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Tissue Engineering the Retinal Ganglion Cell Nerve Fiber Layer

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Abstract

Retinal degenerative diseases, such as glaucoma and macular degeneration, affect millions of people worldwide and ultimately lead to retinal cell death and blindness. Cell transplantation therapies for photoreceptors demonstrate integration and restoration of function, but transplantation into the ganglion cell layer is more complex, requiring guidance of axons from transplanted cells to the optic nerve head in order to reach targets in the brain. Here we create a biodegradable electrospun (ES) scaffold designed to direct the growth of retinal ganglion cell (RGC) axons radially, mimicking axon orientation in the retina. Using this scaffold we observed an increase in RGC survival and no significant change in their electrophysiological properties. When analyzed for alignment, 81% of RGCs were observed to project axons radially along the

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Author Contributions KEK designed and developed the radial scaffold, designed, conducted and analyzed experiments for material, cellular, and transplantation studies, and wrote the paper.

RBM helped design and develop the radial collector and aided in SEM imaging and fiber analysis methods.

PV designed, conducted and analyzed electrophysiology experiments and aided in the writing of those sections of the paper.

JH contributed to the initial conception of the project, developed methods for direct cellular transplantation (Fig. 5A), aided in cellular experiments and aided in the editing of the paper.

ANZ aided in the collection of data for Fig. 1, Fig. 2 and Fig. 4.

DAV aided in the development of a method for quantifying cellular alignment (Fig. 4C) and aided in the collection of data for that figure.

MSU aided in the collection of data for Figs. 1 and 4.

EBL contributed to the initial conception of the scaffold project.

KJM aided in and oversaw the electrophysiology experiments.

FMA contributed to the development of the radial collector and scaffold production.

JLG developed the project, aided in and oversaw all experiments and their analysis. He participated in the writing and editing of this paper.

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scaffold fibers, with no difference in alignment compared to the nerve fiber layer of retinal explants. When transplanted onto retinal explants, RGCs on ES scaffolds followed the radial pattern of the host retinal nerve fibers, whereas RGCs transplanted directly grew axons in a random pattern. Thus, the use of this scaffold as a cell delivery device represents a significant step towards the use of cell transplant therapies for the treatment of glaucoma and other retinal degenerative diseases.

Introduction

The neural retina, like other parts of the mammalian central nervous system (CNS), shows little reparative capacity. Retinal degenerations such as retinitis pigmentosa and macular degeneration in the back of the retina, and glaucoma in the front of the retina, often end with the death of retinal neurons such as rod and cone photoreceptors in the former, or retinal ganglion cells (RGCs) in the latter. In approaches to replace lost cells in the posterior retina, subretinally injected photoreceptors and retinal progenitor cells migrate into the correct lamina of the retina, form local synaptic connections and thereby restore some functionality in animal models.[1, 2] Such an approach for RGC replacement is considerably more difficult, however, given the challenges of local and distal wiring faced by these neurons. Recent steps forward in enhancing RGC migration into the retina after intravitreal cell delivery,[3, 4] sending local dendrites into the inner plexiform layer (IPL),[4] elongating axons into the optic nerve head,[5] and regenerating axons long distances in the injured optic nerve, to the optic chiasm and finally the brain,[6–8] suggest that a transplantation therapy may yet be possible. However, transplanted cells have been unable to direct their axons radially towards the optic disk, perhaps due to the developmental changes in retinal guidance molecules,[9, 10] motivating tissue engineering approaches to mimic retinal neurite patterning. Here we address the radial axon guidance through a newly characterized electrospun scaffold.

Material and Methods

Radial Electrospinning

Poly-D, L-lactic acid (PLA, Purac Biomaterials Inc., PDL20) was dissolved in 1,1,1,3,3,3-hexafluoro isopropanol (HFIP, Chem-Impex International Inc.) to a concentration of 6.6 % (wt/vol). PLA solution was pumped at a continuous feed rate via NE-500 syringe pump (New Era Pump Systems Inc.) and ionized in a 20 gauge blunt tipped needle (Hamilton) by a high voltage power supply (SpellmanHV, 230-30R). A radial collector was constructed with a 1 mm diameter copper wire acting as the central pin and a plastic cup, 1.8 cm diameter, coated around the outside and upper rim with aluminum foil mounted on the central pin, with both the central pin and cup connected to the same ground. Flow rates, voltages and void distances between the needle and the radial collector were varied to create scaffolds with different properties (Figure 1f–g).

SEM Analysis

Scaffolds were characterized for fiber diameter and fiber alignment by scanning electron microscopy (SEM). Samples were sputter coated with gold and imaged at 500× and 5000× magnification under high vacuum using a FEI XL-30 Field Emission Environmental SEM. 15 fiber diameters were measured from 3 regions of interest of 3 scaffolds at each flow rate, voltage and tip-to-collector (evaporation) distance using the measure tool in ImageJ (NIH). Fiber coherency was analyzed on SEM images using the OrientationJ plugin (Biomedical Image Group, Ecole Polytechnique Federale De Lausanne) for ImageJ.[11]

Retinal Ganglion Cell Isolation and Culture

Retinal ganglion cells (RGCs) were purified to >99% by sequential immuno-panning as described previously.[12–14] Briefly, retinas were isolated from early postnatal rat or GFP positive mouse (Jackson Laboratory) litters (postnatal day 2–5), digested using papain and dissociated to single cells using mechanical trituration. After a negative selection to remove macrophages and endothelial cells, RGCs were isolated by Thy1 reactivity. RGCs were cultured at 37°C, 10% CO₂ and 100% humidity in Neurobasal media supplemented with insulin, sodium pyruvate, penicillin/streptomycin, n-acetyl cysteine, triiodo-thyronine, forskolin, Sato, B27 and BDNF and CNTF growth factors at previously published concentrations.[12–14]

PLA scaffolds were cut from radial collectors and sterilized for 20 minutes by UV irradiation. Following sterilization, scaffolds were equilibrated to culture conditions by incubating with laminin (2 µg/ml, Trevigen) in Neurobasal medium (Invitrogen). Control tissue culture plates were coated with poly-D-lysine (PDL, 1mM, Sigma) for 30 minutes, washed and coated with laminin overnight. All samples were seeded with 35,000 RGCs.

Live/Dead Staining

RGCs seeded on PLA scaffolds were incubated with calcein AM (1:500 of 1 mg/ml dissolved in DMSO, Invitrogen) and propidium iodide (1:500 of 1 mg/ml, Invitrogen) in culture media for 30 minutes at 37°C and 10% CO₂. Following incubation, fresh culture medium was added. Samples were imaged using an Axio Observer Z1 inverted fluorescent microscope (Zeiss). Measured values for each culture condition were evaluated for significance using an unpaired student t-test.

Electrophysiology

Whole cell patch clamp recordings were performed on neurons cultured on PLA scaffolds and control tissue culture plates using standard wall borosilicate glass patch pipettes (Warner Instruments) with tip resistances between 4–6 Mohm. The external bath solution contained (in mM) NaCl 140, CaCl₂ 2, MgCl₂ 1, HEPES 5, dextrose 3, and the pipette solution contained (in mM) potassium gluconate 100, CaCl₂ 5, EGTA 10, and HEPES 10. Current clamp recordings were made using an Axopatch 200B amplifier (Molecular Devices), filtered at 10 kHz with a low pass filter (Ithaco), and digitized at 5 kHz (Digidata, Molecular Devices). Pipette resistance was compensated by adjusting pipette offset. After break-in, the cells were held at –60 mV and stimulated with 200 ms current pulses. Traces were analyzed using Clampfit (Molecular Devices). Measured values for each culture condition were evaluated for significance using an unpaired student t-test.

Immunostaining

Samples were fixed for 30 minutes with 4% paraformaldehyde (Electron Microscopy Sciences) diluted in phosphate buffered saline (PBS, pH 7.2). Samples were washed 3× with fresh PBS and then blocked for 30 minutes with 10% goat serum (Invitrogen) with 0.2% triton X-100 (EMD Millipore) to permeabilize the cell membrane. RGCs were stained for βIII tubulin (mAB, 1:500, Covance) overnight at 4°C, washed 3× with PBS and labeled with Alexafluor 488 goat anti-mouse IgG (1:500, Invitrogen). Samples were imaged using a TCS SP5 Inverted Confocal Microscope (Leica) with mosaic images stitched using LAF software (Leica).

Scaffold Cellular Alignment

Imaged scaffolds were divided into 4 sections and all neurites greater than 2 cell bodies in length manually counted for radial alignment. The percentage of aligned neurites in each

section was averaged to produce the cellular alignment for the scaffold. Finally, the cellular alignment for 3 scaffolds, produced on separate days and seeded with RGCs from separate cell isolations were averaged to give radial scaffold cellular alignment, reported with standard error.

Comparison of Scaffold Cellular Alignment to Retinal Explants and 2D Cell Culture

Cellular alignment was calculated on RGCs seeded on scaffolds, control tissue culture plates and retinal explants. Regions of interest (ROI, $n = 6$) were chosen at random, excluding scaffold areas with deformations or holes, and the angle from the center of the scaffold, optic nerve head or center of image for control plates to the center of the ROI was measured. Cellular alignment was calculated using the distribution function of the OrientationJ plugin, which listed the percentage of neurites aligned on each degree from normal. Re-centering the data to the angle measured from the center point, the percent of cells aligned within each 10° from the true radial angle in both directions was summed for each 10° from 0° through 90° . Cellular alignment for RGCs cultured on scaffolds, control plates and retinal explants for each 10° bin was analyzed by one way anova statistical analysis coupled with a post hoc t-test for significance.

Scaffold Transplantation to Retinal Explants

Whole rat retinas were dissected from adult Sprague-Dawley rats, flat mounted on membrane culture inserts and cultured in RGC culture media as described above. 30,000 RGCs isolated from GFP mice were seeded in $50 \mu\text{l}$ either directly onto the explanted retina or onto a laminin-coated radial scaffold. Cells seeded directly on explanted retinas were cultured for 5 days and then fixed as described above. Cells seeded on ES scaffolds were cultured for 3 days and then placed cell-seeded side down onto the flat-mounted retinal explant, with application of $50 \mu\text{l}$ matrigel (BD Biosciences) between. Explants with scaffolds were cultured for an additional 3 days and fixed. Explants with and without scaffolds were immunostained for GFP (1:200, Invitrogen) and $\beta 3$ tubulin (1:500) as described above.

Results

To guide RGC axons, electrospinning was used to create a scaffold from the biodegradable polymer polylactic acid (PLA) using a collector containing a central pole surrounded a ring grounded to the same point (Fig. 1a) to create a 1.2 cm diameter scaffold containing radially oriented fibers with a random fiber orientation over the central pole (Fig 1b–d). [15] Scaffolds produced at varying voltages, feed rates and collecting distances created scaffolds with different mean fiber diameters (Fig 1e) and directional coherencies (Fig 1f). The alignment of the spun fibers increased with the voltage applied. However, scaffolds created at the higher voltage of 20 kV were thinner due to nonspecific grounding during the electrospinning process, which produced too great a pore size to support retinal ganglion cell (RGC) seeding (Fig 1g), while scaffolds produced at the lower voltage of 12 kV included non-uniform, beaded fibers (Fig 1h). Scaffolds produced at 15 kV with a flowrate of 2 ml/hour and a collecting distance of 12 cm yielded the most uniform coherency (Fig 1i) and were used in biological studies.

We next assessed cell viability of RGCs on these PLA scaffolds, which were sterilized and coated with laminin to enhance RGC adhesion during seeding. After 2 days in culture, purified RGCs were analyzed by calcein-AM and propidium iodide staining, to label viable cells and those with disrupted membranes, respectively. When compared to RGCs cultured on polystyrene tissue culture plates coated with poly-D-lysine and laminin (Fig 2a), RGCs seeded on PLA scaffolds (Fig 2b) showed a 50% increased survival (Fig 2c). Thus although

the extracellular matrix ligand is the same in both conditions, RGC survival was higher on the fiber scaffold than in traditional 2-dimensional culture.

Having demonstrated high RGC survival, we next asked whether RGCs cultured on radial PLA scaffolds retained their functional electrophysiological properties. RGCs were analyzed by whole cell patch clamping to study their electrophysiological properties. RGCs were purified from age-matched postnatal litters and seeded on tissue culture plates coated with PDL-laminin and on PLA scaffolds coated with laminin, recording from RGCs with standard rounded morphology and branched neurites (Fig 3a–b). Individual cells from both conditions were patched and their electrical properties assessed by current clamp with increasing pulse amplitudes of 200 msec duration, ranging to suprathreshold (Fig 3c). Various electrophysiological properties including input resistance, capacitance and action potential (AP) threshold potential were measured or calculated. AP characteristics including thresholds, peaks, half-widths and time-to-peak were also compared. Measured values were similar between RGCs cultured in tissue culture plates (n=8) and on PLA radial scaffolds (n=5), with no values being significantly different at the $p < 0.01$ level (Fig 3d). Thus RGCs retain their usual electrophysiological properties on scaffolds, consistent with their normal axon morphology.

With high RGC survival and retention of electrical properties when cultured on the radial PLA scaffold, we next asked whether the radial scaffold could direct axon growth of the seeded RGCs to mimic the extension of RGC axons in the retina, which in rodents extend in radial fashion to the optic nerve head (Fig 4a). Laminin-coated scaffolds cultured with purified RGCs were fixed and stained for neuronal $\beta 3$ tubulin (Fig 4b, 3b). Measuring of axon patterning across whole scaffolds demonstrated $81.1\% \pm 2.8$ of cells following radial alignment (see Methods), similar to that seen in whole retinal explants. The greater axon alignment when compared to the coherence measurement of the scaffold fibers (Fig 1e) was most likely a function of the fibers spun on innermost surface to the collector having a higher degree of alignment than those spun later, combined with the low RGC infiltration into the electrospun scaffolds.[16, 17] While this low infiltration rate is considered a limitation of the scaffold when used in other tissues, RGCs exist in a single cell layer in the retina, allowing this scaffold to mimic that organization. Using the OrientationJ plugin for ImageJ, we compared RGC axon alignment between retinal explants, 2D cell culture plates, and radial scaffolds. RGCs cultured on the radial scaffold were not statistically different from retinal explants at all orientations away from radial, whereas axon growth was statistically different from the random growth seen on tissue culture plates (Fig 4c, $p < 0.005$). Interestingly, axon elongation ended at the high density random PLA fibers found at the scaffold center (Fig 1e), and RGCs seeded on this random fiber zone extended only short processes, less than $110 \mu\text{m}$, or no processes at all (Fig 4d–e), compared to several millimeters for RGCs on the peripheral radial fibers. At higher density, RGC axons fasciculated into axon bundles which still followed radial directionality (Fig 4f). Thus, key properties of RGC axon patterning and organization seen in vivo were retained on the radial scaffold.

Having established that RGCs axons could be oriented on the radial scaffold in culture, we asked if these scaffolds could be used to direct RGC axons to follow the radial pattern of a living retina in a transplant model. RGCs were isolated from early postnatal GFP transgenic mice[18] cultured in vitro on laminin-coated PLA scaffolds for 3 days as above. Then, scaffolds were placed cell side down onto an explanted whole rat retina. A tight scaffold-retinal interface was maintained using the self-aggregating extracellular matrix matrigel. Separately, GFP-positive RGCs without a radial scaffold were seeded on retinal explants. Following culture for 3–5 days, all samples were fixed, stained for GFP and $\beta 3$ -tubulin, and analyzed by confocal imaging for directionality and integration. When seeded directly onto

retinal explants, RGCs did not follow the alignment of the axon bundles of the host retina (Fig 5a). In contrast, RGCs seeded onto radial scaffolds and then transplanted did extend axons along the same radial pattern as the axon bundles of the host retina (Fig 5b). Thus, electrospun scaffolds can deliver properly aligned RGC axons to host retinas.

Discussion

The loss of RGCs caused by retinal degenerative diseases may be addressed by cellular transplantation to replace lost function, however a major challenge for transplanted cells is to direct their axons towards the optic nerve head of the retina. Electrospinning has been used widely technique for creating scaffolds for the tissue engineering of several different tissues including cardiac [19], vascular [20], muscle [21], bone [22], cartilage [23] and neural [24] tissues. Previously, scaffolds capable of aligning axons linearly into tracts have been created for the central and peripheral nervous systems,[25–29] but more complicated axon patterning, such as the radial patterning required for the retina, has not been demonstrated. Using a collector containing a central pole surrounded by a ring grounded to the same point (Fig 1a), the fibers during the spinning process proceeded first to the central grounding pole and were then moved by the normal whipping motion associated with the evaporation of solvent solution radially between the outer grounding ring and the central pole.[15, 30] Interestingly, when RGCs were seeded on these scaffolds, their alignment was greater than the measured alignment of scaffold fibers (Fig 1f). We hypothesize that this discrepancy in alignment is due to fibers on the innermost portion of the scaffold being more radial than fibers in the middle and outer portions of the scaffold. This decrease in the radial alignment of fibers would be caused by fibers which do not return to the central pole during spinning due to the increased fiber buildup and decreased grounding potential when compared to the lightly covered areas of the surrounding collecting ring. Because the RGCs are seeded on the innermost portion of the scaffold, this increases the probability that their axons follow these more radial fibers.

RGCs must maintain their phenotype and function when cultured. On these ES scaffolds, RGCs demonstrated increased survival compared to those on control substrates. Survival of these and other CNS neurons requires multiple molecular signals including peptide trophic factors, small molecules, and substrate interactions[31]; the possibility that specific scaffold fiber topology enhances viability has also been considered.[32] RGCs seeded on these scaffolds also maintained their electrophysiological properties, capable of firing multiple action potentials in response to current injection. This electrophysiological activity suggests that the scaffolded neurons will be capable of relaying signals once they form synaptic connections to targets in the IPL, although this remains to be tested.

When RGC-seeded scaffolds were compared to explanted rat retinas, RGC axons on scaffolds followed a radial alignment similar to the axons in retinal explants, significantly more aligned than the random directions of RGC axons on control, two-dimensional cell culture substrates. This axon alignment was more evident when RGCs were transplanted to retinal explants either through direct injection or via the ES scaffold. On explants which received a direct transplant of GFP-positive RGCs, transplanted RGC axons did not follow the host RGC axon tracts. In contrast, RGCs on scaffolds aligned with axon bundles of host RGCs after transplant. Although the transplanted axons remained attached to the ES scaffolds, the cell bodies of the transplanted RGCs rested directly on the inner limiting membrane of the retina. This may facilitate dendritic ingrowth towards their synaptic partners in the inner plexiform layer, and will be important to examine in future studies. In addition to their radial alignment, RGC axons also fasciculated into bundles when approaching the center of the scaffold and achieving higher density. The process of fasciculation and formation of axon bundles in vivo aids in organization and axon guidance.

[33] In addition, on the scaffold, fasciculation may also provide mechanical support for neurites, preventing damage that could occur during transplantation.

This ES process also formed a random fiber region over the central pole which was observed to stop axon growth of RGCs seeded on the scaffolds. The ability of the scaffolds to stop axon growth at a specific point allows for the alignment of scaffold over optic nerve head where RGC axons in vivo turn to reach targets in the brain. We have observed that RGC neurites can extend away from the scaffold fibers through matrigel, however it is still unknown whether axons would then enter the optic nerve head once implanted following the natural guidance seen in vivo. The optic nerve secretes guidance factors, such as netrin-1, which guide axons to the optic nerve during development and which may aid in this guidance to the optic nerve from the scaffold.[34, 35]

One area where the scaffold does not match the organization of the human retina is in the area of the fovea. In mammals such as rodents, RGC axons project radially directly to the optic nerve (fig 4a), however in humans RGC axons from the temporal retina curve around the fovea as they are guided to the optic nerve. It may be possible to create this effect in future scaffold designs.

While we have shown ex vivo transplantation of scaffolds here, there are several areas which still must be developed before this technique for cell delivery can be translated in vivo, including surgical delivery of the scaffold and attachment of the scaffold on the surface of the retina. Ensuring characteristics such as appropriate elasticity may allow for use in a catheter-like implantation system, similar to that currently used for the insertion of foldable intraocular lenses during cataract surgery[36] and to cell-seeded scaffolds delivered to the subretinal space[37]. The physical attachment of these scaffolds may also require optimization. In these experiments, we used the self-aggregating hydrogel matrigel which transitions from a liquid to a solid at physiological temperature.[38] However, the laminin content found in matrigel may prevent dendritic projections through the hydrogel, as laminin is predominantly found in the ganglion cell layer of the retina and not in the inner plexiform layer into which RGC dendrites project. It will also be important to study the ability of different scaffold constructions to transmit light without significant light scattering, a critical consideration for retinal tissue engineering [39]

Conclusions

We have created a biodegradable electrospun scaffold that mimics the radial axon paths of the nerve fiber layer of the rodent retina. Moreover, our scaffolds increased RGC survival over that of dissociated cultured cells, while allowing the RGCs to retain their electrophysiological properties. Combining these advantages, the scaffolds studied here allowed the majority of RGCs to extend processes along the fibers radially, fasciculating at higher density towards the center, and then stopping at the center point of the scaffold corresponding to the optic nerve head. Finally, by using this scaffold as a cell delivery device, the scaffold was able to orient the axon growth of RGCs to align with axon bundles in the host retina. Together these data suggest that this scaffold may be an important step in RGC transplantation for glaucoma or other optic neuropathies. Combining such tissue engineering with biological approaches that increase axon regeneration through the optic nerve and treatments to facilitate dendritic integration may ultimately address both growth and guidance of the transplanted cells to recreate normal long-distance patterning in the CNS.

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Figure 1.

Production and optimization of the radial electrospun scaffold. A) Diagram of a 1.8 cm diameter radial collector containing a conducting central pole and rim which are grounded to the same source. B) Top view of an electrospun radial scaffold. C and D) SEM image of peripheral radial fiber zone (C) and central random fiber zone (D). E and F) Measured diameter (E) and coherency of alignment (F) of electrospun PLA fibers under different spinning condition including voltages, tip to collector distances and polymer feed flow rate (mean $\pm$ standard error of mean). G) Scaffolds produced at higher voltages contained large pores (*) which were too large to support cells during seeding. H) Scaffolds produce at lower voltages formed non-uniform beaded fibers (arrows). I) Scaffolds produced at 15 kV created densely packed aligned uniform fibers and were used in the subsequent experiments. Scale bars 1B- 5mm; 1C- 50 μ m; 1D- 100 μ m; 1G-I- 10 μ m.

Figure 2. Retinal ganglion cell survival was quantified for cells seeded on control plates (A) and on radial electrospun scaffold (B) using calcein AM and propidium iodide after 3 days in vitro. C) 3 scaffolds and culture plates analyzed for 3 independent experiments demonstrated a significant increase in survival for RGCs seeded on electrospun scaffolds ($p < 0.005$). Scale bars 2A–B 100 μ m.

Figure 3.

Retinal ganglion cells retained their electrophysiological properties when cultured on electrospun scaffolds. A and B) RGCs demonstrated similar morphology whether cultured for 3 days in vitro on control plates (A) and electrospun scaffolds (B) in preparation for electrophysiological recordings. C) Action potentials were elicited by whole cell current clamp from RGCs plated on tissue culture plates (upper trace) and on PLA scaffolds (lower trace). The cells were stimulated with 85 pA current for 200 ms and fired multiple action potentials in response to current injection, demonstrating electrical excitability. D) Electrophysiological properties including input resistance and action potential shape characteristics were assessed using traces from whole cell current clamp experiments. RGCs plated on tissue culture plates and radial electrospun scaffolds exhibited no significant differences in the electrical properties studied. Scale bar 100 μ m.

Figure 4.

Retinal ganglion cells cultured on radial electrospun scaffolds mimic the axonal orientation of the nerve fiber layer of the native retina. A) Rat retinal explant nerve fiber layer labeled with neuronal β 3-tubulin showing the radial direction of axons extending to the optic nerve head. B) RGCs cultured on radial scaffolds and stained for β 3-tubulin exhibited $81.1\% \pm 2.8$ alignment of neurites in radial orientation. C) Nerve fiber layers of rat retinal explants, RGCs cultured on control tissue culture plates and on radial electrospun scaffolds were analyzed for orientation. Neurites were binned for every 10 degrees from radial (mean $\pm$ standard error of mean). Neurite growth on radial scaffolds was significantly more radially aligned when compared to RGCs cultured on control plates for 7 of 9 directional bins ($p < 0.005$). No significant difference was observed for RGCs cultured on radial scaffolds when compared to retinal explants. D and E) Neurites stopped at the random fiber area formed at the central collecting pole (D), with cells seeded on this region failing to extend or only extending short neurites (E). F) In areas of high density growth, neurites fasciculated into bundles, mimicking axon organization seen in vivo. Scale bars 4A: 500 μ m; 4B: 1 mm; 4D: 1 mm; 4E: 100 μ m; 4F: 500 μ m.

Figure 5.

Tissue engineered scaffolds enhance RGC axon orientation on adult rat retinal explants. A) GFP-positive RGC neurites (green) did not align radially to host axon bundles (red) when transplanted directly onto adult rat retinal explants. B) GFP-positive RGC neurites followed the radial patterning of existing axon tracks when transplanted to host retina on an electrospun scaffold. C) Optical cross section of scaffold transplants to retinal explant showing tight alignment of transplanted cells' axons to host retinal axons. (GFP green, $\beta 3$ tubulin red) Scale bar 5A–B: 300 μm ; 5C: 275 μm .