Experimental Implantation and Long-term Testing of an Intraocular Vision Aid in Rabbits

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Objective: To develop an intraocular vision aid to provide artificial vision in severely traumatized eyes, where neuroretinal function could be preserved but irreversible anterior segment opacification resulted in blindness.

Methods: The basis of an intraocular vision aid is in principle a telemetric circuit to bridge the opaque cornea and to allow for artificial light stimulation of the retina. The visual prosthesis comprises an external high-dynamic range complementary metal oxide semiconductor camera and digital signal processing unit and an intraocular miniaturized light-emitting diode array to project the image onto the retina. For in vivo testing of long-term function and biocompatibility, silicone-encapsulated active photodiodes were implanted in 13 pigmented rabbits and were followed up for up to 21 months.

Results: Lens extraction and stable fixation of the device in the ciliary sulcus were successful in all cases. For up to 21 months inductive energy transmission and wireless stimulation of the implants could be maintained. Electrophysiologic data and histology demonstrated a good tissue biocompatibility in the long-term follow-up.

Conclusion: The results demonstrate the general feasibility and biocompatibility to implant and fixate an intraocular light-emitting diode prosthesis. Inductive energy transmission to the intraocular device and wireless light stimulation are assured in the long term but depend on meticulous water-impermeable encapsulation of the delicate microelectronic components.

Clinical Relevance: An intraocular vision aid compound system with a high-resolution light-emitting diode matrix might be a future treatment option to restore vision in blind eyes with severe anterior segment disorders.

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Severe chemical burn injury, explosion trauma, or chronic corneal inflammation may lead to irreversible opacification or scarring of the cornea and shrinkage of the anterior segment. In cases like those, transplantation surgery often fails to restore permanent vision. Some eyes are judged to be inoperable and result in subsequent blindness (Figure 1). In eyes with persistent opacification of the cornea but intact retinal function, artificial vision might be provided by the implantation of a visual prosthesis. With an intraocular light-emitting diode (LED) microarray, it should be possible to project an image to the retina and evoke a visual response.

The concept of an intraocular vision aid (IoVA) has been developed to bridge the opaque cornea by telemetric transmission and to provide ambulatory vision giving a minimum of spatial resolution: a high dynamic range complementary metal oxide semiconductor (CMOS) camera placed outside of the eye perceives the original picture and generates a digital image. This image is converted by a digital signal processing unit into a serial bit stream with a data rate of approximately 1 megabit/s. Image information data and energy supply necessary for maintaining the implant’s function are wirelessly transmitted to the intraocular microelectronic device implanted in the capsular bag. The power supply is based on an inductive transmission circuitry while signal transduction is generated optoelectronically by an infrared LED with a wavelength of 860 nm corresponding to the absorption minimum of the opaque cornea to minimize light scattering. The implanted microarray device consists of a receiver for power supply, a data recovery unit, a miniature LED array flip-chip bonded to a silicon CMOS driver circuit, and micro optics (Figure 2). To assure biocompatibility and maintain water-repellent isolation of...
the microelectronic components, the device is encapsulated in silicone rubber. The implanted LED microarray allows for projecting the image to the intact retina by light stimulation.

Engineering details and functionality on the bench have been described previously and demonstrate the technical feasibility to establish an IoVA with a minimum spatial resolution of $380 \times 300$ pixels.\textsuperscript{1-5} However, several surgical problems remain that need to be solved before its successful application in humans. As a first step, the appropriate surgical technique to implant and fixate the LED microarray has to be determined. Furthermore, wireless long-term stimulation of an implanted microelectronic device has to be tested. Finally, the long-term biocompatibility of the intraocular prosthesis has to be investigated.

**METHODS**

**IMPLANT**

A first prototype with a single active LED was constructed and embedded in silicone. The prosthesis consisted of an inductive receiver coil, a charging condenser for energy storage, and a photodiode (Figure 3). Major focus was assigned to a meticulous silicone embedding to assure water tightness and biocompatibility (Acti Tec Inc, Glienicke, Germany). The implant had a diameter of 11 mm and a depth of 4 mm. Wireless transduction of high-frequency energy was sufficiently achieved by inductive transmission within a distance of 50 mm. To test the biocompatibility and long-term function, the devices were implanted in 13 pigmented rabbits.

**SURGICAL PROCEDURE**

All experiments in this study were performed in accordance with the institutional animal guidelines. Adult rabbits were anesthetized using ketamine hydrochloride (25 mg/kg of body weight) and xylazine hydrochloride (6.5 mg/kg of body weight) by intramuscular injections.

Surgical procedure was started with extracapsular lens extraction or clear cornea phacoemulsification. To prevent fibrinous reaction, 4 mg/mL of dexamethasone dihydrogenphosphate (Dexahexal; Hexal, Holzkirchen, Germany), 0.5 mg/mL of epinephrine (Suprarenin; Aventis, Frankfurt, Germany), and 12 500 IU/mL of heparin sodium (Heparin-Natrium; Braun, Melsungen, Germany) were added to the balanced salt infusion solution. After enlarging the corneal incision up to 13 mm with micro scissors, the device was implanted in the ciliary sulcus, and the incision was sutured with 10-0 nylon. Topical dexamethasone dihydrogenphosphate/gentamicin ointment (Dexamytrex; Mann Pharma, Berlin, Germany) was applied daily for 1 week postoperatively.

**FOLLOW-UP**

Clinical and functional examinations were performed in weekly and later in monthly intervals. The rabbits were sedated with ketamine hydrochloride (12.5 mg/kg of body weight) and xylazine hydrochloride (3.2 mg/kg of body weight) by intramuscular injection. Clinical examination included anterior segment photography and slitlamp evaluation of the cornea, wound status, anterior chamber depth, the presence of iris synchia, neovascularization or atrophy, and the position of the implant. Intraocular pressure (IOP) was measured both with an appplanation tonometer (Tono-Pen XL; Mentor, Norwell, Mass) and an impression tonometer (Schioetz; Winter/Medton, Juengingen, Germany). Ultrasound evaluation (P'ytem; M&C Medizintechnik, Mainhausen, Germany) of the retina was performed to verify retinal attachment. Functional evaluation of the implant was performed by using a transmitter coil in a standardized distance of 50 mm (Figure 4). Consecutive illumination of the intraocular photodiode indicated a successful wire-
less energy transduction. Animals were humanely killed by intracardiac injection of 8 mL of T61 (Intervet, Unterschleisingh, Germany), and the eyes were enucleated and processed for histological examination. For light microscopy, the prosthesis was explanted before formalin fixation and examined separately. In 3 cases, histological cross-sections were performed using the micro-grinding technique to obtain slices of tissue together with the microelectronic components still attached.

ELECTRORETINOGRAPHY

Prior to their humane deaths the rabbits underwent electrophysiological examinations. The sedated animals were dark adapted for 30 minutes. For the scotopic electroretinography, a 1-Hz white xenon single-flash stimulus at a luminance of 9.3 candelas/s/m² and duration of 10 microseconds was used (Photistic Stimulator PS33 Plus; Grass, Warwick, RI). The recordings were obtained with a corneal electrode (ERG-Jet; MicroComponents, Grenchen, Switzerland) and a reference gold electrode (Grass) at the forehead. Both connected earlobes served as ground. The signal was filtered and amplified (100-Hz high pass filter, 50-Hz notch filter, 100 000 × amplification) using a Grass RPS312RM Amplifier. The data were processed and converted with an analog-to-digital data acquisition board (PCI-MIO-16XE-50; National Instruments, Austin, Tex) in a desktop computer (PC compatible). The signal was acquired at a 1-kHz sampling rate and 32 responses were averaged (Labview 7.0, National Instruments). The b-wave amplitude was measured from the trough of the a-wave. The averaged recordings of the operated on eye were calculated as a relative b-wave ratio of the fellow eye. Failure was defined as a b-wave ratio of less than 0.8.

RESULTS

SURGICAL RESULTS AND FOLLOW-UP

In total, a visual prosthesis was successfully implanted in 13 rabbit eyes. The first series of eyes were operated on using primary extracapsular lens extraction and there were complications due to a shallow anterior chamber, massive vitreous pressure, fibrinous reaction, and anterior synchia (Table). In 1 eye, iris incarceration into the wound cleft and progressive wound dehiscence led to persistent hypotony (rabbit 3). In the following series, the operative technique was consecutively improved by the use of extensive oculopression and primary phacoemulsification with secondary enlargement. This led to a deeper anterior chamber, and the addition of heparin, dexamethasone, and epinephrine to the infusion solution successfully prevented the development of fibrin (Table). Postoperatively, a moderate inflammatory reaction dissolved within the first 2 weeks and the IOP stabilized between 11 and 20 mm Hg. The rabbits were observed for up to 21 months. Slitlamp examination showed a clear cornea, and only a slight fibrosis with moderate neovascularization was seen around the suture area. No eye exceeded an IOP of 20 mm Hg or showed signs of persistent hypotony (except rabbit 3). The iris plane was anteriorly bowed with a shallow peripheral chamber angle, but no relevant anterior synchia, iris atrophy, incarceration, or wound dehiscence was seen. Ultrasound examination revealed a partial retinal detachment after 14 months in 1 case (rabbit 5) that was not progressive. During the follow-up time, all rabbits showed a stable prosthesis with the microelectronic components visible through the pupil. No decentration or dislocation of the implant occurred (Figure 4A).

FUNCTIONAL TESTING

For functional testing, wireless stimulation of the intraocular LED was performed by transmitting high-frequency energy. In the first lot, the water-resistant coating of the 3 implanted photodiodes was defective owing to inappropriate sterilization procedures resulting in functional loss during the first weeks. By using gas sterilization, the silicone embedding remained watertight and the following 10 implants showed a stable signal transmission within the maximum follow-up time of 21 months (Figure 4B).

ELECTRORETINOGRAPHY

Prior to euthanization, electrophysiological testing (Figure 5) was performed after a mean implantation time of 12.2 months. One animal (rabbit 5) showed a diminished b-wave ratio of 0.39 after a follow-up time of 14 months. Ultrasound examination and subsequent histology disclosed a partial retinal detachment as the underlying cause. The remaining animals showed a mean...
The presence of lymphocytes still indicated a moderate chronic inflammatory reaction, but no inflammatory signs were visible after 6 months.

Within the capsular bag, metaplastic lens epithelial cells showed normal anatomical configuration and an intact epithelium. Typical signs for persistent hypotony such as epiciliary membranes, atrophy of the nonpigmented ciliary epithelium, or cystic vacuolization of the pigmented ciliary epithelium were not present. Except in 1 case where the retina was partially detached (rabbit 5), the retinal structure was well preserved (Figure 6). Tissue sections with the prosthesis still in place revealed a well-centered device implanted in the sulcus being supported by the intact capsular bag. However, the large dimension of the implant, mainly owing to its extensive silicone coating, may exert chronic pressure on the ciliary body. Subsequently, anterior shift of the iris resulted in peripheral iridocorneal touch and anterior synchiae in some cases (Figure 6F). No atrophy of the ciliary body was detectable.

**BIOCOMPATIBILITY**

Findings from histological examination of the enucleated eyes showed moderate signs of postoperative inflammation in the first weeks. During the first week, acute inflammatory cells were identified around the capsular bag together with free erythrocytes embedded in fibrin clots. After 3 weeks, the fibrin was completely resolved. The presence of lymphocytes still indicated a moderate chronic inflammatory reaction, but no inflammatory signs were visible after 6 months.

Within the capsular bag, metaplastic lens epithelial cells indicated regenerative proliferation. Ciliary body and iris showed normal anatomical configuration and an intact epithelium. Typical signs for persistent hypotony such as epiciliary membranes, atrophy of the nonpigmented ciliary epithelium, or cystic vacuolization of the pigmented ciliary epithelium were not present. Except in 1 case where the retina was partially detached (rabbit 5), the retinal structure was well preserved (Figure 6). Tissue sections with the prosthesis still in place revealed a well-centered device implanted in the sulcus being supported by the intact capsular bag. However, the large dimension of the implant, mainly owing to its extensive silicone coating, may exert chronic pressure on the ciliary body. Subsequently, anterior shift of the iris resulted in peripheral iridocorneal touch and anterior synchiae in some cases (Figure 6F). No atrophy of the ciliary body was detectable.

In recent years, numerous intelligent implants in the field of biotechnology have received increasing attention as a possible future strategy to replace tissue at the neuroperceptive interface. Inspired by the successful clinical application of cochlear implants, the feasibility of a visual prosthesis was advocated and promoted, intensified research activity in this new ophthalmological subspeciality. Several research groups focused on the development of retinal implants designed to provide artificial vision to patients suffering from dystrophic retinal diseases.6-9 Those promising results led to a fundamental understanding of implantation and fixation techniques,10 cellular responses at the bioelectronic interfaces, and biocompatible coating of the microelectronic components.11

The concept of an IoVA aims to introduce the advancing knowledge of intraocular biotechnology to a well-defined subgroup of ocular diseases. Irreversible anterior segment opacification might be bridged by wireless signal and energy transmission to an intraocular LED microarray. One prerequisite is the application of minimally invasive implantation techniques to substantially reduce the potential damage to the most delicate structures of the injured eye and to enhance the long-term bio-compatibility of the device. Our preliminary results demonstrate a safe and biocompatible way to implant and fixate the prosthesis, but the anatomical peculiarities of the rabbit eye may cause a number of surgical challenges.

### Table. Tabulated Synopsis of Techniques and Outcome in 13 Implanted Rabbits

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Follow-up Time</th>
<th>Technique</th>
<th>OC</th>
<th>EDH</th>
<th>Early Complications</th>
<th>Telemetric Function</th>
<th>Late Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 wk</td>
<td>ECCE</td>
<td>+</td>
<td>−</td>
<td>SC, VP, FR, AS</td>
<td>− (day 7)</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>1 wk</td>
<td>ECCE</td>
<td>+</td>
<td>−</td>
<td>SC, VP, FR, AS</td>
<td>− (day 4)</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>3 wk</td>
<td>ECCE</td>
<td>+</td>
<td>−</td>
<td>SC, VP, FR, AS, INC</td>
<td>− (day 12)</td>
<td>WD, persistent hypotony</td>
</tr>
<tr>
<td>4</td>
<td>6 mo</td>
<td>ECCE</td>
<td>+</td>
<td>−</td>
<td>SC</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>21 mo</td>
<td>ECCE</td>
<td>+</td>
<td>−</td>
<td>SC, FR, AS</td>
<td>+</td>
<td>CNV, RD, ERG decline</td>
</tr>
<tr>
<td>6</td>
<td>21 mo</td>
<td>Phacoemulsification</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>15 wk</td>
<td>Phacoemulsification</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>15 wk</td>
<td>Phacoemulsification</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>18 mo</td>
<td>Phacoemulsification</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>CNV</td>
</tr>
<tr>
<td>10</td>
<td>12 mo</td>
<td>Phacoemulsification</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>11</td>
<td>12 mo</td>
<td>Phacoemulsification</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>12</td>
<td>6 wk</td>
<td>Phacoemulsification</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+ (Gastric infection)</td>
</tr>
<tr>
<td>13</td>
<td>6 wk</td>
<td>Phacoemulsification</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+ (Gastric infection)</td>
</tr>
</tbody>
</table>

Abbreviations: AS, peripheral anterior synechia; CNV, corneal neovascularization; EDH, epinephrine, dexamethasone dihydrogenphosphate, and heparin sodium added to infusion solution; ECCE, extracapsular cataract extraction; ERG, electroretinography; FR, fibrinous reaction; INC, iris incarceration; OC, oculopression; RD, retinal detachment; SC, shallow anterior chamber; VP, substantial vitreous pressure intraoperatively; WD, wound dehiscence; −, absent; +, present.

* A gastric infection in 2 rabbits was unrelated to the operation.

(± SD) b-wave ratio of 1.09±0.13 with little variation (range, 0.93-1.19).

**COMMENT**

In recent years, numerous intelligent implants in the field of biotechnology have received increasing attention as a possible future strategy to replace tissue at the neuroperceptive interface. Inspired by the successful clinical application of cochlear implants, the feasibility of a visual prosthesis was advocated and promoted, intensified research activity in this new ophthalmological subspeciality. Several research groups focused on the development of retinal implants designed to provide artificial vision to patients suffering from dystrophic retinal diseases.6-9 Those promising results led to a fundamental understanding of implantation and fixation techniques,10 cellular responses at the bioelectronic interfaces, and biocompatible coating of the microelectronic components.11

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lenges. The described operative techniques with small-incision surgery, secondary enlargement, oculopression, and the use of heparin and epinephrine in the infusion solution are recommended and substantially facilitate the procedure. Although the implant exceeds the rabbit’s lens volume, pushing forward the iris and flattening the anterior chamber, stable fixation in the sulcus is possible. This apparently minor detail is highly important as the capsular bag is the only natural intraocular structure capable of supporting and stabilizing a large microelectronic device. Alternative fixation techniques applied in the retinal implant project have shown that they still bear considerable risks.

Furthermore, one should take into account that the IoVA prototype has a weight of 300 mg (age-dependent human lens wet weight, 180-300 mg) and is subject to significant centrifugal force due to eye movements. In this context, positioning the IoVA implant near the rotational center of the eye, just anterior to the vitreous face, may minimize the angular acceleration force and stabilize the ocular contents, thereby decreasing the probability of complications such as iritis, cystoid macular edema, and retinal detachment.

However, as successful implantation surgery is limited by the anatomy given, the prosthesis should not exceed a certain size. With a diameter of 11 mm and a depth of 4 mm, our prototype completely filled the rabbit’s ciliary sulcus that has a mean diameter of 13 mm. The large dimension of the implant in proportion to the rabbit eye was confirmed by the grinding preparation (Figure 6F). Human anatomy of the ciliary sulcus is similar and will have the same limitations.

This means that future designs should not exceed the current dimensions since severely injured eyes after explosion trauma or chemical burn are known to be highly susceptible to persistent hypony. Additional pressure on the ciliary body and iris might provoke chronic irritation. Rabbit eyes in particular show a strong sensitivity to changes in aqueous humor formation resulting in an early and significant drop of the mean IOP level if the ciliary body function is compromised. In our series, persistent hyptony was found in only 1 eye with progressive wound dehiscence. In the other eyes, the IOP remained stable and no histological signs of ciliary body insufficiency were present. Although histologic examination revealed a prolonged moderate inflammatory reaction around the capsular bag for several weeks, no significant cellular alteration occurred in the long-term, indicating a good biocompatibility of the implant. By using optimized implantation techniques, there was no iris synechia development or increase in outflow resistance in the anteriorly bowed iris plane. The flat appearance of the ciliary body in the grinding preparation represents the normal shape of the ciliary body, which is poorly developed in rabbits, with a diameter of only 0.3 mm at its thickest part. Standard histological examination results revealed no signs of atrophy in the ciliary body stroma or processes (Figure 6). Further, electrophysiological testing showed no signs of adverse effects on the retina in the long-term (Figure 5).

The technical feasibility of establishing a miniaturized wireless circuitry for signal and energy transduction has been shown previously. However, proper function of this telemetric circuit depends on a water-resistant coating of the implant. Although silicone material is known to have watertight characteristics, some authors described a vapor transmission rate of 1.73 to 3.11 g/m² per day that is highly dependent on the sterilization procedure. Using heatless gas sterilization, a persistent isolation can be achieved to maintain long-term function of intraocular microelectronic components for almost 2 years.

In summary, the presented results are the first to demonstrate the general feasibility of being able to persis-
tently fix an IoVA prototype at the level of the capsular bag and to maintain wireless energy transmission to the intraocular device in the long term. Currently, a multi-LED array is constructed yielding to a spatial resolution of $32 \times 32$ pixels. Perceptual studies after patterned stimulation have to be awaited to see whether the IoVA compound system with image acquisition, data transfer, and a high-resolution LED matrix will result in a sufficient artificial vision in those otherwise blind patients.

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