Subretinal Semiconductor Microphotodiode Array

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**BACKGROUND AND OBJECTIVE:** To examine the function of a semiconductor microphotodiode array (SMA) surgically implanted in the subretinal space.

**MATERIALS AND METHODS:** Positive-intrinsic layer-negative (PiN) or negative-intrinsic layer-positive (NiP) SMAs were surgically placed into the subretinal space of rabbits through a pars plana incision and a posterior retinotomy. The implants required no external connections for power and were sensitive to light over the visible and infrared (IR) spectrum; IR stimuli were used to isolate implant-mediated responses from the activity of native photoreceptors. A stimulator ophthalmoscope was used to superimpose IR stimuli on the implant and adjacent retinal areas, and responses were recorded during the postoperative recovery period. SMA responses were also evaluated in vitro. The animals were given lethal anesthetic overdoses, and the retinas were examined histologically.

**RESULTS:** The in vitro implant response consisted of an electrical spike, followed by a small-amplitude DC offset that followed the time course of the IR stimulation, and an overshoot at the stimulus offset. The SMAs placed in the subretinal space retained a stable position and continued to function throughout the postoperative period. The SMA responses recorded in vivo included additional slow-wave components that were absent from the in vitro recordings. These responses reverted to the in vitro configuration following the death of the animal. There was a significant loss of retinal cells in areas underlying the implant, and the retina appeared normal away from the implant and surgical site.

**CONCLUSION:** SMAs can be successfully implanted into the subretinal space and will generate current in response to light stimulation during an extended period of time.


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**INTRODUCTION**

The possibility that visual function may be restored in eyes compromised by various retinal disorders has become an active area of investigation. At the retinal level, the general approach of transplanting normal tissue into the retina has been evaluated by a number of investigators. Although transplanted cells have been demonstrated to survive such surgical procedures, the restoration of visual function has yet to be shown.
Studies have demonstrated that visual sensations or "phosphenes" may be elicited by electrical stimulation of the cortex or eyeball and that reproducible electrical potentials are associated with electrical stimulation of the retina. Based on this research, several groups have explored the possibility that electrical stimulation may be used to restore visual function. Dobelle et al. demonstrated that stimulation of the visual cortex resulted in light sensations, or phosphenes, in two blind subjects. More recently, Schmidt et al. reported that phosphenes were reliably elicited in a subject blinded by glaucoma when the visual cortex was stimulated using surgically implanted electrodes. At the level of the inner retina, Humayun et al. found that phosphenes were perceived by patients blinded by retinitis pigmentosa after electrical stimulation of the nerve fiber layer. In animals, such stimulation has also been shown to evoke reproducible visual evoked potentials.

Although these results are encouraging, a fundamental limitation exists as to the degree of spatial resolution and photic sensitivity that might be achieved from electrical stimulation of the inner retina or the more central visual system. These limits are imposed because the nerve fiber layer and the central visual cortex stimulation will theoretically bypass the initial processing stages that are present in the outer retina, where resolution and sensitivity limits of the retina are established.

An alternative approach that may not bypass the initial retinal processing stages is to electrically stimulate the outer retina or remnants of this structure. Although this approach is necessarily limited to disorders that selectively affect the outer retina, this situation is encountered in retinitis pigmentosa, age-related macular degeneration, and many hereditary retinal degenerations for which there are presently no therapeutic remedies. However, it is yet to be shown that it is possible to implant a device into the subretinal space or that such a device would continue to function for an extended period of time. The present study was conducted to address these issues.

**MATERIALS AND METHODS**

**Animals and Surgical Procedures**

In this study, we used adult rabbits, maintained on a 12:12 light:dark cycle. All the procedures were approved by the local Animal Care and Use Committee and were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Two types of semiconductor-based microphotodiode arrays (SMAs), positive-intrinsic-negative (PIN) and negative-intrinsic-positive (NiP), were fabricated using standard photomask and etch techniques. The SMAs were 250 μm in thickness and ranged in diameter from 1.5 to 3.0 mm. Each SMA microphotodiode subunit subtended 20 μm × 20 μm, and each was physically and electrically separated from adjacent subunits by 10 μm of channel stop. The resultant pixel density was ~1100 subunits/mm². The SMAs had no external connections and were powered solely by incident light, responding from 500 to 1100 nm.

Surgery was performed under anesthesia with intramuscular ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg). The ocular surface was anesthetized with 0.5% proparacaine hydrochloride, and the pupil was dilated with 2.5% phenylephrine hydrochloride and 1% tropicamide. A 4-mm-long sclerotomy was made 4 to 5 mm posterior to the limbus in the superior quadrant. Intravitreally, a localized separation of the retina was created at the posterior pole by injecting a small amount of balanced salt solution into the subretinal space. The creation of a retinotomy near the edge of the bleb allowed the SMA to be inserted into the subretinal space. The retina was then flattened over the implant, and the sclerotomy was closed with an 8-0 nylon suture.

**Electrophysiology**

Anesthesia and pupil dilation were accomplished as described previously. After the application of 1% proparacaine hydrochloride, an ERG-jet contact lens electrode, wetted with 1% methylcellulose, was placed on the cornea. A needle electrode placed subcutaneously on the forehead served as a reference lead, and the ear was grounded. Responses were differentially amplified (0.5–3000 Hz), averaged, and stored using a signal-averaging system (UTAS-2000, LKC Technologies, Gaithersburg, MD).

A Maculoscope (Doran Instruments, Littleton, MA) generated the focal stimulus, which had a duration of 11 milliseconds. On the retinal surface, the stimulus subtended a circular spot of ~1 mm in diameter and was presented at 1 Hz. The stimulus illumination was switchable between white light and IR light (IR; Kodak, Rochester, NY; Wratten 87C;
< 0.1% below 780 nm). The white light was used to orient the Maculoscope illumination, and the IR light was used for the actual implant stimulus to exclude visible-light responses mediated by native photoreceptors. In addition, conventional, full-field flash electroretinograms were recorded to white flashes presented in darkness using a LKC ganzfeld.

In Vitro SMA Response
To establish the baseline response of an SMA to the IR stimuli used, NiP and PiN implants were tested in vitro. The SMA was embedded at a 45° angle on a paraffin button and placed at the bottom of a 33 × 14-mm Petri dish filled with saline. Needle electrodes on either side of the SMA were used to record the response to the IR stimulus delivered from the Maculoscope, which was placed perpendicular to the SMA at a distance of 3 cm. The recording protocol was otherwise identical to that used for the in vivo recordings.

Histology
Eyes were enucleated following the anesthetic overdose. The anterior portion of the eye was dissected away, and the eyecup was fixed in 1% glutaraldehyde-4% formaldehyde phosphate-buffered solution. After overnight fixation, the eyecup was washed in phosphate buffer. Because crystalline silicon cannot be sectioned by conventional microtomes, the implants were gently removed from the subretinal space. Pieces of the eyecup were embedded in epoxy, sectioned, and stained with toluidine blue for light microscopy.

RESULTS
An SMA implant is shown in Figure 1. At low magnification (Fig. 1A) it is not possible to resolve the individual microphotodiode subunits. At a higher magnification (Fig. 1B), the individual 20 × 20-μm subunits are clearly seen, as well as the 10-μm channel block that separates adjacent subunits. Figure 1C shows a fundus photograph taken -10 months after the surgical implantation. This retina shows little reaction to the implant, even after this relatively long period. Although the retina overlying most of the SMA implants remained clear, patches of fibrosis were observed over some of the devices. It is unclear whether this fibrosis was a result of the implantation surgery or was a secondary effect of the implant itself. Pigmentary changes at the retinal pigment epithelium.
(RPE) level surrounding the implants were also seen in several animals. However, no inflammatory reaction, retinal detachment, neovascularization, or secondary hemorrhage was observed in any of the eyes that received implants. Intraoperative vitreous blood, when present after surgery, generally cleared within 1 to 2 months. In one case, the position of the implant shifted postoperatively during reabsorption of the surgically induced subretinal bleb, but remained stable after that period.

Figure 2 shows the in vitro electrical activity of the PiN and NiP SMAs in comparison with the retinal responses after implantation of the SMAs into the subretinal space. For each condition, two responses are overlaid. In the in vitro control recordings, an early SMA electrical spike was seen at the onset of the stimulus, caused by the initial high impedance of the saline to current flow. The implant-produced voltage response then leveled to a stable DC offset as the saline impedance decreased and persisted for the duration of the stimulus. On termination of the stimulus, a small overshoot of opposite polarity was recorded, followed by a return of the waveform to baseline. This overshoot corresponds to the discharge of the polarized layer of water molecules on the electrode after termination of the implant response. As expected, the response of the PiN SMA (left-hand waveforms) was opposite in polarity but otherwise similar to that of the NiP SMA (right-hand waveforms).

Although the polarities of the retinal responses recorded in vitro corresponded to those recorded in vivo, the in vivo responses were wider and longer in duration than those recorded in vitro. In addition, the in vivo responses were often more complex. For example, the NiP response often included two distinct negative peaks, as shown in Figure 2. When present, this waveform characteristic was a persistent feature of the response. Responses to IR stimulation of areas adjacent to the implant were not different from noise recordings.

Because the retinal responses analyzed here have no clear relation to standard electroretinograms or other retinal recordings, it was necessary to develop a convention for analyzing the waveforms quantitatively. The approach that we adopted is diagrammed in Figure 3 for a PiN and a NiP response. Three amplitude parameters were developed. The "peak" response amplitude was measured from the prestimulus baseline to the initial positive or negative peak. The "trough" response amplitude was measured from base-

Figure 2. Responses of positive-intrinsic layer-negative (PiN) or negative-intrinsic layer-positive (NiP) semiconductor microphotodiode arrays (SMAs) to infrared stimulation. Upper tracings show responses made with the SMA placed in saline. Middle records show responses made following implantation of the SMA into the subretinal space. Two repeats are shown for each condition. Lower tracing shows the time course of stimulus presentation as delivered by the Maculoscope (Doran Instruments, Littleton, MA). Calibration equals 20 milliseconds horizontally and 20 μV vertically.

line to the positive or negative overshoot that occurs at flash offset. The amplitude of the response at a fixed point in time was also measured. This response, designated "AMP50%" was measured from baseline to the amplitude at 5.5 milliseconds following stimulus onset (i.e., half of the total stimulus duration of 11 milliseconds). The midpoint of stimulus duration was chosen for convenience; however, our general observation of waveform changes indicates that measurements made at other time points within the period of stimulus duration would yield a similar pattern. In addition, an amplitude-independent measure of response duration was obtained. This measure, designated "T50%," corresponds to the duration between the rising and the falling limbs of the response at half-peak amplitude.

Figure 4 presents measures of T50% made during the postoperative recovery period. A symbol was assigned to the results obtained for each rabbit, and the results were normalized to the final value achieved in each case. When plotted in this manner, it is apparent that the recovery process was similar across experimental subjects and that several weeks were required before the implant response stabilized. A similar pattern of postoperative change was observed for each of the amplitude measures. When a stable level was attained, the implants continued to function throughout the duration of the study.
Figure 3. A schematic diagram of the manner in which implant responses were evaluated. Four measures were made. The "peak" response was measured from prestimulus baseline to the initial peak. The "trough" response was measured from the prestimulus baseline to the trough following stimulus offset. AMP₂₅ was measured from the prestimulus baseline to the midpoint of the response (i.e., at 5.5 milliseconds following stimulus onset; total stimulus duration was 11 milliseconds). T₅₀ reflects the duration of the response at the half-maximum amplitude.

Figure 5A shows a comparison of responses recorded from two rabbits before and after the animals were killed. The responses for animals implanted with PiN and NiP SMAs are shown; two repeats are shown for each condition. The postmortem waveforms are narrower and of shorter duration, and they approximate the saline responses shown in Figure 2. In Figure 5B, the changes in each response measure after sacrifice are compared, and data are expressed relative to the average baseline response made after the postoperative recovery was complete. Although the postmortem peak and trough amplitude measures did not change from baseline, there was a significant decline in AMP₂₅ (t₃ = 7.81; P < .05) and in T₅₀ (t₃ = 6.15; P < .05), that is, the measures that are designed to capture the waveform changes observed at the later portion of the implant response.

The electrical activity of the semiconductor implant itself would not be expected to be affected by these manipulations. To examine what had changed, we subtracted the postmortem response from the baseline. As shown in Figure 5A, the resulting "difference waveforms" consisted of slow waves, peaking at 25 to 30 milliseconds. The polarity of the difference waveforms was dependent on implant polarity, being positive for PiN implants and negative for NiP implants.

Figure 6 gives a comparison of histologic sections made at two locations within an implanted retina. The retina appeared normal in all respects at retinal locations away from the surgical and implant sites (Fig. 6A).
Abnormalities were seen in retinal sections taken from areas overlying the implant (Fig. 6B). At the level of the outer retina, there was a complete loss of photoreceptor outer segments and significant damage to the inner segments and outer nuclear layer. The inner retina was disrupted with a decline of inner nuclear and ganglion cell layer densities and thinning of the inner plexiform layer. In addition, there was evidence of macrophages lining the internal limiting membrane, possibly cells from the retinotomy site.

**DISCUSSION**

The present results indicate that it is possible to place an SMA device into the subretinal space and that the device will continue to function in that location for an extended period of time. The device requires only the power supplied by incident light to generate a recordable current. Although many important issues remain to be resolved, the present data allow us to address several key issue areas.

With respect to issues of biocompatibility, the results indicate that the SMA device is detrimental to retinal areas directly overlying the implant site in the rabbit model, but it does not impair retinal function at locations away from that location. Thus, although patches of retinal fibrosis were observed in some animals, the overall ophthalmoscopic appearance of the fundus was grossly normal in implanted eyes (Fig. 1C). Consistent with this impression, histologic
analysis of the retina indicated a normal appearance away from the implant site. The retinal thinning observed over the implant may reflect that the solid disc implants interfered with the myriad activities that occur within the interphotoreceptor matrix and/or blocked choroidal nourishment to the inner retina; the latter effect may be minimized by using animals with an inner retinal circulation. Finally, beyond a modest reduction in amplitude, white flash electroretinograms recorded from implanted eyes were comparable to those from the fellow eyes in which no operations were performed. This indicates that retinal areas away from the implant also functioned normally.

Focal IR stimuli were used to isolate SMA-mediated responses from those of the native retina. Implant responses were obtained up to the longest postoperative period examined. In addition, SMAs remained photoelectrically active when bench tested after removal, and deterioration was not observed on either the gold surface electrodes or the silicon substrate. The longevity of the SMAs may be due to the fact that silicon and gold do not readily enter into chemical reactions with biologic tissues. In addition, SMAs operate with relatively low stimulating charge and current densities, a feature that could increase their functional life.

IR stimulation of an SMA placed in the subretinal space elicited a retinal response that was qualitatively and quantitatively different from that recorded under in vitro conditions from an implant placed in saline. The in vivo implant responses were wider than the in vitro responses. In addition, the in vivo NIP responses often included a double-negative peak that was never observed under in vitro conditions (Fig. 3). Following the death of the rabbit, the retinal response in an implanted eye reverted to the in vitro saline configuration (Fig. 5A). When the retinal response obtained postmortem was subtracted from the premortem baseline recording, a slow wave with a peak occurring at 25 to 30 milliseconds after the onset of the stimulus was isolated. Because the native rabbit retina is not sensitive to IR stimulation, this wave must reflect some aspect of SMA-induced activity. Although the origin of this wave remains a mystery, we plan to conduct further experiments that bear directly on this question.

In summary, the present study evaluated several features of the SMA implant approach to replicating retinal function. The results indicate that the SMA device will continue to function in the subretinal space for an extended period of time. If the device were able to activate the visual pathway, it may be possible to restore a degree of visual function to retinas that are damaged in diseases such as age-related macular degeneration and retinitis pigmentosa. The spatial resolution of the SMA devices used in our study was ~1100 pixels/mm². However, present-day semiconductor fabrication techniques could increase pixel density by more than a factor of five. SMAs could also be fabricated as individual separate microphotodiodes that are placed in a liquid vehicle for injection into the subretinal space or that are incorporated within a dissolvable biocompatible polymer sheet prior to subretinal implantation. Certainly, many issues relating to biocompatibility and efficacy of SMAs will need to be resolved before human application. However, the present results indicate that electrical stimulation of the retina from the subretinal space may be an approach toward restoring vision to patients who suffer from outer retinal disorders.

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REFERENCES


