Electronic Supplementary Information:

As a qualitative measure of the surfaces available for neurite outgrowth on aligned fibers, we compared neurite densities from explants on aligned fibers and collagen films. A radially expanding circular grid at 200 μm increments was overlaid on top of mosaic images. The radial sections representative of neurite outgrowth longer than 800 μm were extracted, thresholded to eliminate background noise, and the neurites were traced, and their areas calculated using ImageJ.

There was no significant difference in neurite density (p<0.05) between explants grown on either substrate, indicating that the dense aligned fibers do not obstruct neurite outgrowth (Figure S3A).

Supplementary figures:

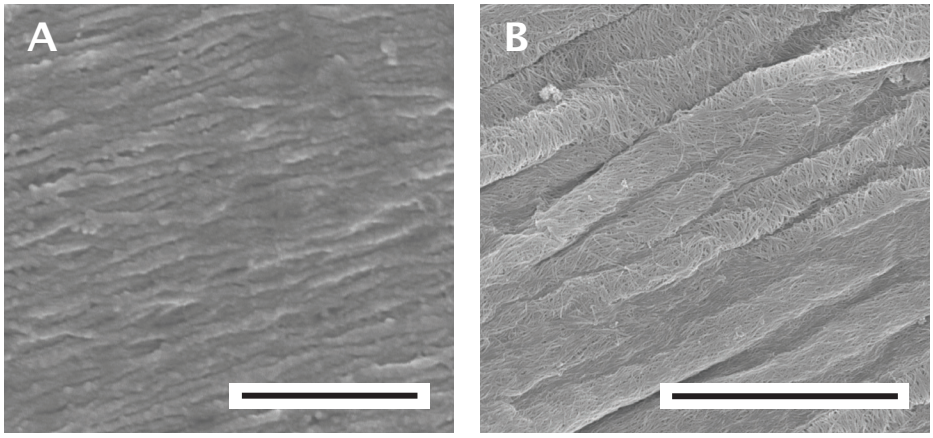

Figure S1. Hierarchical structure of iso-electrically focused fibers without post processing

Representative SEM micrographs showing the orientation and fibrillar arrangement of iso-electrically focused collagen fibers not subjected to further buffer treatments (dried). (A) At high magnification, there is parallel arrangement of the nanofibrils, and alignment with the long axis of the construct. (B) At lower magnification, there is bundling of nanofibrils into larger micron-sized fibrils. Scale bars A= 500 nm, B= 5 μm.

Figure S2

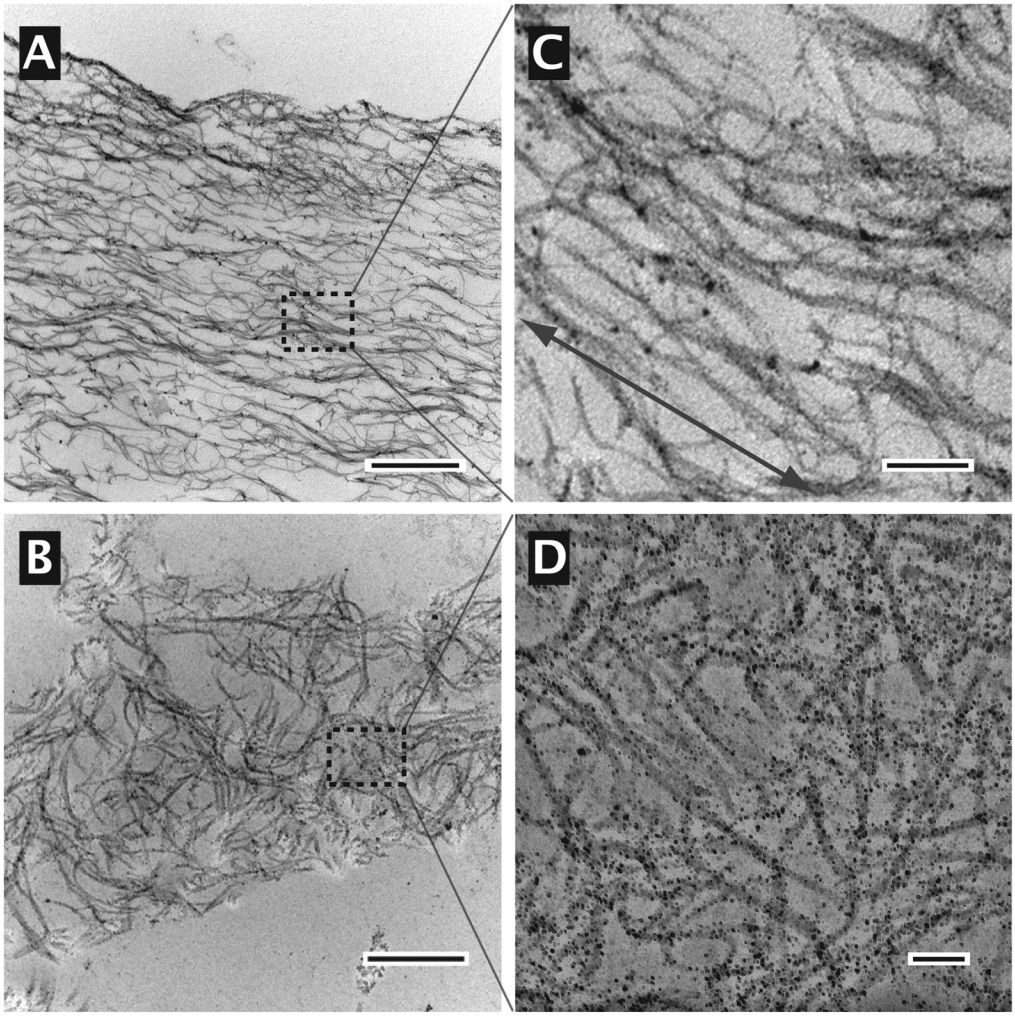

Figure S2. Nanofibrillar structure and morphology of collagen fibrils

(A-D) TEM micrographs of 90 nm horizontal cross-sections of collagen hydrogels (A and C) with and (B and D) without iso-electric focusing. (A) There is a high packing density of collagen fibrils after iso-electric focusing, as well as uniaxial orientation with the long fiber axis indicated by a double arrowed line. (B) In contrast to aligned hydrogels, random hydrogels exhibit a random fibrillar structure and increased spacing between fibrils. (C and D) are magnifications of A and B, respectively. Scale bars (A, B) = 500 nm, (C, D) = 100 nm.

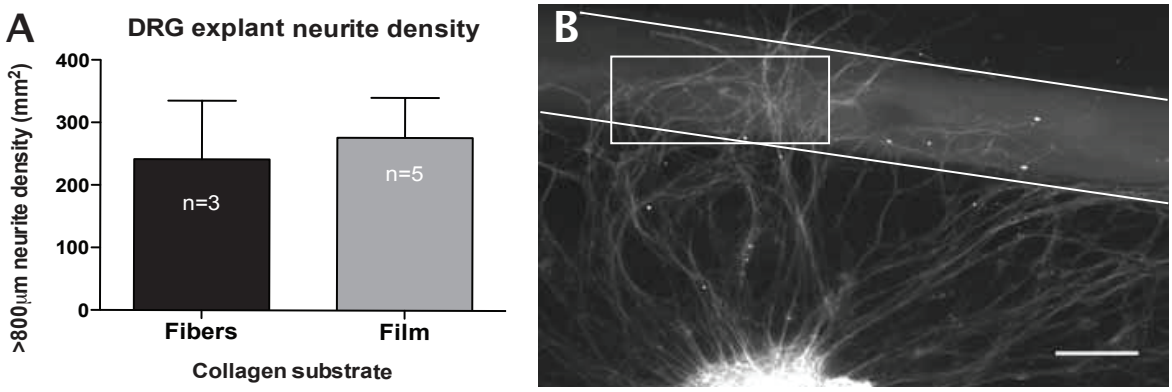

Figure S3. Aligned collagen fibers direct neurite migration

(A) Quantification of neurite outgrowth from embryonic DRG explants (3 days *in vitro*) using a radially expanding circular grid to calculate neurite density in mm^2 of neurites longer than 800 μm. There is no significant difference in neurite density between DRG explants grown on aligned collagen fibers and collagen films. (B) Representative fluorescent image of βIII tubulin stained embryonic DRG explant seeded outside an aligned collagen hydrogel and allowed to grow onto the fiber. The white box indicates a change in neurite trajectory to follow paths of underlying fibers (indicated by white lines). Scale bar for B is 100 μm