

Laminin-coated electrodes improve cochlear implant function and post-insertion neuronal survival

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Abstract—The benefits of Cochlear implant (CI) technology depend among other factors on the proximity of the electrode array to the spiral ganglion neurons. Laminin, a component of the extracellular matrix, regulates Schwann cell proliferation and survival as well as reorganization of actin fibers within their cytoskeleton, which is necessary for myelination of peripheral axons. In this study we explore the effectiveness of laminin-coated electrodes in promoting neuritic outgrowth from auditory neurons towards the electrode array and the ability to reduce acoustic and electric auditory brainstem response (i.e. aABR and eABR) thresholds. *In vitro*: Schwann cells and neurites are attracted towards laminin-coated surfaces with longer neuritic processes in laminin-coated dishes compared to uncoated dishes. *In vivo*: Animals implanted with laminin-coated electrodes experience significant decreases in eABR and aABR thresholds at selected frequencies compared to the results from the uncoated electrodes group. At 1 month post implantation there were a greater number of spiral ganglion neurons and neuritic processes projecting into the scala tympani of animals implanted with laminin-coated electrodes compared to animals with uncoated electrodes. These data suggest that Schwann cells are attracted towards laminin-coated electrodes and promote neuritic outgrowth/guidance and promote the survival of spiral ganglion neurons following electrode insertion trauma. © 2019 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: spiral ganglion neurons, Schwann cells, laminin, neuritic outgrowth/guidance, cochlear implants.

INTRODUCTION

Cochlear implants are surgically implantable devices that can restore hearing by bypassing deteriorated auditory hair cells (HC) and delivering electrical signals directly to the auditory nerve. The benefits of cochlear implant (CI) technology depend on the number of excitable spiral ganglion neurons (SGN) remaining and the distance between the electrode and these neurons, located in the modiolus (Wilson and Dorman, 2008). A close contact between the electrode array and neurons is ideal in order to lower the threshold required for excitation and prevent spacial current spread that results in impaired frequency resolution (Frick et al., 2017). Among new designs, perimodiolar electrodes aim to bring the

electrode closer to the neuronal target allowing for a decrease in signal spread and power consumption. However, the perimodiolar electrode is not suitable for all patients due to variations in cochlear anatomy and in addition it may flex during the insertion process causing unwanted mechanical trauma. The scientific community is in search of a technology that brings the electrode contacts into a closer relationship with the SGNs. Engineers focus on designing electrodes with different stiffnesses, lengths, and diameters for a better reach to their neuronal targets while biologists are working towards developing coatings and scaffolds that may help direct SGN neuritic projections to reach the electrode contacts (Cai et al., 2016). The use of chronic electrical stimulation and/or growth factors and neurotrophins seem beneficial for SGN survival (Ylikoski et al., 1998; Roehm and Hansen, 2005; Miller et al., 2007; Pettingill et al., 2007; Budenz et al., 2012; Leake et al., 2013; Budenz et al., 2015; Schwieger et al., 2015), however unspecific instillation of neurotrophins into the scala tympani may result in neurite sprouting and regrowth along the array making indiscriminate contact with electrodes (Leake et al., 2013). Although the instability of neurotrophins may be bypassed by the use of viral vectors (Staecker and Garnham, 2010) the risk of tumorigenesis remains a limitation for their use in the clinical practice

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Abbreviations: aABR, acoustic auditory brainstem response; eABR, electric auditory brainstem response; CAP, cochlear action potentials; CI, cochlear Implant; DAPI, 4',6-diamidino-2-phenylindole; Dexa, dexamethasone; DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; EIT, electrode insertion trauma; EDTA, ethylenediaminetetraacetic acid; HC, hair cell; OC, organ of Corti; PBS, phosphate buffered saline; PNS, peripheral nervous system; SC, Schwann cell; SGN, spiral ganglion neuron; TCD, tissue culture dish.

(Fransson et al., 2015). Growth cone environments can be created by chemical gradients (Anderson et al., 2006; Ceschi et al., 2014; Hadler et al., 2016; Li et al., 2017), topographic features (Clarke et al., 2011; Reich et al., 2012; Tuft et al., 2013; Mattotti et al., 2015), adhesive patterns and altering the stiffness of the matrix (Arreaga-Salas et al., 2015). Laminin, a component of the extracellular matrix, is known to regulate Schwann cells (SC) proliferation and survival as well as reorganization of actin fibers in their cytoskeleton, which is necessary for myelination of peripheral axons (Akassoglou et al., 2002; Previtali et al., 2003; Chernousov et al., 2008; Yu et al., 2009; Kwiatkowska et al., 2016). Schwann cells are the glial cells of the peripheral nervous system (PNS). During development of the PNS, SC differentiate into highly specialized myelinating and nonmyelinating cells (Kim et al., 2013). The mechanical trauma associated with the insertion of the cochlear electrode triggers a strong neuroinflammatory and proliferative response at the site of injury (Bas et al., 2015). Schwann cells rapidly produce pro-inflammatory cytokines, chemokines and cell adhesion molecules that recruit and activate other inflammatory cells to the cochlea. Schwann cells are able to dedifferentiate into a specialized type of cell with proliferative and repairing functions, and are different from those found in the developing nerve (Kim et al., 2013).

Laminin-coated CI electrodes may provide a trophic environment and a scaffold for these unique SC that support SGN adhesion, survival and neurite growth towards the electrode array.

EXPERIMENTAL PROCEDURES

Animals

For the *in vitro* section, 16 Sprague Dawley rat pups (four ears/group, the experiment was replicated once) 3 or 4 days old (P3–P4) were used (Charles River Laboratories, Wilmington, MA, USA). For the *in vivo* animal model of electrode insertion trauma (EIT)-induced hearing loss 22 male Brown rats (Charles River, MA) of 3 months of age were used, 10 rats were used for a histology study at 10 days post-CI and 12 rats were used for electrophysiology and histology studies at 4 weeks post-CI. The animals were fed a standard pellet diet and kept on a 12 h light/dark cycle in the Division of Veterinary Resources of University of Miami and were fed sterilized standard diet and water *ad libitum*. The animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC, protocol# 17-223) of the University of Miami and was in full compliance with published National Institute of Health (NIH) guidelines for the care and use of laboratory animals (Guide for the Care and Use of Laboratory Animals, 8th Edition, The National Academies Press, Washington, DC, 2011).

In vitro neuritic growth and guidance study

P3–P4 rat pups were anesthetized with ice for 30 min and then decapitated. The otic capsules were dissected using a surgical microscope and placed in cold and sterile phosphate

saline buffer (PBS). Four organs of Corti, including SGNs, were randomly assigned to each experimental group: 1) Non-treated plastic surface; 2) Tissue culture dish (TCD), 3) Laminin (1 mg/ml, Gibco-Thermo Fisher Scientific, MA, USA) micro-patterned TCD (TCD + Laminin), 4) Laminin micro-patterned TCD + Dexamethasone (2 µg/ml) (TCD + Laminin + Dexa). The explants were cultured for 4 days at 37 °C in serum-free culture media consisting of Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with glucose (final concentration at 6 g/l), 1% of N-1 supplement (Sigma Aldrich, St. Louis, MO, USA), penicillin G (30 U/ml) and either saline or Dexamethasone (2 µg/ml, D1756, Sigma Aldrich). Following the incubation period, the explants were fixed and stained for tubulin β3 (neurons), S100 (Schwann cells) and DAPI (nuclei). The length of the neurite outgrowth was calculated using the Simple neurite tracer plugin (Longair et al., 2011) for ImageJ. The experiment was independently replicated.

In vivo CI-electrode insertion

Ten naïve rats (5 rats/group) were used for histology-only studies at 10 days post-implantation with electrode analogs. Twelve naïve rats (6 rats/group) were used for electrophysiology and histology studies with an end-time point of 4 weeks post-implantation. For the cochlear implantation procedure, rats were anesthetized with Isoflurane (0–5%). Ketorolac (5 mg/kg) was used for analgesia and it was administrated immediately prior to surgery and at 12 h post-surgery. The eye blink and toe pinch withdrawal responses were used to assure a surgical plane of anesthesia. Artificial tears ointment was used to prevent dry eye. The skin behind the ear was shaved and cleaned with iodine and local anesthesia was achieved with 1% lidocaine subcutaneous injection. A post-auricular incision was made and the bulla tympanum was exposed. A small bullostomy was performed and the bony wall of the cochlea was visualized. For the histology-only study a 0.2-mm diameter nylon fishing line (Cajun Line; W.C. Bradley Co., OK, USA) was used as electrode analog to simulate EIT. For the electrophysiology and histology study, sterile silicone electrode array (provided by MEDEL and originally designed for guinea pigs but suitable for use in adult rats, Bas et al., 2016) that either contained no additive or was pre-coated with laminin (1 mg/ml) was inserted through the round window into the scala tympani of the cochlea to a depth of approximately 2 mm. Once the placement and stability of the electrode array were established, the defect in the ventro-lateral wall of the bulla was sealed with tissue glue. A burr hole was made at 1 mm anterior to the lambdoid suture and in close proximity to the inferior colliculus and a stainless steel screw was epidurally implanted in order to record acoustic ABRs (aABRs), cochlear action potentials (CAPs), electric ABRs (eABR) and impedances from the electrodes, a connector was secured to the skull with three additional screws and dental cement. The post-auricular incision was closed with nylon sutures and cleaned with iodine. The animals were returned to a clean cage and allowed to recover from the anesthesia. For both the histology-only and electrophysiology and histology studies

the experimental groups were: 1) Silicone-No Laminin (CI) and 2) silicone-Laminin pre-coated (CI-Laminin). In the electrophysiology and histology study initially six animals/ condition were used ($N = 6$), however two animals from the CI-Laminin group were lost. One animal died during the anesthesia and one animal was withdrawn from the experiment due to an impaired electrode with an out of range impedance. The number of animals at the end of the experiment was $N = 6$ for the CI group and $N = 4$ for the CI-Laminin group. Right ears were selected as experimental ears with contralateral ears used as un-implanted controls.

Auditory function studies

A commercial system (Intelligent Hearing Systems (IHS), FL, USA) was used for the electrophysiological hearing assessments that consisted in: 1) aABRs recorded from subdermal scalp needle electrodes for the pre-trauma hearing threshold baselines; 2) high amplitude aABRs recorded via the stainless steel screw placed in contact with the dura; 3) CAPs, 4) impedance resistance and 5) eABR were recorded via the electrode array (for detailed information of the aABR and CAP settings see [Bas et al., 2016](#)). Acoustic stimuli of 1, 4, 8, 16 and 32 kHz were used to assess cochlear function (aABR and CAP). For the eABRs an IHS Low-current electrical stimulator was used with the settings recommended by the manufacturer with a stimulus of 75 μ s biphasic (25 μ s positive, 25 μ s neutral and 25 μ s negative), a rate of 19.3/s, a limit of max intensity of 700 μ A, and filters 30–3000 Hz, and the electrode array was used as transducer.

Histology studies

Rats were euthanized with CO₂ inhalation followed by decapitation and collection of their cochleae at 10 days and 4 weeks post-CI implantation. All cochleae were fixed by immersion in freshly prepared 4% paraformaldehyde and kept at 4 °C for 2 days. Subsequently, they were decalcified in EDTA 10% in PBS (pH 6) with gentle rotation for 1 week at room temperature, and then rinsed three times in PBS. The cochleae were then placed in sucrose gradient ranging from 5% to 30% and embedded in OCT compound media (Tissue-Tek, Sakura Finetek USA, Inc., CA, USA) and allowed to freeze at -20 °C. Cryo-sections were cut (15–20 μ m) parallel to the central plane of the cochlea's modiolus and mounted on plus slides.

Immunostaining for Schwann cells and auditory neurons

Slides from contralateral unimplanted and electrode array implanted cochleae were washed in PBS and kept in a blocking-permeabilizing media (normal serum, 1% Triton x-100 in PBS) for 1 h. After this time, samples were incubated at 4 °C overnight with the following primary antibodies: rabbit anti-S100 (ab868, Abcam, MA, USA) and mouse anti-Tubulin β3 (801,201, BioLegend, CA, USA) or mouse anti-p75^{NGFR} (clone 192, [Andersen et al., 2016](#)). The slides were washed three times with PBS and incubated for 90 min at room temperature with the secondary antibodies anti-mouse IgG Alexa 488 and anti-rabbit IgG Alexa 555 (Invitrogen,

CA, USA). After three more washes the specimens were stained with DAPI (Sigma Aldrich, MO, USA), washed and cover-slipped with anti-fade mounting media. Three consecutive slides/ cochlea containing an average of four sections/ slide were observed under a Zeiss LSM 700/ confocal upright microscope. ImageJ was used to analyze the images.

Statistics

One-way ANOVA followed by Tukey's Multiple Comparison test was utilized for the axonal length *in vitro* study and SGN body counts. Two-way ANOVA followed by Bonferroni test was used to analyze aABRs, eABRs, CAPs and impedances and to compare the number of cells positive for p75, S100 and Tubulin β3 in the CI and CI-Laminin adult cochleae histology sections (*in vivo* studies). Two-way ANOVA results for the electrophysiology tests are expressed in the text as F(df, residual). Counts from 8 to 10 sections/ cochlea were averaged and the means were compared between groups. In all graphs the results are expressed as mean values ±S.D. For correlation analyses Pearson's test was used.

RESULTS

In vitro neurite growth and guidance study

Longer SGN neurites were observed in the organ of Corti (OC) explants cultured in laminin micropattern coated surfaces (426 μ m) compared to uncoated tissue culture dish surfaces (165 μ m, $p < 0.001$, [Fig. 1 A and B](#)).

The length of the neurites was not affected by the type of plastic used (114 μ m vs 165 μ m, $p > 0.05$). Dexamethasone was included in the *in vitro* part of this study because of its beneficial use in cochlear implantation to prevent inflammation and preserve residual hearing. The presence of this corticosteroid in the culture media did not affect the neurite outgrowth from the OC explants (TCD + laminin 426 μ m vs TCD + laminin + Dexa 493 μ m, $p > 0.05$).

Auditory function studies

Animals included in the 4-week study and implanted with laminin coated electrodes experienced a significant decrease in acoustic ABR thresholds at selected frequencies, 8 kHz F(1,32) = 8.109, $p = 0.0076$; 16 kHz F(1,32) = 7.409, $p = 0.0104$; 32 kHz F(1,32) = 9.933, $p < 0.0035$ (see [Fig. 2](#)). Time did not affect significantly the aABR results ($p > 0.05$, pre-CI values were excluded to analyze the effect of time in the results). For data visualization purposes and without affecting statistics, electric ABR thresholds are represented as shift values where eABR value at time zero (post-CI) is subtracted from each absolute value. Over time both groups experienced an increase in eABR threshold shifts (the effect of time was significant F(4,40) = 61.40, $p < 0.0001$) however eABR values in the laminin-coated electrodes group remained lower than in those from animals with uncoated electrodes (F(1,40) = 14.10, $p = 0.0006$, see [Fig. 3](#)). CAPs were not significantly affected (data not shown). Over time, a significant increase in impedances was observed in the laminin-coated electrodes compared to the uncoated electrodes.

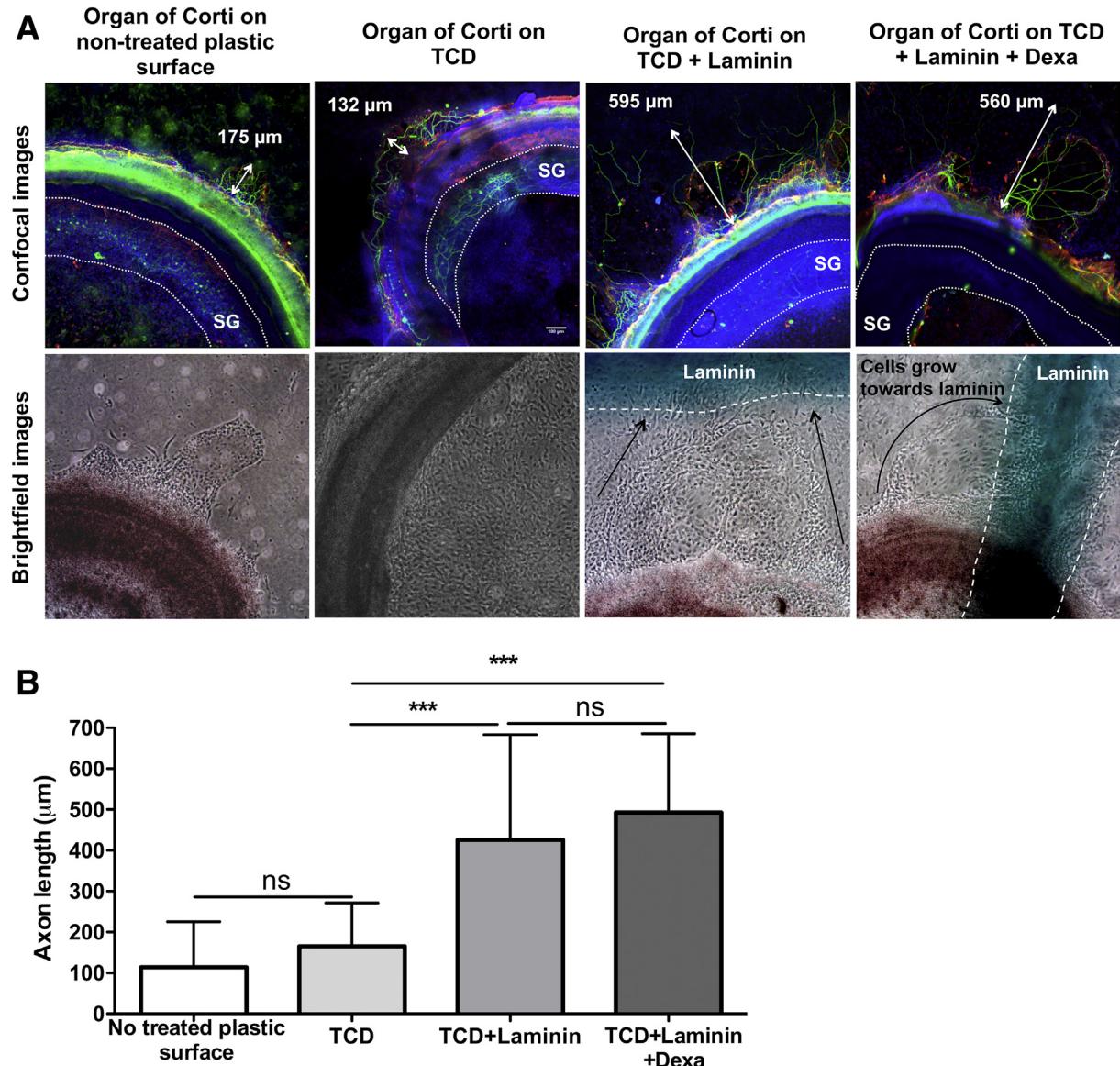


Fig. 1. (A) Top row: immunofluorescence confocal imaging of OC whole mounts cultured under different conditions, from left to right: non-treated (i.e. uncharged surface) plastic dish, positive charged surface-tissue culture dish (TCD), laminin-micropatterned TCD (laminin trays show in blue and are delineated by white dashes) and laminin-micropatterned TCD with dexamethasone in media (2 µg/ml). OC was stained for tubulin β3 (neurons, green), S100 (non-specific Schwann cell marker, red) and DAPI (nuclei, blue). The bottom row shows bright field microscopic images. Note different orientation of the specimens between confocal and bright field images to include the laminin micro-strip and the organ of Corti in the same image. Migration of cell bodies towards the laminin micro-strip (black arrows) was noticed in the TCD+ Laminin group. (B) Analysis of total neurite length for the four different culture conditions (mean ± SD). N = 4 organ of Corti explants/ group; the experiment was independently replicated.

Laminin coating affected the impedances results significantly ($F(1,40) = 19.69$, $p < 0.0001$) and the effect of time was significant $F(4,40) = 2.930$, $p = 0.032$; see Fig. 4). Due to a low size sample, post-hoc Bonferroni test did not show statistically significant differences when comparing aABRs, eABRs and impedances between groups at specific time points.

Immunostaining for Schwann cells and auditory neurons

At 10 days post-Cl, laminin-coated analog electrodes attracted Schwann cells and neurons towards the electrode

analog. An increase in cells positive for S100, a marker for SC ($p < 0.01$); p75, a marker for dedifferentiated SC ($p < 0.001$); and Tubulin β3, a neuronal marker ($p < 0.05$) was observed in the tissue ensheathing the laminin-coated electrodes (see Figs. 5, 6 and 7). Schwann cells align in Bunger tracks in a radial fashion to provide a scaffold for neurites to grow around the electrode; these guidance structures were identified only in the Cl-Laminin group (Figs. 5G, H, I and 6G, H, I white arrows). Additionally, SC and neurites appeared to project into the scala tympani through the osseous spiral lamina (see Figs. 5F, I and 6F, I, white arrowheads).

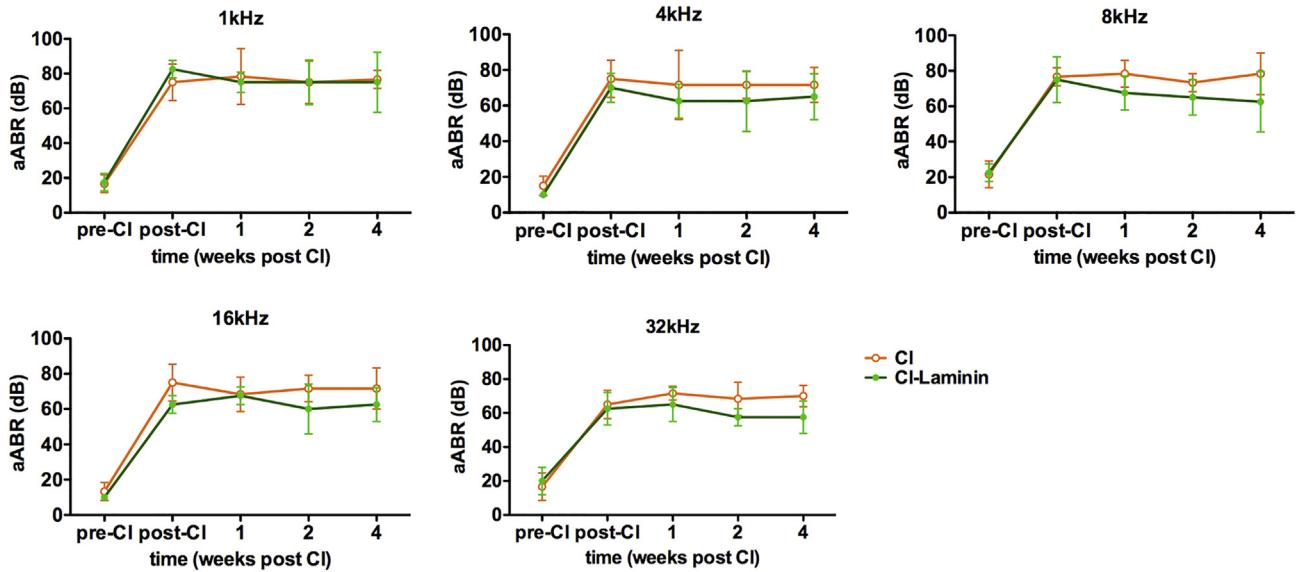


Fig. 2. Acoustic ABR (aABR) thresholds at 1, 4, 8, 16 and 32 kHz over time for animals implanted with uncoated electrodes (CI) and laminin-coated electrodes (CI-Laminin). CI: N = 6 animals, CI-Laminin: N = 4 animals. Mean \pm SD, paired t-test (aABR thresholds were compared at each time point), two tailed.

Cochleae from the CI uncoated group experienced a decrease in spiral ganglion neurons compared to the CI-Laminin group at 10 days and 4 weeks post-implantation (see Figs. 5D and 8D, asterisks). The number of SGN cell bodies at 4 weeks post-implantation was significantly larger in the CI-Laminin implanted ears compared to the CI implanted ears ($p < 0.05$) and comparable to numbers of SGN observed in the contralateral cochleae ($p > 0.05$) (see Fig. 9). Additionally, CI-Laminin cochleae at 4 weeks showed neurite processes projecting into the scala tympani (see Fig. 8G, I and I', white arrows).

For the correlation analysis all the electrophysiology results were transformed to relative or shift values. The analysis shows a negative correlation between SGN counts and eABR thresholds shifts on week 4 ($r = -0.728$, $p = 0.017$), meaning that a decrease in the SGN population correlated with an increase in the eABR threshold shift (see Fig. 10A). Note that correlation but not causation was demonstrated. No correlation was established between SGN counts and the aABR threshold shift at 32 kHz on week 4 ($r = -0.198$,

$p = 0.583$) and between impedance and either eABRs or aABRs threshold shifts at 32 kHz on week 4 (see Fig. 10B).

DISCUSSION

Mechanical trauma associated with the insertion of a CI electrode is known to trigger a neuro-inflammatory response and fibrotic tissue deposition around the electrode array, initially triggered by an attempt from the body to protect, restore and heal damage to the affected organ (Bas et al., 2012; Seyyedi and Nadol, 2014; Bas et al., 2015; Zhang et al., 2015; Bas et al., 2016; Wilk et al., 2016; Ishai et al., 2017). Nevertheless, the presence of a foreign body leads to a process of chronic tissue remodeling which results in scarring. Fibrous tissue growth, electrode placement and the integrity of SGNs are factors that can affect the electrode–neuron interface and therefore interfere with the activation of the auditory nerve by the CI (Pfingst et al., 2015a,b). Cochlear implants are designed to electrically stimulate different subgroups of SGN along the cochlear partition resulting in a tonotopic pattern of stimulation (Wilson and Dorman, 2008; Boulet et al., 2016). Ideally, the electrode array should be placed in close proximity to the modiolus of the cochlea to

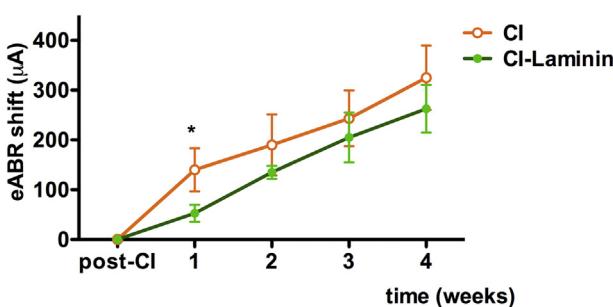


Fig. 3. Electric ABR (eABR) thresholds shift at day 1 (post-Cl surgery) and 1, 2 and 4 weeks post-implantation for animals implanted with uncoated electrodes (Cl) and laminin-coated electrodes (Cl-Laminin). Cl: N = 6 animals, Cl-Laminin: N = 4 animals. Mean \pm SD, paired t-test, two tailed.

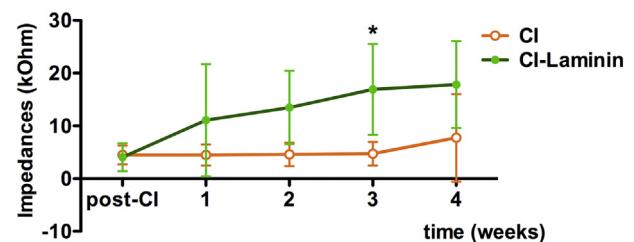


Fig. 4. Electrode impedances at day 1 (post-Cl surgery) and 1, 2 and 4 weeks post-implantation for animals implanted with uncoated electrodes (Cl) and laminin-coated electrodes (Cl-Laminin). Cl: N = 6 animals, Cl-Laminin: N = 4 animals. Mean \pm SD, paired t-test, two tailed.

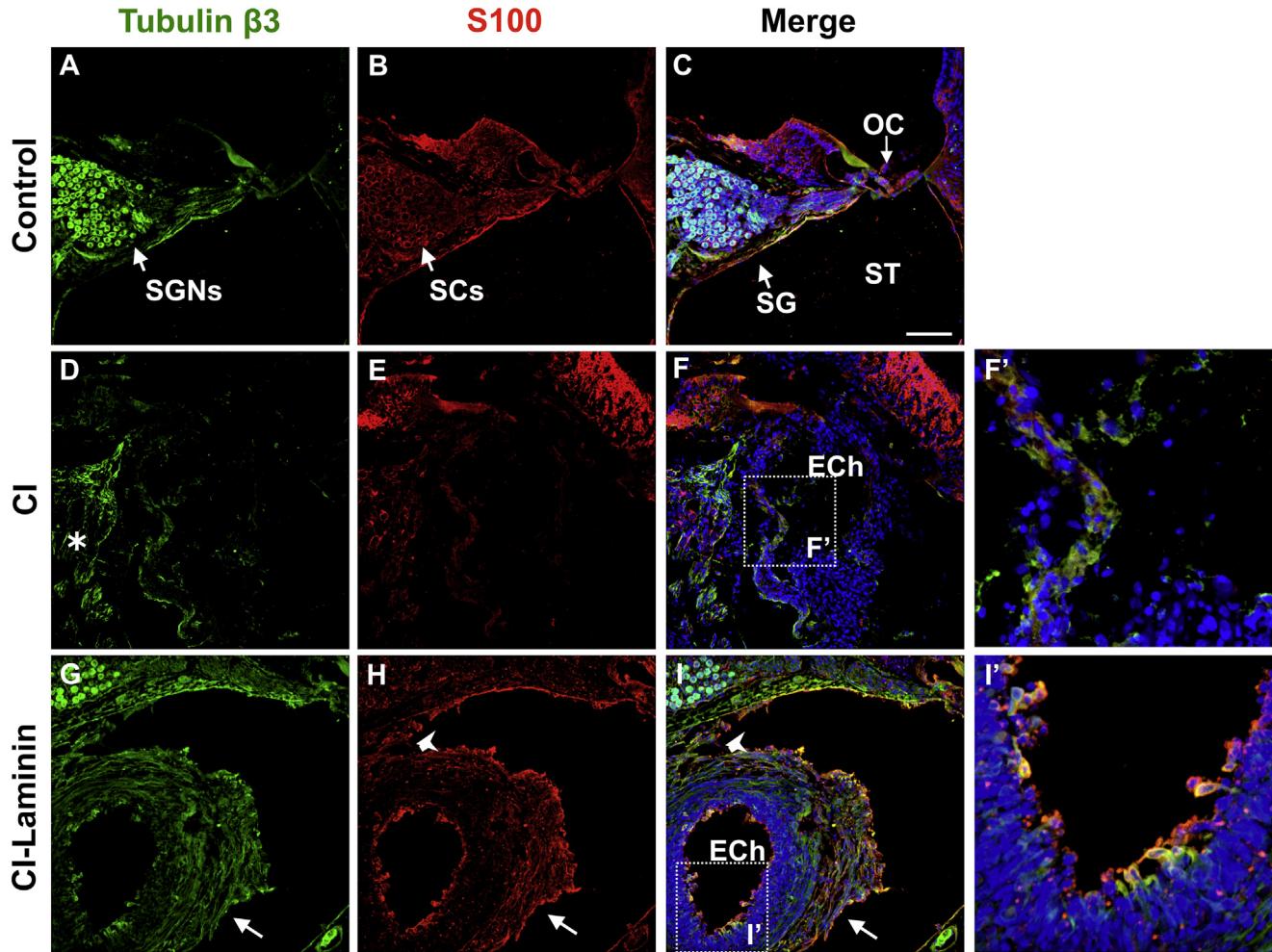


Fig. 5. Immunofluorescence confocal imaging of cochlear cross-sections at 10 days post-implantation. Representative micrographs correspond to the basal turn of contralateral cochleae (A–C), and implanted cochleae with either uncoated electrode analog (CI, D–F, F' magnification of F) or laminin-coated electrode analog (CI-Laminin, G–I, I' magnification of I) at 10 days post-implantation. Asterisk marks a less dense population of SGNs in CI group (Fig. 5D). Bungner tracks were identified only in the CI-Laminin group of animals (Fig. 5G, H, I white arrows). Schwann cells and neurites were identified projecting into the scala tympani through the osseous spiral lamina (Fig. 5H, I white arrowhead). Tubulin β 3 (neurons, green), S100 (non-specific Schwann cell marker, red) and DAPI (nuclei, blue). SGNs, Spiral ganglion neurons; SCs, Schwann cells; OC, Organ of Corti; SG, Spiral ganglion; ST, Scala tympani; ECh, Electrode channel.

achieve more specific stimulation of neuronal targets; however, close proximity positioning can be compromised by the presence of fibrous tissue growth, which creates a physical gap. A different approach to improve the electrode–neuron interface is to promote the growth of neurites towards the electrode (Pettingill et al., 2007; Wilson and Dorman, 2008; Leake et al., 2013; Tuft et al., 2013; Hadler et al., 2016; Frick et al., 2017). While our study cannot establish a correlation between impedances (as result of fibrous tissue deposition around the electrode) and aABR and/or eABR, it does support the findings of previous studies that show a larger electrophysiological response to electrical stimulation in ears with greater populations of surviving SGNs (Landry et al., 2011; Pfingst et al., 2015a, b). In our hands, cochleae implanted with laminin-coated electrodes showed higher densities of SGN cell bodies and resulted in lower eABR and aABR thresholds compared to thresholds observed in animals implanted with uncoated electrodes. *In vitro*, laminin

micropatterns promoted outgrowth and guidance of SGN neurites; importantly the neurite outgrowth was not affected by the addition of dexamethasone. The use of corticosteroids peri- and post-surgical implantation of CI has been beneficial in limiting the inflammatory response triggered by EIT and the deposition of fibrotic tissue on the electrode array (Liu et al., 2015; Bas et al., 2016; Lo et al., 2017; Plontke et al., 2017). Laminins have shown pro-survival effects if different organs, for example in starved mammary epithelial cells through the internalization of β 4-integrin and laminin and the activation of the mTORC1 pro-survival pathway (Muranen et al., 2017). In inflammatory bowel disease, increased expression of laminins reinforces the intestinal basement membrane and provides a physical barrier and an attenuation of inflammation (Spenle et al., 2014). Therefore, it is possible that laminin may have a protective effect on the sensory epithelial cells, which could explain its effect on the improvement of acoustic ABRs.

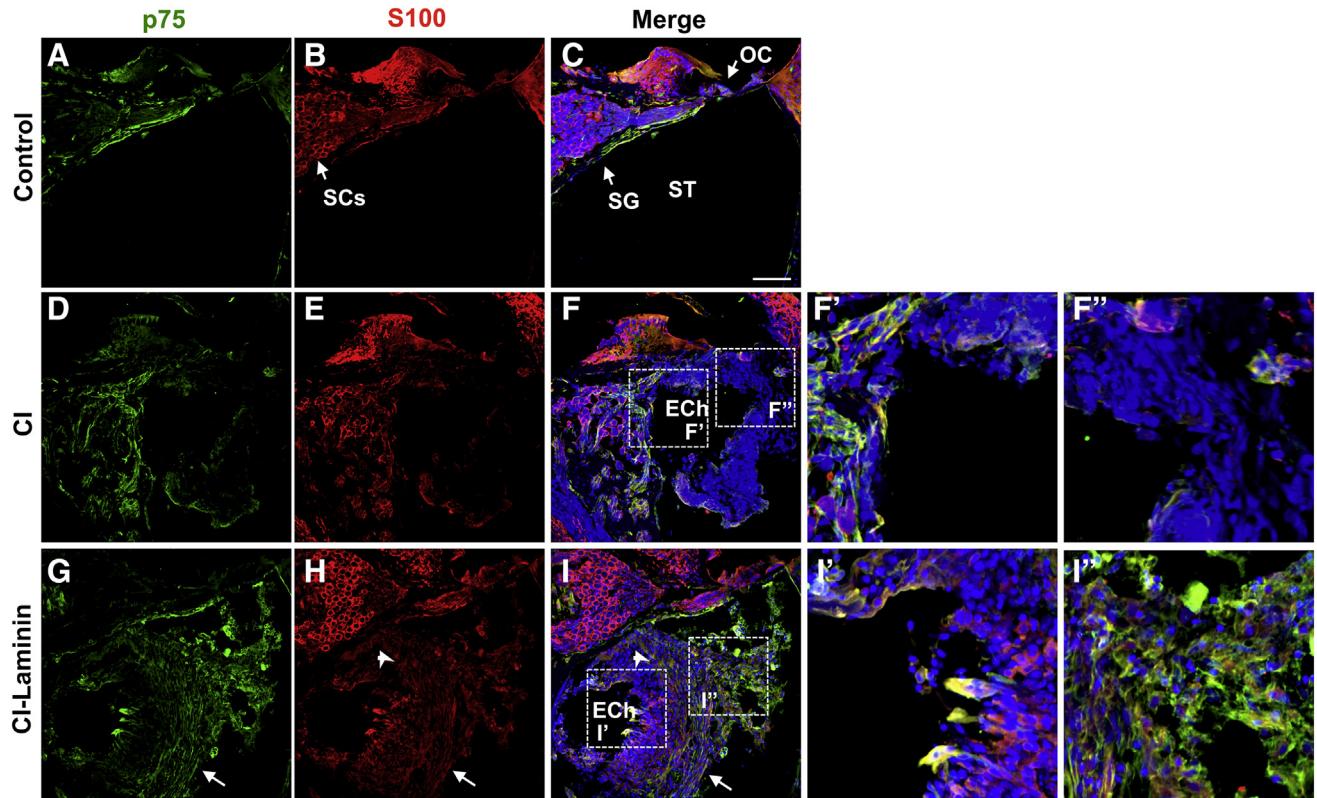


Fig. 6. Immunofluorescence confocal imaging of cochlear cross-sections at 10 days post-implantation. Representative micrographs correspond to the basal turn of contralateral cochleae (A–C), and implanted cochleae with either uncoated electrode analog (CI, D–F, F' and F'' magnification of F) or laminin coated electrode analog (CI-Laminin, G–I, I' and I'' magnification of I) at 10 days post-Cl. Bungner tracks were identified only in the CI-Laminin group (Fig. 6G, H, I white arrows). White arrowheads point at the projection of SCs into the scala tympani through the osseous spiral lamina (Fig. 6H, I). P75 (activated Schwann cells, green), S100 (non-specific Schwann cell marker, red) and DAPI (nuclei, blue). SCs, Schwann cells; OC, Organ of Corti; SG, Spiral ganglion; ST, Scala tympani; ECh, Electrode channel.

Although previous studies (Rask-Andersen et al., 2012; Fransson et al., 2015) suggest a weak correlation between the number of SGN within the cochleae of deceased patients and their historical CI function, recent studies show that larger distances between the electrode and neurons, either by a physical gap between the electrode and the modiolus, or by

the pattern of neural survival along the cochlea, result in the need for higher stimulation thresholds (Long et al., 2014). However, these authors did not endorse the hypothesis that fibrous tissue and bone growth interfere with speech understanding (Long et al., 2014). Laminin contains EGF-like structures that exhibit growth factor-like function on neighboring cells (Engel, 1989), which promote cell proliferation and may explain the progressive increase in impedances due to an accumulation of fibroblasts within the Bungner tracks. Although no correlation could be established between impedances and eABRs, an increase in fibroblasts proliferation around the electrode may result in a reduced eABR improvement, despite the preservation of large numbers of SGN in animals implanted with laminin-coated electrodes compared to those receiving uncoated electrodes.

Following a lesion to the peripheral nervous system, Schwann cells enter into a process of proliferation and dedifferentiation into progenitor-like SC to initiate the repair of the affected nerve. During this regeneration process, SC align in Bungner tracks, i.e. tube like structures, to provide trophic support and scaffolding for neurite growth and guidance (Parrinello et al., 2010; Boerboom et al., 2017). Once the repair process is finished SC exit the cell cycle and differentiate again into myelinating and non-myelinating SC (Kim et al., 2013). Bungner tubes may shrink and be replaced by scar tissue if the axons fail to regenerate (Campbell, 2008).

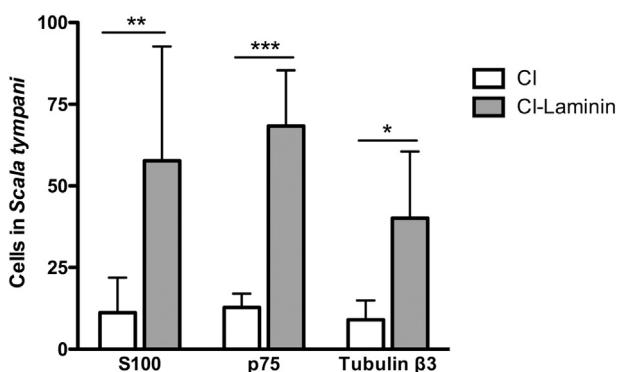


Fig. 7. Analysis of cells located in the scala tympani implanted with uncoated electrode analog (CI) or laminin-coated (CI-Laminin) electrode analog at 10 days post-implantation. The graph shows cell counts for the immunostaining markers S100, p75 and Tubulin β 3 (mean \pm SD). Two-way ANOVA followed by Bonferroni test, counts from 8 to 10 sections/ cochlea were averaged and the means were compared between groups. N = 5 cochleae/ group.

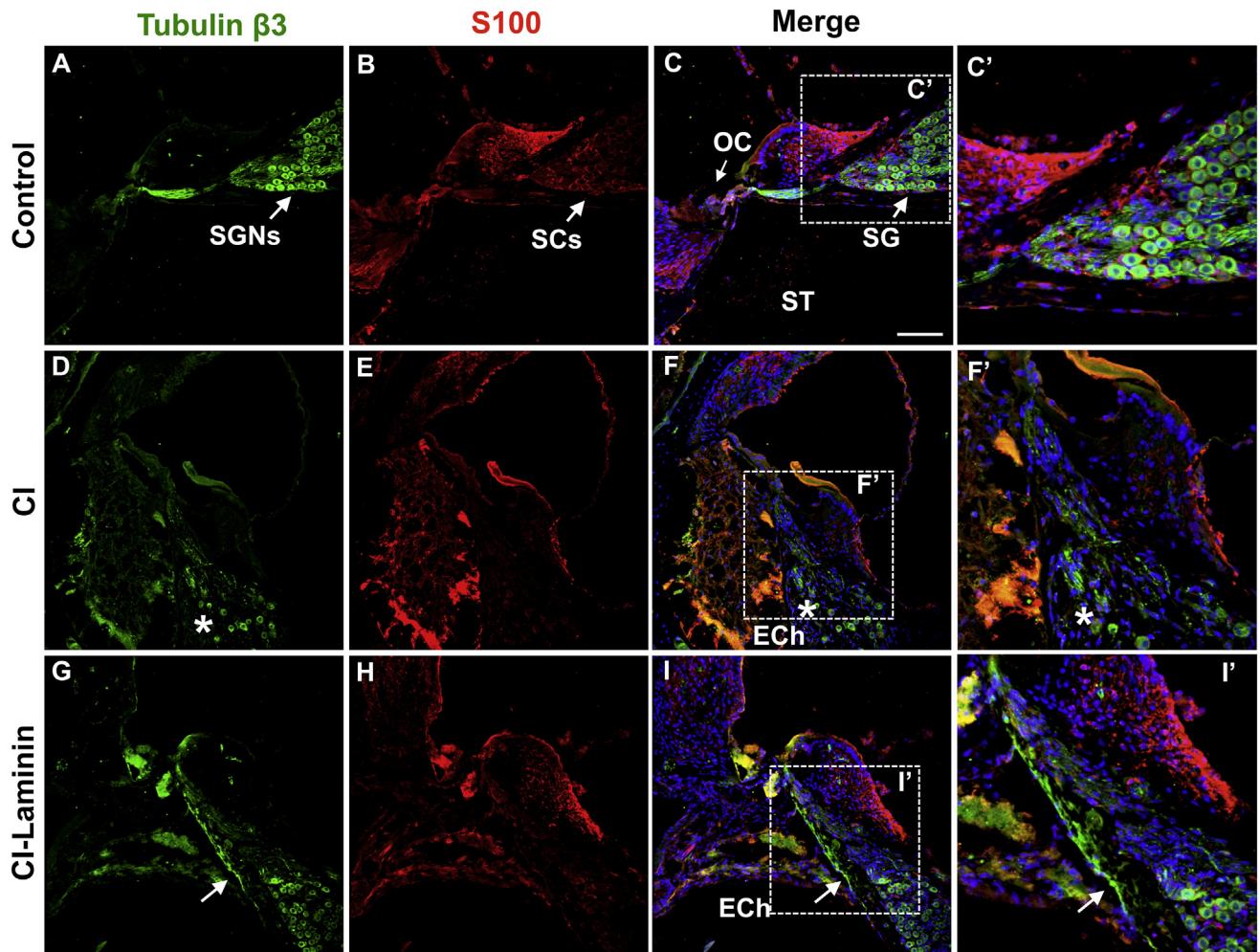


Fig. 8. Immunofluorescence confocal imaging of cochlear cross-sections at 4 weeks post-implantation. Representative micrographs correspond to the basal turn of contralateral cochleae (A–C and its magnification C'), and implanted cochleae with either uncoated silicone electrode (CI, D–F and its magnification F') or laminin-coated silicone electrode (CI-Laminin, G–I and its magnification I') at 4 weeks post-Cl with rat adapted electrodes. Asterisk (D, F and F') indicates a loss of neurons. White arrow (G, I and I') points at neurite processes projecting into the scala tympani where the electrode was located. Tubulin β 3 (neurons, green), S100 (non-specific Schwann cell marker, red) and DAPI (nuclei, blue). SGNs, Spiral ganglion neurons; SCs, Schwann cells; OC, Organ of Corti; SG, Spiral ganglion; ST, Scala tympani; ECh, Electrode channel.

In our study, laminin-coated CI electrodes promoted SC adhesion and growth which in turn provided the pathway for the SGN neurites to grow towards the electrode array. This observation supports previous findings (Whitton et al., 2009) that consider a neuronal–glial cell interaction rather than direct neuronal interaction with ECM proteins, in the case of our study that ECM molecule would be laminin. The ring pattern created by SC (i.e. Bungner tracks) around the CI-laminin electrodes suggests that the direction of the neurites may be perpendicular to the electrode, which would favor a tonotopic pattern of cochlear stimulation. Morphologically, SC and the tip of the neurites that project through the ossaceous spiral lamina into the scala tympani are hypertrophic. Other authors have reported these observations (see Landry et al., 2013 for a review) and suggested that the cell enlargement may be the result of ectopic neural remodeling induced by neurotrophin treatment (Leake et al., 2013). While

we did not use neurotrophins or growth factors, laminin-coated electrodes showed an increase in de-differentiated or repair SC, which are known to activate and recruit immune cells to the lesion site (Bas et al., 2015), as well as to up-regulate production of neurotrophic factors and cell surface proteins that aid with neuronal survival and elongation of axons (see Jessen and Mirsky, 2016 for a review in repair SC).

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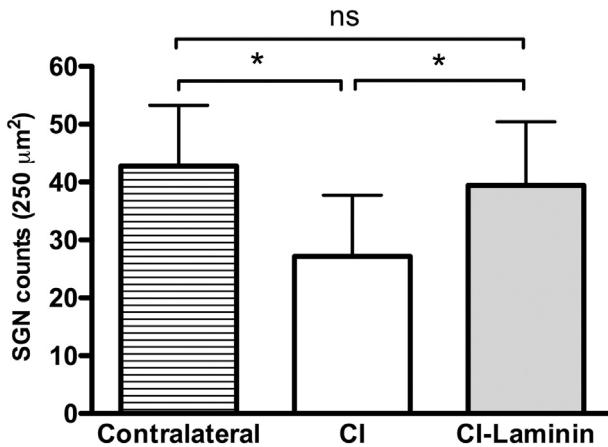


Fig. 9. Spiral ganglion neuron counts located in the basal turn of cochleae implanted with uncoated (CI) and laminin-coated (CI-Laminin) silicone electrodes at 4 weeks post-Cl (region of interest is $250 \mu\text{m}^2$). The graph shows cell counts positive for tubulin B3 (mean \pm SD). One-way ANOVA followed by Tukey's Multiple Comparison test. Counts from 8 to 10 sections/ cochlea were averaged and the means were compared between groups. Contralateral ear: N = 5 cochleae, CI: N = 6 cochleae, CI-Laminin: N = 4 cochleae.

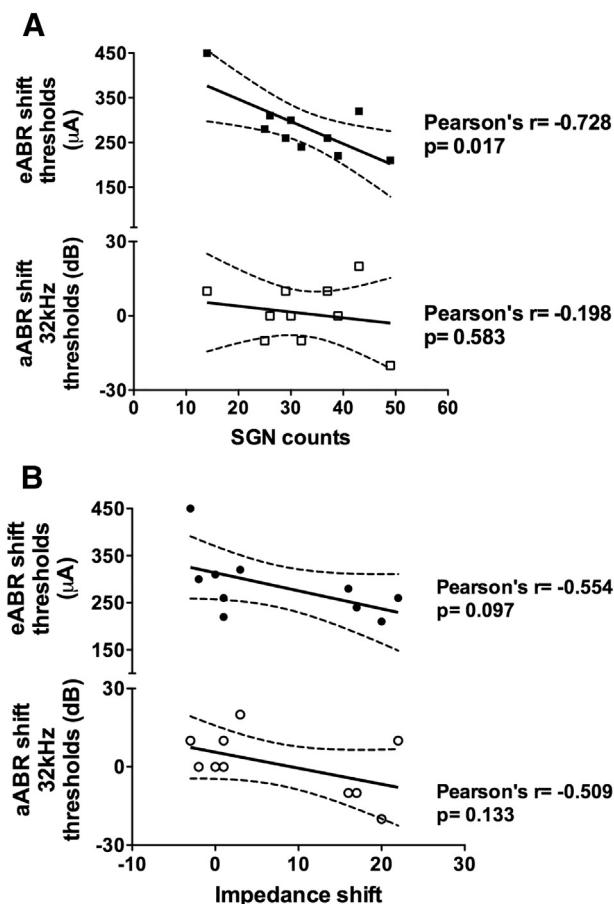


Fig. 10. (A) Pearson's correlation test for SGN counts-eABR thresholds shift (top) and SGN counts-aABR thresholds shift at 32 kHz (bottom) at 4 weeks post-Cl. (B) Pearson's correlation test for impedance shift-aABR thresholds shift at 32 kHz (bottom) and impedance shift-eABR thresholds shift (top) at 4 weeks post-Cl. Dotted lines delimit 95% confidence interval.

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