Integration of Perforated Subretinal Prostheses With Retinal Tissue

Adewumi N. Adekunle¹, Alice Adkins², Wei Wang³, Henry J. Kaplan³, Juan Fernandez de Castro³, Sang Joon Lee^{3,4}, Philip Huie^{5,6}, Daniel Palanker^{5,6}, Maureen McCall^{3,7}, and Mabelle T. Pardue^{1,2}

¹ Department of Ophthalmology, Emory University, Atlanta, GA, USA

² Center for Visual and Neurocognitive Rehabilitation, Atlanta VA Medical Center, Atlanta, GA, USA

³ Department of Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY, USA

⁴ Department of Ophthalmology, Kosin University, Busan, Korea

⁵ Department of Ophthalmology, Stanford University, Palo Alto, CA, USA

⁶ Hansen Experimental Physics Laboratory, Stanford University, Palo Alto, CA, USA

⁷ Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY, USA

Correspondence: Mabelle T. Pardue, Research Service (151Oph), Atlanta VA Medical Center, 1670 Clairmont Road, Decatur, GA 30033, USA; email: mpardue@emory.edu

Received: 3 February 2015

Accepted: 3 July 2015

Published: 14 August 2015

Keywords: retina; prosthetic; biocompatibility; retinitis pigmentosa; migration

Citation: Adekunle AN, Adkins A, Wang W. Integration of perforated subretinal prostheses with retinal tissue. *Trans Vis Sci Tech.* 2015;4(4):5. doi:10.1167/tvst.4.4.5

Purpose: To investigate the integration of subretinal implants containing full-depth perforations of various widths with rat and pig retina across weeks of implantation.

Methods: In transgenic P23H rhodopsin line 1 (TgP23H-1) rats and wild-type (WT) pigs, we examined four subretinal implant designs: solid inactive polymer arrays (IPA), IPAs with 5- or 10- μ m wide perforations, and active bipolar photovoltaic arrays (bPVA) with 5- μ m perforations. We surgically placed the implants into the subretinal space using an external approach in rats or a vitreoretinal approach in pigs. Implant placement in the subretinal space was verified with optical coherence tomography and retinal perfusion was characterized with fluorescein angiography. Rats were sacrificed 8 or 16 weeks post-implantation (wpi) and pigs 2, 4, or 8 wpi, and retinas evaluated at the light microscopic level.

Results: Regardless of implant design, retinas of both species showed normal vasculature. In TgP23H-1 retinas implanted with 10- μ m perforated IPAs, inner nuclear layer (INL) cells migrated through the perforations by 8 wpi, resulting in significant INL thinning by 16 wpi. Additionally, these retinas showed greater pseudo-rosette formation and fibrosis compared with retinas with solid or 5- μ m perforated IPAs. TgP23H-1 retinas with bPVAs showed similar INL migration to retinas with 5- μ m perforated IPAs, with less fibrosis and rosette formation. WT pig retina with perforated IPAs maintained photoreceptors, showed no migration, and less pseudo-rosette formation, but more fibrosis compared with implanted TgP23H-1 rat retinas.

Conclusions: In retinas with photoreceptor degeneration, solid implants, or those with 5- μ m perforations lead to the best biocompatibility.

Introduction

Retinitis pigmentosa (RP) and age-related macular degeneration (AMD) are among leading causes of blindness in developed nations, with the prevalence of AMD rising with increased life expectancy.¹ The pathophysiology of these diseases involves death of photoreceptors and associated late stage retinal remodeling.² Electronic visual prosthetics are one potential strategy to restore visual function, particu-

larly once photoreceptor degeneration is complete. Two approaches are used with retinal prosthetics: epiretinal,^{3,4} where the device is placed in proximity to the retinal ganglion cells (RGCs); and subretinal,^{5–8} where the device is placed in proximity to the inner nuclear layer (INL). Epiretinal electrodes stimulate primarily the RGCs, but can also affect cells/synapses in the INL/inner plexiform layer,⁹ whereas the subretinal implants target primarily the INL neurons (bipolar, horizontal, and amacrine cells),^{10,11} although at higher settings they can also affect RGCs.¹¹

Table 1. Number of TgP23H-1 Rats and WT Pigs Successfully Implanted With Each Device Type

	0- μ m IPA	5- μ m IPA	10- μ m IPA	5- μ m bPVA
TgP23H-1 rat eyes (unsuccessful surgeries)	6 (1)	19 (4)	18 (3)	4 (0)
WT pig eyes	0	2	2	5

To optimally stimulate retinal neurons, all implants should be located in close proximity to the target tissue and be highly biocompatible.^{8,12–14} Our previous work assessed the short- and long-term integration of solid subretinal implants^{5,15–18} or short-term integration of subretinal implants with trenches or pillars^{8,12,19,20} with retinas in various species with and without photoreceptor degeneration. In this study we assess migration of retinal tissue through perforations of various sizes over extended periods of time in rats and pigs, and compare biocompatibility of these designs with solid (nonperforated) implants.

Methods

Animals

The TgP23H-1 transgenic rat model expresses a mutant mouse rhodopsin protein with a proline to histidine substitution at position 23 that leads to rapid photoreceptor death (Steinberg RH, et al. *IOVS* 1996;37:ARVO Abstract 698). Homozygous, Sprague Dawley TgP23H-1 (provided by Mathew LaVail, PhD; University of California at San Francisco School of Medicine, Beckman Vision Center) that were bred to wild-type (WT) Long-Evans rats and heterozygous, pigmented TgP23H-1 rats were used in all experiments. Pigmented TgP23H-1 rats demonstrate ONL thinning as early as P30 and less than 50% thickness remaining relative to WT by P90 (Lowe RJ, et al. *IOVS* 2005;46:ARVO E-Abstract 2300). Rats were maintained in cyclic light conditions (12 hour light/dark; 25–200 lux) at the Atlanta VA Medical Center and given access to food and water ad libitum. A total of 49 eyes from 30 TgP23H-1 rats were implanted at 8 to 11 weeks of age with one of four implant designs (described below; Table 1). Retinas were assessed histologically 8 or 16 weeks later.

Domestic WT pigs were implanted at 1 to 3 months of age and followed for 2, 4, or 8 weeks. A total of nine eyes from seven pigs were implanted with the implant designs described here (Table 1). Pigs were housed and implanted at the University of Louisville in cyclic light conditions (12 hour light/dark). All methods were conducted in accordance

with institutional guidelines and the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

Implants

Four implant designs were tested: inactive polymer arrays (IPAs) with three perforation widths (0, 5, or 10 μ m), and active bipolar photovoltaic arrays (bPVA) with 5- μ m perforations (Fig. 1). Fabrication of IPA devices has been previously described.¹⁹ Briefly, IPA devices were composed of SU-8 (Micro-Chem Corp., Newton, MA), a light-activated polymer with high structural strength¹⁹ and coated in parylene-C (poly-dichloro-diparaxylylene), a Food and Drug Administration–approved polymer commonly used for implantable devices. The final dimensions of the IPA devices were 0.8×1.2 mm and 30 μ m in thickness (Fig. 1A). All implants were sterilized using gas or liquid methods before implantation.

Fabrication and specifications of bPVA devices have been previously described.^{10,12} Briefly, each photovoltaic cell of the bPVA was comprised of three-photodiodes that produced biphasic electrical pulse when illuminated by pulsed light (Fig. 1B). bPVAs had 5- μ m perforations to electrically isolate pixels¹⁴ and promote movement of extracellular milieu between adjacent pixels.⁸ bPVA electrodes were sputter coated with iridium oxide on top of platinum base layer. The final dimensions of the

Figure 1. Representative IPA and bPVA implants. (A) IPA device with 70- μ m pixels separated by 5- μ m perforations. (B) bPVA implants with 70- μ m pixels separated by 5- μ m perforations. Insets show magnified region of the implant. Scale bar: 100 μ m.

bPVA device were 0.8×1.2 mm, and 30 μ m in thickness.

Surgery

TgP23H-1 rats were randomly implanted with one of four subretinal devices, as previously described.²¹ Briefly, animals were anesthetized (ketamine 60 mg/kg; xylazine 7.5 mg/kg), and the globe rotated inferiorly using a superior, transscleral traction suture. Next, an approximately 1-mm incision was made through all layers of the globe and the retina was allowed to spontaneously detach from the retinal pigmented epithelium (RPE) over 10 minutes. Finally, the implant was gently guided into the subretinal space by an intraocular lens manipulator. Implants were positioned in the superior retina approximately 1.5 mm from the optic nerve head. Antibiotic ointment was applied to the area of incision. Implant placement was confirmed via fundoscopy and spectral-domain optical coherence tomography (SD-OCT) at 2 weeks and then at least monthly.

Pig eyes were implanted with subretinal devices using a vitreoretinal approach, similar to one described previously.^{5,22,23} Briefly, after initial sedation with tiletamine HCl and zolazepam HCl (2.0–8.8 mg/kg Telazol; Zoetis, Inc., Florham Park, NJ), sedation was maintained by intubated anesthesia (1.5–2.0% isoflurane mixed with oxygen). After pupil dilation and relaxation of accommodation with 2.5% phenylephrine and 1% tropicamide, a three-port 20-G pars plana vitrectomy was performed. Small neurosensory retinal blebs were created with 30 to 50 μ L BSS Plus (Alcon, Fort Worth, TX) injected into the subretinal space through a 39-G blunt tip needle. The opening of the bleb was widened using vitrectomy scissors and the implant device gently manipulated into the subretinal space. In pig eyes, 1 to 3 devices were implanted per eye in different locations.

In Vivo Imaging

Fundus exams and in vivo examination of retinal cross sections were obtained with a scanning laser ophthalmoscope (SLO) and SD-OCT (Heidelberg Spectralis HRA+OCT, Heidelberg Engineering, Heidelberg, Germany and Envisu SD-OCT Preclinical, Bioptigen, Inc., Morrisville, NC) to confirm subretinal placement of the implant 8 days after implantation and to verify stability of the device at 2-week intervals during the follow-up period. Rats and pigs were anesthetized as described above, eyes dilated with 1% tropicamide (Bausch & Lomb, Tampa, FL)

and corneas anesthetized with 0.5% tetracaine hydrochloride (Alcon). Rats were placed on a heating pad (maintained at 37°C) and multiple images obtained over and around the implanted device. Vascular effects of subretinal implants in rats were documented using fluorescein angiography (2.5-mL injected intraperitoneal of 10% percent fluorescein, AK-Fluor; Akorn, San Jose, CA).

Histology and Statistical Analysis

At 8 or 16 weeks post-implantation (wpi) rats were anesthetized followed by a lethal dose of sodium pentobarbital (Euthasol; Virbac, Fort Worth, Texas). Anesthetized pigs were euthanized with beuthanasia (1 mL/5 kg). All eyes were enucleated and fixed in 2% paraformaldehyde/2.5% glutaraldehyde and embedded in resin (Embed 812/DER 736; Electron Microscopy Sciences, Fort Washington, PA). Posterior eyecups were sectioned at 0.5- μ m thickness and stained with toluidine blue. Histological cross sections were viewed on a light microscope and a subset of rat eyes ($n = 22$) were quantified for total INL thickness and migration of INL neurons through the implant perforations. The latter was quantified as the thickness of INL nuclei layer under the implant (RPE side) or over the implant (RGC side). For each implant type, three to seven rat retinas were quantified, with five regions measured across the length of the implanted region. One-way ANOVA was performed (SigmaStat 3.5, Richmond, CA) to compare differences in INL thickness between the different implant types with Holm-Sidak multiple comparisons. The number of rat retinas with pseudo-rosettes and fibrosis were counted across the different implant designs in individual retinal sections (Table 2).

Results

Implanted Devices Remain Stable in the Subretinal Space

All devices were placed in the superior retina and subsequent SLO and OCT imaging confirmed that each device remained near its placement site (Figs. 2A–C). Each remained within the subretinal space and produced minimal tissue disruption (Figs. 2D, E). Fundus examinations indicated that all surgically induced neurosensory retinal detachments resolved within 1 week. In all rats and pigs, retinal vasculature overlying and distal to the implant was normal and none of the retinas showed ischemia or neovascular-

Table 2. Presence of Pseudo-rosettes and Fibrosis in TgP23H-1 Rat and WT Pig Retinas Implanted With Different Implant Designs

		0- μ m IPA	5- μ m IPA	10- μ m IPA	5- μ m bPVA
TgP23H-1 rats	Pseudo-rosette	0/3	3/8	5/8	1/3
	fibrosis	0/3	5/8	8/8	0/3
WT pigs	Pseudo-rosette		0/2	0/2	2/5
	fibrosis		2/2	2/2	4/5

Number of retinas affected/total retinas examined.

ization (Fig. 2B). As expected,^{24,25} TgP23H-1 rat retinas (Fig. 2D) showed photoreceptor degeneration. A thinner photoreceptor layer also occurred in WT pig retina overlying the implant (Fig. 2E).

IPAs With 10- μ m Perforations Show Excessive Inner Retinal Cell Migration

At 8 wpi, we compared TgP23H-1 rat retinal morphology across subretinally implanted IPA designs. Figure 3 shows representative sections from retinas with solid, 5-, and 10- μ m perforated IPAs and bPVAs with 5- μ m perforations at 8 wpi. In all eyes, the IPA was positioned in the subretinal space, between the retina and RPE and in close proximity to both. The degree of INL cell migration through the

implant toward the RPE varied with the perforation width (Figs. 3B–D). Solid IPAs precluded INL cell migration (Fig. 3A), and those with 5- μ m perforations allowed minimal INL cell migration (Fig. 3B). In contrast, we found significant INL cell migration through the 10- μ m perforations of IPAs toward the RPE (Fig. 3C). As a result, retinal thickness on the RPE side of IPA was significantly greater compared to 5 μ m or solid devices (Fig. 4A; One-way ANOVA $F(3, 14) = 19.71$, $P < 0.001$) and total retinal thickness was significantly greater due to the disruption of INL lamination with migration (Fig. 4C; Two-way ANOVA $F(3, 14) = 6.70$, $P < 0.01$). Retinas implanted with bPVA implants with 5- μ m perforations were similar to retinas implanted with solid or 5- μ m IPAs (Figs. 4A–C). Survival of the INL cells did not depend on the presence of perforations, as the INL thickness of solid and 5- μ m perforated IPAs were similar regardless of whether INL thickness was evaluated only above the implant (RGC side; Fig. 4B) or as total INL thickness (both above and below the implant; Fig. 4C).

Further analysis of the INL migration at 8 and 16 weeks wpi in 5- and 10- μ m IPAs showed significantly greater INL thickness on the RPE side with 10- μ m perforations versus 5- μ m perforations at 8 wpi, but not at 16 wpi (Figs. 3E–H, 4D; Two-way ANOVA $F(1, 14) = 19.81$, $P < 0.001$). Thinning of the INL on the RGC side was evident by 16 weeks in retinas

Figure 2. Representative fundus imaging (A, C), fluorescein angiography (B), and OCT imaging (D, E) of implanted TgP23H-1 rats (A, B, D) and WT pig (C, E). (A) At 2 weeks after implantation with an active bPVA with 5- μ m perforations, the position of the implant superior to the optic nerve (white arrow) was confirmed by fundus imaging in TgP23H-1 rats. (B) Fluorescein angiography showed normal retinal macro- and microvasculature overlying the implant (black arrows indicate implant border) in the same retina. (C) The position of the active bPVA with 5- μ m perforations was confirmed in a WT pig via fundus imaging (white arrow indicates optic nerve head; black arrow indicates subretinal implant). (D) OCT image of a TgP23H-1 rat implanted with a 5- μ m perforated IPA device (white arrow) for 8 weeks (black arrow indicates INL). (E) OCT image of WT pig implanted with a 5- μ m perforated bPVA (white arrow indicates subretinal implant; black arrow indicates INL). Due to the higher optical density of crystalline silicon relative to ocular tissue, the bPVA implant appears about 2 $\times$ thicker than it is in reality. Asterisk indicates vitreous side of OCT image. For a reference scale, the implant devices are 0.8×1.2 mm, and 30 μ m in thickness.

Figure 3. Retinal cell migration depends on perforation width and duration of implantation. Representative histological images of TgP23H-1 rat retinas implanted for 8 weeks with (A) solid IPAs (B) IPAs with 5- or (C) 10- μ m perforations or (D) bPVAs implants having 5- μ m perforations. The second row shows TgP23H-1 rat retinas implanted with 5- (E, F) or 10- μ m (G, H) perforations for 8 (E, G) or 16 (F, H) wpi. Perforated devices show INL cells on the RPE side of the implant (arrows in B, C, E–H) suggesting migration of the INL. (C) The double arrows indicate significantly more INL nuclei on the RPE side in retinas with 10- μ m perforated IPAs. (H) By 16 wpi, the INL layer on the RGC side of the retinas implanted with 10- μ m perforated IPAs was significantly thinner than retinas implanted with 5- μ m perforated IPAs for a similar duration or retinas examined at 8 wpi. The black artifacts in the active bPVA section (D) are remnants of the bPVA device. Arrowhead: INL nuclei within the perforations. Scale bar: 50 μ m. GCL, ganglion cell layer; ONL, outer nuclear layer.

implanted with 10- μ m perforated devices (Figs. 3G, H, 4E; Two-way ANOVA $F(1, 14) = 7.18$, $P < 0.05$). The migration of the INL affected total INL thickness (above and below the implant) of the 10- μ m perforated devices only at 8 weeks (Figure 4F; Two-way ANOVA $F(1, 14) = 19.81$, $P < 0.001$).

Other Morphological Changes in Retinas With Perforated IPAs

In TgP23H-1 rat retinas, perforation width also affected the formation of both pseudo-rosettes (photoreceptor nuclei in the outer nuclear layer that form partial or full circles) and glial fibrosis (Figs. 3, 5; Table 2). Retinas implanted with solid IPAs showed neither pseudo-rosette formation nor glial fibrosis, whereas 40% of retinas with 5- μ m perforated IPAs and 63% of 10- μ m perforated IPAs had pseudo-rosettes. Similarly, 40% and 100% of retinas implanted with these IPAs showed fibrosis, respectively. Finally, 33% of retinas implanted with 5- μ m perforated active bPVAs showed pseudo-rosette formation and none had fibrosis (Table 2).

Subretinal Implantation in Pigs

A variety of vitreoretinal forceps were tested in the vitreoretinal surgery to implant IPAs in WT pigs, and all achieved successful placement with minimal tissue disruption (Fig. 6B). At 8 wpi, retinal lamination was normal and although only a few eyes were analyzed, there was a striking absence of pseudo-rosette formation in the WT pig with 5- and 10- μ m IPAs, compared with 40% in pig retinas implanted with 5- μ m perforated active bPVAs (Table 2). Nearly all implanted WT pig retinas showed fibrosis with the perforated devices tested (Table 2). Overall, WT pigs showed less pseudo-rosette formation and greater fibrosis than TgP23H-1 rats implanted with the same implant designs (Table 2).

Discussion

Close proximity between neurons and electrodes reduces stimulation thresholds and cross-talk between neighboring electrodes in retinal prosthetics.^{8,20} Among the strategies proposed to improve tissue

Figure 4. Cell migration depends on perforation width and duration post-implantation. INL thickness on the RPE side of the implant (A, D), INL thickness of the RGC side of implant (B, E) and total INL thickness (C, F) of TgP23H-1 rats after implantation with various subretinal perforated devices after 8 weeks (A–C) or comparing 8 and 16 weeks post-implantation (D–F). (A) Retinas implanted with 10- μm perforated IPA devices showed significant migration of INL to the RPE side of the implant (one-way ANOVA $F(3, 14) = 19.72$, $P < 0.001$) compared with solid devices or 5- μm perforated IPAs. (B) No significant differences in INL thickness on the RGC side of the implant were found. (C) Total INL thickness (both retinal and RPE sides of the device) was significantly greater for retinas implanted with 10- μm perforated IPAs (one-way ANOVA $F(3, 14) = 6.70$, $P < 0.01$). (D) INL thickness in retinas with 10- μm IPA devices was significantly greater on the RPE side of the implant only at 8 wpi (two-way ANOVA $F(1, 14) = 7.28$, $P < 0.05$). (E) By 16 wpi, retinas implanted with 10- μm perforated IPAs showed significantly thinner INL on the RGC side (two-way ANOVA $F(1, 14) = 7.18$, $P < 0.05$). (F) The migration of the INL neurons increased total retinal thickness with the 10- μm perforated IPAs at 8 wpi (two-way ANOVA $F(1, 14) = 19.81$, $P < 0.001$). Error bars: represent SEM. Multiple comparisons *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Figure 5. Representative pseudo-rosettes and fibrosis observed in a TgP23H-1 rat implanted with 10- μm perforated IPA device. Pseudo-rosettes of photoreceptor nuclei were observed in the ONL (asterisks). Fibrosis was observed around the implant (arrow).

Figure 6. Representative histological images of TgP23H-1 rat (A) and WT pig retinas (B) implanted with active bPVAs having 5- μm wide perforations. Active bPVA showed close proximity of the INL due to cell migration across the implant without significant retinal disruption when implanted using an external (A) or vitreoretinal (B) approach. The black artifacts are remnants of the bPVA device after histological sectioning.

apposition between INL cells and subretinal implant electrodes in the degenerated retina,^{19,20,26} we have examined the integration of the subretinal implant having perforations of 3 to 40 μm in diameter with retinal tissue in vitro and during short term (<9 days) in vivo experiments.¹⁹ We found significant INL cell migration through the openings when gaps exceed 5 μm in diameter.¹⁹ In the current study we extended our follow-up to 8-16 wpi, and again observe 70% more migration of the INL cells towards RPE through the 10- μm wide perforations than through 5- μm perforations (Fig. 4A). Because INL cells are the primary target of subretinal stimulation, this migration moves the cells away from the electrode stimulation and would be detrimental for prosthetic restoration of sight. We therefore conclude that if perforations are included they should be sufficiently narrow to preclude excessive migration.

Perforations Do Not Improve INL Biocompatibility

Previous studies have shown that wild-type retinas implanted with subretinal devices undergo photoreceptor degeneration in the region immediately overlying the device.^{5,18} The death of photoreceptors was attributed to the blockage of choroidal blood flow to the outer retina due to the solid nature of the subretinal implant. The inclusion of perforations was hypothesized to increase the health of the retina overlying the device. Our results show that INL thickness on the stimulating side of the IPA in retinas with solid IPAs is the same as IPAs with 5- μm perforations (Fig. 4). This indicates that the INL is less sensitive to the blockage of choroidal blood flow than the photoreceptors. Furthermore, the retinas with solid IPAs do not form pseudo-rosettes or undergo glial fibrosis, compared with IPAs with perforations. While the mechanism of retinal pseudo-rosette formation is unclear, higher rates have been observed in several retinal pathologies including retinal detachment,²⁷ retinal transplantation,²⁸ retinal trauma,²⁹ RP,^{30,31} AMD,³² and subretinal polymer implantation.²⁶ Additionally, we mainly observed pseudo-rosette formation in degenerating retinas in which photoreceptors were still present and migration was minimal, suggesting that death of photoreceptors may be necessary for migration through the perforations. Finally, we cannot rule out the possibility that the differences in rosette formation and fibrosis were due to the different implant materials in the IPA and bPVA devices. However, our previous studies of

Royal College of Surgeons (RCS) rats,^{17,19,33} mer-KO mice,¹⁵ and WT cat retinas^{5,16,18} implanted with nonperforated bPVAs have not observed rosette formation and others have shown parylene to be biocompatible in pigs,²⁶ dogs,³⁴ rabbits,³⁵ and RCS rats.¹⁹

Gliosis and fibrosis have also been reported with other subretinal implants in both pigs^{26,36} and rats,¹⁹ and appear to be dependent on implant material. While we found less fibrosis formation in 5- μm perforated bPVA compared with 5- μm perforated IPA devices (Table 2), the basal electrical activity produced by the bPVAs makes a direct comparison of implant material difficult.

Perforations Permit Migration of Inner Retinal Neurons

Similar to other studies, we found the retina readily conformed to subretinal implants with 3-dimensional shapes.^{19,20} In this study, we showed that the long-term biocompatibility of the retinal-implant interface may be compromised when perforation width is similar or larger than the neuron soma size (Figs. 3, 4). IPAs with 10- μm perforations permit significant migration of the INL cells in TgP23H-1 rats by 8 weeks (Figs. 4A, 4D). While this did not immediately affect the INL on the RGC side of the implant, the total INL thickness was increased (Fig. 4F), indicating that tight stacking of INL neurons in normal and unimplanted retinas was disrupted (Fig. 3). In fact, the INL cell density was variable, as seen by comparing the micrographs in Figure 3. However, this variability in INL packing density was not associated with different implant types. By 16 weeks, the INL cells on the RGC side of the implant were significantly reduced. Such a loss should be a disadvantage for implant signaling to the remaining retina.

The migration of cells from the INL was only observed in TgP23H-1 rat retinas implanted with perforated IPAs and not by the photoreceptors in WT pigs. The perforation width is large enough to accommodate photoreceptors, thus, it appears that photoreceptors do not respond to the same cues and actively migrate through the perforations. Alternatively, this disparity could be due to differences in the degenerating retina and the WT retina, with the former undergoing structural plasticity more easily. The thinning of the INL on the RPE side of the implanted TgP23H-1 rats between 8 and 16 weeks suggests cell death (Fig. 4D). Because subretinal

prosthetics are designed for patients with advanced photoreceptor degeneration, this INL cell migration using perforated IPAs is disadvantageous to the ultimate functional signaling capability particularly with long-term implantation.

Active Devices Provide Additional Benefits

When we compared TgP23H-1 rats implanted with inactive 5- μ m perforated IPAs and active 5- μ m perforated bPVAs, we saw similarity in the minimal migration of INL cells through the gaps (Figs. 3, 4). However, the addition of electrical activity in bPVAs significantly improved biocompatibility. There was no pseudo-rosette formation and little or no glial fibrotic response. Electrical stimulation from subretinal implants has previously been shown to have neuroprotective effects on the degenerating retina, slowing the rate of photoreceptor degeneration.^{17,37,38} In these experiments, active subretinal devices implanted into RCS rats housed under normal laboratory conditions provided significantly more protection to degenerating retinas than inactive subretinal devices. We speculate that an electrically-induced increase in expression of a neuroprotective agent, such as fibroblast growth factor 2,³⁷ may reduce inflammation and/or injury responses. Thus, a device design with low tonic activity might be beneficial to the recipient retina. Since the bPVA design already has been shown to provide spatially relevant input in a central visual structure,^{10,39} it will be important to determine whether any of the changes in the IPA design lead to improved function. Evaluation of longer post-implantation times, similar to those we used previously,^{5,18} are also needed for active 5- μ m perforated bPVAs to examine: long-term biocompatibility, when migration through perforations reaches equilibrium and how migration changes the implant-retina interface and ultimately visual function.

Acknowledgments

The author thank Douglas W. Emery for excellent technical assistance.

Funding was provided by the National Institutes of Health (NIH; R01-EY-018608), R01 EY14070 (MAMC), NIH HLO76138-08 (JPFdC); the Air Force Office of Scientific Research (FA9550-04); NIH CTSA (UL1 RR025744, Stanford Spectrum fund); a Stanford Bio-X IIP grant; and Research to Prevent Blindness (UofL DOVS; Emory Eye Center).

Also a Research Career Scientist Award from the Department of Veterans (MTP), and a KY Research Challenge Trust Fund (HJK).

Disclosure: **A.N. Adekunle**, None; **A. Adkins**, None; **W. Wang**, None; **H.J. Kaplan**, None; **J. Fernandez de Castro**, None; **S.J. Lee**, None; **P. Huie**, None; **D. Palanker**, P (US 7,058,455); **M. McCall**, None; **M.T. Pardue**, None

References

1. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet*. 2006;368:1795–1809.
2. Jones BW, Marc RE. Retinal remodeling during retinal degeneration. *Exp Eye Res*. 2005;81:123–137.
3. Humayun MS, Dorn JD, da Cruz L, et al. Interim results from the international trial of Second Sight's visual prosthesis. *Ophthalmology*. 2012; 119:779–788.
4. Jensen RJ, Ziv OR, Rizzo JF. Responses of rabbit retinal ganglion cells to electrical stimulation with an epiretinal electrode. *J Neural Eng*. 2005;2:S16–S21.
5. Chow AY, Pardue MT, Chow VY, et al. Implantation of silicon chip microphotodiode arrays into the cat subretinal space. *IEEE Trans Neural Syst Rehabil Eng*. 2001;9:86–95.
6. Rizzo JF III. Update on retinal prosthetic research: the Boston Retinal Implant Project. *J Neuroophthalmol*. 2011;31:160–168.
7. Zrenner E. Fighting blindness with microelectronics. *Sci Transl Med*. 2013;5:210ps216.
8. Mathieson K, Loudin J, Goetz G, et al. Photovoltaic retinal prosthesis with high pixel density. *Nat Photon*. 2012;6:391–397.
9. Jepson LH, Hottowy P, Mathieson K, et al. Focal electrical stimulation of major ganglion cell types in the primate retina for the design of visual prostheses. *J Neurosci*. 2013;33:7194–7205.
10. Fransen JW, Pangeni G, Pardue MT, McCall MA. Local signaling from a retinal prosthetic in a rodent retinitis pigmentosa model in vivo. *J Neural Eng*. 2014;11:046012.
11. Boinagrov D, Pangratz-Fuehrer S, Goetz G, Palanker D. Selectivity of direct and network-mediated stimulation of the retinal ganglion cells

- with epi-, sub- and intraretinal electrodes. *J Neural Eng.* 2014;11:026008.
12. Loudin JD, Simanovskii DM, Vijayraghavan K, et al. Optoelectronic retinal prosthesis: system design and performance. *J Neural Eng.* 2007;4:S72.
 13. Palanker D, Vankov A, Huie P, Baccus S. Design of a high-resolution optoelectronic retinal prosthesis. *J Neural Eng.* 2005;2:S105–S120.
 14. Wang L, Mathieson K, Kamins TI, et al. Photovoltaic retinal prosthesis: implant fabrication and performance. *J Neural Eng.* 2012;9:046014.
 15. Mocko JA, Kim M, Faulkner AE, Cao Y, Ciavatta VT, Pardue MT. Effects of subretinal electrical stimulation in mer-KO mice. *Invest Ophthalmol Vis Sci* 2011;52:4223–4230.
 16. Pardue MT, Ball SL, Phillips MJ, et al. Status of the feline retina 5 years after subretinal implantation. *J Rehabil Res Devel.* 2006;43:723–732.
 17. Pardue MT, Phillips MJ, Yin H, et al. Neuroprotective effect of subretinal implants in the RCS rat. *Invest Ophthalmol Vis Sci.* 2005;46:674–682.
 18. Pardue MT, Stubbs EB Jr, Perlman JI, Narfstrom K, Chow AY, Peachey NS. Immunohistochemical studies of the retina following long-term implantation with subretinal microphotodiode arrays. *Exp Eye Res.* 2001;73:333–343.
 19. Butterwick A, Huie P, Jones BW, Marc RE, Marmor M, Palanker D. Effect of shape and coating of a subretinal prosthesis on its integration with the retina. *Exp Eye Res.* 2009;88:22–29.
 20. Palanker D, Huie P, Vankov A, et al. Migration of retinal cells through a perforated membrane: implications for a high-resolution prosthesis. *Invest Ophthalmol Vis Sci.* 2004;45:3266–3270.
 21. Ball S, Pardue M, Chow A, Chow V, Peachey N. Subretinal implantation of photodiodes in rodent models of photoreceptor degeneration. In: Hollyfield J, Anderson R, LaVail M eds. *New Insights into Retinal Degenerative Diseases*. New York, City: Kluwer/Plenum; 2001:175–180.
 22. Ross JW, Fernandez de Castro JP, Zhao J, et al. Generation of an inbred miniature pig model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci.* 2012;53:501–507.
 23. Scott PA, Fernandez de Castro JP, Kaplan HJ, McCall MAA. Pro23His mutation alters prenatal rod photoreceptor morphology in a transgenic swine model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci.* 2014;55:2452–2459.
 24. Garcia-Ayuso D, Ortin-Martinez A, Jimenez-Lopez M, et al. Changes in the photoreceptor mosaic of P23H-1 rats during retinal degeneration: implications for rod-cone dependent survival. *Invest Ophthalmol Vis Sci.* 2013;54:5888–5900.
 25. Machida S, Kondo M, Jamison JA, et al. P23H rhodopsin transgenic rat: correlation of retinal function with histopathology. *Invest Ophthalmol Vis Sci.* 2000;41:3200–3209.
 26. Montezuma SR, Loewenstein J, Scholz C, Rizzo JF. Biocompatibility of materials implanted into the subretinal space of Yucatan pigs. *Invest Ophthalmol Vis Sci.* 2006;47:3514–3522.
 27. Erickson PA, Fisher SK, Anderson DH, Stern W, Borgula GA. Retinal detachment in the cat: the outer nuclear and outer plexiform layers. *Invest Ophthalmol Vis Sci.* 1983;24:927–942.
 28. Sharma RK, Bergstrom A, Ehinger B. Influence of technique and transplantation site on rosette formation in rabbit retinal transplants. *Acta Ophthalmol Scand.* 1997;75:3–10.
 29. Lahav M, Albert DM, Craft JL. Light and electron-microscopic study of dysplastic rosette-like structures occurring in disorganized mature retina. *Albrecht Von Graefes Archiv Klin Exp Ophthalmol.* 1975;195:57–68.
 30. Milam AH, Jacobson SG. Photoreceptor rosettes with blue cone opsin immunoreactivity in retinitis pigmentosa. *Ophthalmology.* 1990;97:1620–1631.
 31. Tulvatana W, Adamian M, Berson EL, Dryja TP. Photoreceptor rosettes in autosomal dominant retinitis pigmentosa with reduced penetrance. *Arch Ophthalmol.* 1999;117:399–402.
 32. Curcio CA, Medeiros NE, Millican CL. Photoreceptor loss in age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 1996;37:1236–1249.
 33. Ciavatta VT, Mocko JA, Kim MK, Pardue MT. Subretinal electrical stimulation preserves inner retinal function in RCS rat retina. *Mol Vis.* 2013;19:995–1005.
 34. Weiland JD, Humayun MS, Eckhardt H, et al. A comparison of retinal prosthesis electrode array substrate materials. *Conf Proc IEEE Eng Med Biol Soc.* 2009;2009:4140–4143.
 35. Yu W, Wang X, Zhao C, Yang Z, Dai R, Dong F. Biocompatibility of subretinal parylene-based Ti/Pt microelectrode array in rabbit for further artificial vision studies. *J Ocul Biol Dis Inform.* 2009;2:33–36.

36. Christiansen AT, Tao SL, Smith M, et al. Subretinal implantation of electrospun, short nanowire, and smooth poly(epsilon-caprolactone) scaffolds to the subretinal space of porcine eyes. *Stem Cells Int.* 2012;2012:454295.
37. Ciavatta VT, Kim M, Wong P, et al. Retinal expression of Fgf2 in RCS rats with subretinal microphotodiode array. *Invest Ophthalmol Vis Sci.* 2009;50:4523–4530.
38. DeMarco PJ Jr, Yarbrough GL, Yee CW, et al. Stimulation via a subretinally placed prosthetic elicits central activity and induces a trophic effect on visual responses. *Invest Ophthalmol Vis Sci.* 2007;48:916–926.
39. Mandel Y, Goetz G, Lavinsky D, et al. Cortical responses elicited by photovoltaic subretinal prostheses exhibit similarities to visually evoked potentials. *Nat Comm.* 2013;4:1980.