [1]. When scaffolds are seeded with cells, fiber-alignment directs the ordered deposition of ECM and so improves mechanical properties with in vitro culture [2]. However, when formed from a slow degrading synthetic material, scaffolds suffer from a low rate of cell infiltration [2]. For example, 1mm thick mesenchymal stem cell (MSC)-seeded fiber-aligned scaffolds comprised of synthetic poly(e-caprolactone) (PCL) were sparsely populated in the inner third, even after 10 weeks in culture. Conversely, nanofibrous scaffolds formed from biologic polymers, such as type I collagen and gelatin, are infiltrated much more rapidly [4]. These biomimetic scaffolds, however, have poor mechanical properties upon hydration (modulus <1 MPa compared to ~20MPa for PCL) [5] and so are unsuitable for implantation in cases where load-bearing is required. To address this issue, we hypothesized that judicious combinations of type I collagen and PCL nanofibers would enhance infiltration while maintaining mechanical properties. In this study, we employed a multi-jet electrospinning method to create two distinct fiber populations in the same scaffold, at two different levels of biomimetic inclusion, and evaluated mechanical properties and MSC infiltration with time in culture.

RESULTS: Accellular composite scaffolds (Low and High) showed positive staining with PSR throughout the depth after hydration, indicative of successful fixation of collagen in the composite structure (data not shown). The presence of collagen in the composite decreased the modulus on day 21, with Low and High constructs showing significantly lower modulus than PCL (p<0.05, Fig. 1A). As expected, higher collagen content was quantified in Low and High composites compared to PCL controls (p<0.05, Fig. 1C). With longer periods of culture, all seeded constructs increased in mechanical properties by day 63 (Fig. 1A). PCL constructs increased in DNA, collagen and GAG content with time, while Low composite constructs showed increasing DNA and GAG content, with collagen remaining unchanged. Composites containing high collagen levels increased in DNA content, did not increase in GAG, and slightly decreased in collagen with time (Fig. 1B-D), presumably a result of the electrospun collagen leaching from the composite system. Comparisons between groups at day 63 showed that PCL scaffolds had higher DNA and GAG content (p<0.05). By day 63, cross-sections showed that PCL constructs achieved the greatest amount of cell infiltration and matrix deposition, followed by the Low composite constructs. Composites with highest collagen content showed the poorest cell infiltration.

DISCUSSION: Rapid cell colonization is essential for clinical application of nanofibrous scaffolds for regenerative applications. Cellular infiltration occurs rapidly in electrospun biomimetic scaffolds compared to synthetic ones [4]. However, the poor mechanics of these protein-based scaffolds remains problematic. To address this concern, we fabricated composite PCL/collagen type I nanofibrous scaffolds and evaluated their biomechanical properties, cell infiltration capacity, and functional maturation with time in culture. Composite systems retained significant mechanical properties, even at high (20%) levels of collagen fiber inclusion. However, contrary to our hypothesis, the presence of collagen type I fibers at this level did not improve cell infiltration. In fact, the reverse was observed, with the poorest infiltration and matrix deposition observed with the highest concentration of collagen. While the mechanism underlying these findings remains to be determined, it is possible that the increased propensity for cells to adhere to collagen fibers limits migration, or that the genipin crosslinking limits fiber degradation. Both effects would restrict cells to the periphery of the scaffold and so limit their ability to produce distributed matrix. Future studies will elucidate the mechanism by which this limitation occurs in order to optimize biomimetic-biosynthetic composites for rapid construct maturation.


ACKNOWLEDGEMENTS: This work was supported by the NIH (AR056624) and the Penn Center for Musculoskeletal Disorders.