**RETNAL DISEASE**

A bioengineered retinal pigment epithelial monolayer for advanced, dry age-related macular degeneration

Amir H. Kashani, Jane S. Lebkowski, Firas M. Rahhal, Robert L. Avery, Hani Salehi-Had, Wei Dang, Chih-Min Lin, Debbie Mitra, Danhong Zhu, Biju Thomas, Sherry T. Hikita, Britney O. Pennington, Lincoln V. Johnson, Dennis O. Clegg, David R. Hinton, Mark S. Humayun

Retinal pigment epithelium (RPE) dysfunction and loss are a hallmark of non-neovascular age-related macular degeneration (NNAMD). Without the RPE, a majority of overlying photoreceptors ultimately degenerate, leading to severe, progressive vision loss. Clinical and histological studies suggest that RPE replacement strategies may delay disease progression or restore vision. A prospective, interventional, U.S. Food and Drug Administration–cleared, phase 1/2a study is being conducted to assess the safety and efficacy of a composite subretinal implant in subjects with advanced NNAMD. The composite implant, termed the California Project to Cure Blindness–Retinal Pigment Epithelium 1 (CPCB-RPE1), consists of a polarized monolayer of human embryonic stem cell–derived RPE (hESC-RPE) on an ultrathin, synthetic parylene substrate designed to mimic Bruch’s membrane. We report an interim analysis of the phase 1 cohort consisting of five subjects. Four of five subjects enrolled in the study successfully received the composite implant. In all implanted subjects, optical coherence tomography imaging showed changes consistent with hESC-RPE and host photoreceptor integration. None of the implanted eyes showed progression of vision loss, one eye improved by 17 letters and two eyes demonstrated improved fixation. The concurrent structural and functional findings suggest that CPCB-RPE1 may improve visual function, at least in the short term, in some patients with severe vision loss from advanced NNAMD.

**INTRODUCTION**

Age-related macular degeneration (AMD) is a leading cause of severe visual impairment in the developed world, affecting 10 to 20% of adults older than 65 years (1–4). AMD is categorized into neovascular or non-neovascular forms (NVAMD and NNAMD, respectively), depending on whether choroidal neovascularization is present or absent. In NNAMD, vision loss is highly correlated with loss of retinal pigment epithelium (RPE) in geographic regions of the macula and is referred to as geographic atrophy (GA) (5). The exact cause of GA is not clear, although several pathophysiological processes are implicated (6). The Age-Related Eye Disease Study (AREDS), a major clinical trial sponsored by the National Eye Institute, demonstrated that antioxidant therapy can decrease the rate of progression of intermediate NNAMD (6). Nevertheless, these studies have demonstrated that stem cell–based therapies are safe and have potential for treatment of AMD.

In addition to RPE dysfunction, alteration of Bruch’s membrane structure and function is likely an important factor in the development of GA (13, 14). Bruch’s membrane demonstrates pathologic changes with age that have been shown to affect RPE metabolism and attachment (15). These changes can be partially reversed by reconstitution of the extracellular matrix composition of Bruch’s membrane (16). In comparative studies, we have demonstrated that hESC-RPE cultured on a synthetic parylene substrate survive longer than a suspension of hESC-RPE cells after subretinal injection in the nude rat (17). Therefore, RPE replacement alone, without a supportive scaffold, is most likely insufficient for sustained structural and functional improvements in GA.

We have developed an approach for treatment of GA associated with NNAMD by surgically implanting a polarized monolayer of hESC-RPE on a nonbiodegradable, synthetic parylene substrate, henceforth termed the California Project to Cure Blindness–Retinal Pigment Epithelium 1 (CPCB-RPE1), into the area of GA. Parylene is the trade name for a variety of chemical vapor–deposited poly(p-xylene) polymers and has the highest U.S. Pharmacopeia (USP) rating of...
Class VI (medical-grade safety). This synthetic substrate was designed to mimic the structural and functional properties of Bruch’s membrane by providing a substrate for RPE adhesion in a polarized monolayer and a diffusion barrier similar to Bruch’s membrane (18). We have previously reported the safety, survival, and functionality of hESC-RPE monolayers cultured on this synthetic substrate in a rodent model of retinal degeneration (19) and the feasibility of subretinal implantation of hESC-RPE cultured on this synthetic substrate in minipigs (20, 21). Here, we report the results of five subjects that were enrolled in a first-in-human phase 1/2a trial to assess the safety and efficacy of this composite subretinal implant for treating advanced NNAMD and severe vision loss. Our results support the safety, anatomic integration, and functional activity of this implant as a potential treatment for severe vision loss from NNAMD.

Fig. 1. CPCB-RPE1 investigational implant. (A and B) Low-magnification (actual size 3.5 mm × 6.25 mm) (A) and high-magnification (B) color photographs of California Project to Cure Blindness–Retinal Pigment Epithelium 1 (CPCB-RPE1). Scale bar, 50 μm. (C) Schematic of the synthetic parylene substrate for human embryonic stem cell–derived RPE (hESC-RPE). The parylene membrane is 6 microns thick with submicrometer-thick circular regions and a smooth, nonporous anterior surface that promotes cell adherence and tightly spaced pattern of ultrathin circular regions that have molecular exclusion characteristics similar to Bruch’s membrane to facilitate nutrient and growth factor diffusion. Arrows show the bidirectional diffusion.

RESULTS

Baseline examination findings

Five subjects were enrolled in the study at the date of this analysis, and four subjects were successfully implanted with the composite implant, CPCB-RPE1, and custom insertion forceps (Fig. 1, A to C, and fig. S1). Table 1 summarizes the characteristics of the subjects enrolled in the clinical trial and their visual outcomes.

Baseline fundus photographs and clinical examination confirmed that each subject had a large area of GA exhibiting decreased pigmentation and involving the fovea (two representative cases are shown in Fig. 2, A and B). Baseline microperimetry sensitivity testing and multifocal electroretinography (mfERG) were not reliable for any meaningful analysis because of the subjects’ poor baseline visual acuity and were discontinued for this cohort of subjects after initiation of the trial.

Table 1. Baseline subject characteristics and postoperative testing results. Text in boldface indicates eyes implanted with CPCB-RPE1. L, left eye; R, right eye; F, female; M, male; ETDRS, Early Treatment of Diabetic Retinopathy Severity Score; LogMAR, logarithm of the minimum angle of resolution; Unstable, less than 75% of fixation events occurred within a 4° retinal locus; Stable, 75% or more of fixation events occurred within a 4° locus; ELM, external limiting membrane; ND, fixation testing was not carried out because the implant was not delivered in subject 123; +/-, the presence or absence of ELM band on OCT.

<table>
<thead>
<tr>
<th>ID-Eye</th>
<th>Age, sex</th>
<th>Preoperative</th>
<th>Postoperative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ETDRS (LogMar)</td>
<td>ELM</td>
</tr>
<tr>
<td>204L</td>
<td>85, F</td>
<td>21 (1.28)</td>
<td>–</td>
</tr>
<tr>
<td>204R</td>
<td></td>
<td>50 (0.70)</td>
<td>–</td>
</tr>
<tr>
<td>123L</td>
<td>85, M</td>
<td>29 (1.12)</td>
<td>–</td>
</tr>
<tr>
<td>123R</td>
<td></td>
<td>24 (1.22)</td>
<td>–</td>
</tr>
<tr>
<td>125L</td>
<td>84, F</td>
<td>3 (1.64)</td>
<td>–</td>
</tr>
<tr>
<td>125R</td>
<td></td>
<td>67 (0.36)</td>
<td>–</td>
</tr>
<tr>
<td>303L</td>
<td>84, M</td>
<td>32 (1.06)</td>
<td>–</td>
</tr>
<tr>
<td>303R</td>
<td></td>
<td>54 (0.62)</td>
<td>–</td>
</tr>
<tr>
<td>128L</td>
<td>69, F</td>
<td>7 (1.56)</td>
<td>–</td>
</tr>
<tr>
<td>128R</td>
<td></td>
<td>56 (0.58)</td>
<td>–</td>
</tr>
</tbody>
</table>
eye and fellow eye, respectively. Using a 15-letter ETDRS change as a benchmark for substantial visual change, four of the five study subjects showed no substantial change from baseline in the study eye (22). Subject 128 improved by 17 letters at day 60, and this visual acuity improvement was maintained through day 120 (Table 1 and Fig. 3, A and C). There was no significant change in the visual acuity in the contralateral, nonstudy (fellow) eye in any subject (Fig. 3B).

Postoperative OCT findings

Optical coherence tomography (OCT) images provide high-resolution, cross-sectional images of retinal structure at tissue and cellular level (5, 11, 12, 20). In the normal retina, the presence of the RPE is demonstrated by a single, bright hyperreflective band that forms the boundary between the neurosensory retina and choriocapillaris. The integration of the RPE with overlying retina is also demonstrated on OCT by one or more hyperreflective lines [external limiting membrane (ELM) and ellipsoid zone band] anterior to the RPE band. Baseline images demonstrated no evidence of an RPE monolayer and minimal or no evidence of an outer nuclear layer throughout the entire region of GA (Fig. 4, A to F). Similarly, there was no evidence of an ELM within the region of GA (Fig. 4, C to F). In contrast, postoperative images of the same subject illustrate the placement of the CPCB-RPE1 in the area of GA (Fig. 4, G and H).

Postoperative OCT images also demonstrated hyperreflective outer retinal bands anterior to the implant, which confirmed that the hESC-RPE of the implant was present and integrating with the overlying retinal tissue. Specifically, two hyperreflective bands (likely representing RPE and ELM) were observed in at least some regions overlying the CPCB-RPE1 (Fig. 4, I to L). These two hyperreflective bands were continuous with and isointense to the normal host RPE and ELM (Fig. 4, I to L). There was no evidence of an ELM band within the area of GA at baseline (representative images from subject 128 are shown in Fig. 4, C to F). Similar postoperative findings were observed on at least two separate exam dates and in at least two or more focal retinal locations overlying the implant in two other subjects (Fig. 5, A to F, and fig. S2). Because of relatively poor fixation, automated registration between baseline and postoperative OCT exams from different dates was not reliable. However, a detailed manual assessment of approximately the same retinal region...
was possible over several visits using anatomic landmarks to compare images. In this assessment, the ELM associated with the CPCB-RPE1 was noted both in areas within and outside the GA overlying the implant [Figs. 4 (I to L) and 5 (E and F) and fig. S2], suggesting that these anatomic features represent the implanted hESC-RPE and reappearance of the ELM in regions within and around the GA overlying the implant.

**Postoperative fixation testing**

Fixation testing assesses a subject’s ability to visually fixate on a specific location. Normal subjects fixate on an object using the fovea, but subjects with severe vision loss from AMD often demonstrate unstable fixation outside the fovea or no fixation at all (23). Here, two of the five study subjects improved from unstable to stable fixation after implantation of CPCB-RPE1 (Table 1). This finding suggests that the presence of hESC-RPE monolayer of the implant supports visual function in the overlying and previously nonfunctional retina. Figure 6 illustrates representative fundus photographs (Fig. 6A) and fixation data for subject 303 (Fig. 6, B and C). This subject had the best baseline visual acuity in the cohort and was able to complete preoperative and postoperative fixation testing with stable fixation. At 60 days after implantation, subject 303’s implant covered the area of GA (Fig. 6D), and fixation improved in several ways. First, the locus of fixation was directly over the implant and in the middle of the GA (Fig. 6E). In contrast, preoperative fixation was at the edge of the GA (Fig. 6B). Second, the distribution of fixation events detected by the microperimeter in the central 2° field improved from 62 to 100% for preoperative and postoperative conditions, respectively (Fig. 6, C and F). The implant position and fixation improvements were maintained at day 120 after implantation visit (Fig. 6, G to I).

A similar improvement was noted in subject 128 at day 60 and was maintained at day 120 (fig. S3) and was concurrent with the 17-letter improvement in visual acuity in that subject (Fig. 7). Figure 7 summarizes the fixation data from preoperative and postoperative fixation trials for all implanted subjects. Although the sample size was not sufficiently powered to detect significant changes, there was a trend toward an increase in the mean percentage of fixation events (±SD) detected within a 2° (32 ± 42% baseline versus 65 ± 38% after the implant; two-tailed t test, \( P = 0.10 \) and \( n = 4 \)) and a 4° (51 ± 41% baseline versus 82 ± 29%; two-tailed t test, \( P = 0.07 \) and \( n = 4 \)) retinal locus after CPCB-RPE1 implantation compared to baseline (preoperative) for the same eye. This trend became significant for both 2° and 4° retinal loci if it was assumed that the study eyes would only improve (one-tailed \( t \) test, \( P = 0.05 \) and \( P = 0.03 \), respectively). In contrast, there was no improvement in the mean percentage of fixation events (±SD) detected in either the 2° (47 ± 38% versus 45 ± 38%; \( n = 4 \)) or 4° (76 ± 26% versus 76 ± 29%; \( n = 4 \)) retinal location. Normal subjects fixate on an object using the fovea, but subjects with severe vision loss from AMD often demonstrate unstable fixation outside the fovea or no fixation at all (23). Here, two of the five study subjects improved from unstable to stable fixation after implantation of CPCB-RPE1 (Table 1). This finding suggests that the presence of hESC-RPE monolayer of the implant supports visual function in the overlying and previously nonfunctional retina.

**Adverse events**

In all subjects, including the subject who did not receive the implant, there were no unanticipated severe adverse events related to the implant, surgical procedure, or immunosuppression. The nonimplanted subject is reported here as part of an intent-to-treat analysis. There was one event reported by the clinical investigator as an anticipated serious adverse event possibly related to the surgery involving a subretinal hemorrhage. This was reported during routine postoperative follow-up. The subject (303) reported no subjective changes in vision associated with the hemorrhage. During the last follow-up visit, this hemorrhage had substantially resolved, and the CPCB-RPE1 implant was unaffected (Fig. 5). This subject received a single intravitreal injection of bevacizumab. Mild to moderate subretinal hemorrhages were reported as adverse events in all other cases both intraoperatively (movie S1) and postoperatively (Figs. 2 and 6). However, in all mild and moderate cases, the hemorrhages resolved without any intervention. There were no other ocular serious adverse events reported for any subject.

There were two unrelated systemic serious adverse events. Subject 123 was hospitalized and successfully treated for a preexisting condition (rectal prolapse) within 1 month after ocular surgery. This was deemed an unrelated serious adverse event. Subject 125 was hospitalized for 9 kilogram weight loss 6 months after surgery due to difficulty eating. The subject was appropriately treated and discharged with recovery of the lost weight. The cause of the weight loss is under investigation but not thought to be related to the implant or surgery. Overall, there were no systemic or ocular safety concerns for implantation of the CPCB-RPE1 in these first five subjects.
DISCUSSION

We show results of the first five subjects enrolled in a first-in-human, phase 1/2a study of CPCB-RPE1 for the treatment of severe vision loss and GA associated with NNAMD. There was no evidence of safety concerns in any subject. Four subjects maintained vision, and it is notable that there was a 17-letter improvement in vision for subject 128 that was sustained over three visits. This improvement was also associated with improved fixation over the implant. In addition, fixation in the study eyes demonstrated significant improvement compared to the nonimplanted fellow eyes. Last, all implanted subjects demonstrated anatomic changes in the outer retina that were consistent with reappearance of the RPE and ELM overlying the implant. Overall, these concurrent anatomic and functional improvements provide evidence for the short-term safety of CPCB-RPE1 (at least up to day 120) and preliminary evidence of potential efficacy.

Because our sample size is small, we cannot determine whether this improvement is statistically or clinically significant. However, the 17-letter improvement in visual acuity for subject 128 is a reasonable and accepted standard for visually significant changes using standardized visual acuity testing (24). In addition, this subject reported subjective improvements in vision that corroborate the objective test results. In contrast to the stable or improved vision in CPCB-RPE1 implanted eyes, visual acuity in the fellow eye was modestly worse in three of five of the subjects over the same period. Natural history studies of GA demonstrate that ~30% of eyes lose vision within 2 years and ~50% do so by 4 years (25). The fellow eyes of the implanted subjects, albeit not perfect, can serve as controls and are a reasonable indicator of the natural course of the disease in the subjects. The contrast between the visual stability or improvement in CPCB-RPE1 implanted eyes and continued vision loss in nonimplanted fellow eyes further suggests that the CPCB-RPE1 implant might provide therapeutic effects. Note that the visual acuity in the worse eye of subjects with GA may improve if the better-seeing eye suddenly becomes worse. This is thought to occur due to...
photoreceptor inner and outer segments. This also supports the hypothesis that a similar process is occurring anywhere in the implanted region where viable dormant photoreceptors are present. We do not have histological data to confirm the identity of these bands definitively, but a reasonable interpretation of the available evidence suggests that at least one of these bands that has reappeared is a reconstituted ELM overlying the donor RPE in the CPCB-RPE1. A significant body of human histopathologic literature demonstrates that the ELM degenerates and is absent in the area of GA (29–31). In addition, histopathologic data from animal models of retinal degenerations also demonstrate robust and dynamic ELM remodeling (32). Last, OCT evidence of ELM reformation in macular hole surgery has been correlated with improved visual performance (33, 34). Therefore, the reappearance of the ELM may be an important prognostic sign for future visual recovery after CPCB-RPE1 implantation.

There are several limitations to our report, including the fact that our study is not fully enrolled, the sample size is small, ethical considerations preclude a formal control group or histological evaluation of tissue, and the subjects represent relatively late stages of disease. Nevertheless, the data from the first five subjects in our study demonstrate concurrent structural and functional changes that are suggestive of efficacy. Larger prospective studies are warranted and are being pursued. In addition, the lack of short-term safety concerns in these first five subjects reassures that the delivery method and CPCB-RPE1 implant might be a viable option for treating AMD in humans.

MATERIALS AND METHODS

Study design

A prospective, interventional, U.S. Food and Drug Administration (FDA)–cleared, phase 1/2a study is being conducted to assess the safety and potential efficacy of a composite subretinal implant in subjects with advanced NNAMD. The study was approved by the Institutional Review Board of the University of Southern California and the Western Institutional Review Board. An Investigational New

---

**Fig. 5. Preoperative and postoperative OCT images in subject 303.**

(A) Preoperative infrared fundus photograph. Red bracketed lines serve as anatomic landmarks that identify a focal region of GA that is separate from a much larger region of GA superior to it and is highlighted by a yellow circle. (B) Postoperative infrared fundus photograph at day 120 showing the implant location (green square) covering almost the entire region of GA including the focal region of GA within the yellow circle. (C and D) Low-magnification OCT cross sections through the bold green arrows shown in (A) and (B). Green lines indicate scans performed on this subject. The red brackets indicate the same region before implantation without ELM (C) and 120 days after implantation with ELM (D). This region was annotated using the OCT software tools that automatically coregister the OCT scan with the infrared fundus photograph above. (E and F) Higher magnification of OCT regions in dashed red boxes from (C) and (D). There is absence of the ELM (E) in the preoperative scan and reappearance of the ELM (F) in the postoperative scan in the same region. Blue arrows indicate the location of ELM band.
Drug (IND) application was cleared by the FDA for a prospective, single-arm, phase 1/2a study to recruit and enroll up to 20 subjects to assess the safety and potential efficacy of the investigational implant (CPCB-RPE1). The IND application also included a custom-designed and manufactured surgical insertion forceps for delivery of the CPCB-RPE1 into the subretinal space (fig. S1) (21). The substrate was engineered by one of us (M.S.H.) in collaboration with Y.-C. Tai at California Institute of Technology and is manufactured by Camtek LLC. The hESC-RPE cells are generated, banked, differentiated, and grown on the substrate under current good manufacturing practice (cGMP) conditions at City of Hope. The insertion forceps was designed by one of us (M.S.H.) and manufactured by Synergetics under cGMP. All surgeries were conducted at the Outpatient Surgery Center of the University of Southern California, Keck School of Medicine. Written informed consent was obtained from the patients, and the study was conducted in accordance with the tenets of the Declaration of Helsinki. A data monitoring and safety committee was assigned for the study to review all adverse events. The study was registered in the ClinicalTrials.gov database before enrollment was initiated (NCT02590692). The stopping rules for the study, as

---

**Fig. 6. Preoperative and postoperative fundus photographs and fixation testing in subject 303.** (A and B) Preoperative color fundus photograph (A) and preoperative fixation testing (B). The red cross represents the location of fixation target. The fine blue dots visible in the magnification (top right) indicate the location of individual fixation attempts. (C) Preoperative distribution of fixation events within 2° and 4° circles. (D) Postoperative day 60 color fundus photograph demonstrating the location of CPCB-RPE1 (black dashed lines) in relation to GA (white dashed circle). (E and F) Postoperative day 60 fixation testing (E) and distribution of fixation events (F) located overlying the implant. (G) Postoperative day 120 color fundus photograph. (H and I) Postoperative day 120 fixation testing (H) and distribution of fixation events (I). Blue arrow indicates a vascular landmark that can be seen in all images for reference.
defined in the clinical protocol, were as follows: (i) development of an expanding mass; (ii) accelerated loss of visual acuity in the implanted eye; (iii) development of any serious adverse pathology associated with the delivery, immunosuppression, or use of the implant that warrants enucleation of the eye; and (iv) surgical delivery–related events involving the device, implant, and surgical procedure that leads to the failure of the implant delivery. Secondary and exploratory endpoints included efficacy as assessed by visual acuity and visual function measures including microperimetry (Nidek MP1S, Nidek Technologies) and mfERG (Veris, Electro-Diagnostic Imaging Inc.). A detailed clinical protocol is available in Appendix 1.

All subjects underwent baseline screening consisting of a comprehensive ophthalmic examination, a general physical examination, and diagnostic imaging. Key inclusion criteria for subjects included age ranging from 55 to 85 years and history of advanced NNAMD, GA, pseudophakia, and severe vision loss with best-corrected visual acuity of 20/200 or worse in the study eye. Study criteria mandated that the study eye be the worse-seeing eye. Subjects with history of any other vision-threatening disease, including NVAMD or health conditions that would prevent general anesthesia, were excluded from the study. Other key exclusion criteria include history of active malignancy within the previous 5 years, history of enrollment in another clinical trial within the previous 3 months, history of active or untreated infectious disease, or any history of immunosuppression or dysfunction. A detailed list of inclusion and exclusion criteria is available in the clinical protocol (Appendix 1) and ClinicalTrials.gov database (NCT02590692).

Visual acuity testing was performed using the ETDRS criteria and charts and reported as absolute letter score. Retinal anatomy was assessed using clinical examination, fundus photography, and OCT. Retinal function was assessed using microperimetry and mfERG. All subjects underwent implantation of a single CPCB-RPE1 on day 0. In all cases, the foveal region was targeted for treatment. Details of surgical methods and a representative surgical video are available as movie S1. Each enrolled subject received immunosuppression using tacrolimus (0.075 mg/kg per day; Astellas Pharma US Inc.) from days −8 to 60 to achieve a therapeutic trough range of 3 to 10 ng/ml. Tacrolimus dosing was tapered by successive 50% increments starting on day 42 until it was stopped on day 60.

The primary outcome measure for the study was safety, as assessed by frequency and severity of adverse events within 1 year of CPCB-RPE1 implantation that are related to the implant, the surgical procedure, or the immunosuppression. Postoperative evaluation of subjects and image interpretation was conducted by one or more independent vitreoretinal specialists unaffiliated with the University of Southern California over the course of the first year. Adverse events were determined using standard ophthalmological examinations to assess integrity of the eye and potential new pathologies. Secondary endpoints assessed potential improvements in visual acuity, visual function, and retinal anatomy.

**Optical coherence tomography**

OCT was performed using a commercially available Heidelberg Spectralis (Heidelberg Engineering Inc.) with image registration and eye-tracking capabilities. All subjects underwent baseline and follow-up OCT raster scans in duplicate at postoperative visits. Because of the poor fixation ability of all subjects, automated image registration was not reliable. However, OCT images of the implant region were still available, with manually guided OCT images taken at each visit. The presence or absence of retinal features was assessed by the site principal investigator using the best-quality scan available and was confirmed by a second investigator. For all retinal features that were evaluated on OCT, the presence of the retinal feature (ELM or RPE) overlying the CPCB-RPE1 implant was assessed if the quality of the scan was sufficient to demonstrate the same or similar features in the normal adjacent retina.

**Microperimetry fixation testing**

Fixation was assessed using a microperimetry device (Nidek MP1S, Nidek Technologies) and was performed on each subject at least at one baseline visit and repeated on postoperative visits (days 60, 90, 120, 180, 270, and 365) in duplicate. Subjects were first asked to perform fixation testing using the largest fixation target available for 15 s. Fixation was graded as one of two possible outcomes, “Unstable” or “Stable,” based on the number of fixation events that were detected within a 4° retinal locus. Unstable fixation was assigned to subjects with less than 75% of fixation events detected within a 4° retinal locus. Stable fixation was assigned to subjects with 75% or more of fixation events detected within a 4° retinal locus. Sensitivity testing was attempted after the assessment of the fixation stability but in no case was sensitivity testing reliably performed in this cohort of subjects. In addition, fixation was also assessed to determine the change in the average percentage of fixation events within 4° and 2° loci between preoperative and postoperative testing reported with SD.

**Investigational implant**

CPCB-RPE1 is a composite implant that consists of a monolayer of hESC-RPE cells seeded and grown on an ultrathin parylene substrate (Fig. 1). Each CPCB-RPE1 implant measures 3.5 mm × 6.25 mm with circular ultrathin areas of 0.3 to 0.4 μm in thickness supported.
by thicker (6 μm) parylene peripherally. The thickness and design of the parylene substrate were intended to mimic the diffusion properties of the native Bruch’s membrane (Fig. 1C) (18). In addition, the implant has a handle for grasping and loading into the custom insertion forceps device (Fig. 1A and fig. S1). About 100,000 mature, polarized, and pigmented hESC-RPE cells are present on each CPCB-RPE1 implant. Culture conditions for production of the implant have been described previously (19, 20).

**Investigational surgical insertion forceps**

The custom insertion forceps were manufactured specifically for handling and delivery of the CPCB-RPE1 and have been described previously (21, 35). The insertion forceps consist of a handle, a thumbwheel, and a shaft that houses a retractable forceps for grasping, folding, and loading the CPCB-RPE1 (fig. S1). The custom insertion forceps protect the CPCB-RPE1 from damage during the surgical delivery into the eye and facilitate delivery through small scleral and retinal incisions (~1 mm versus unfolded implant that would require ~4-mm incision). The insertion forceps are designed for single use and delivery (folding and unfolding) of the CPCB-RPE1 into the subretinal space, as illustrated in fig. S1.

**Surgical procedure**

All surgeries were performed by A.H.K. and M.S.H. A representative surgical video is available in the Supplementary Materials (movie S1). General anesthesia was performed for all surgeries. Subjects were prepped and draped in the usual fashion for pars plana vitrectomy (PPV). Only one eye was prepped and implanted for each subject. Twenty-three–gauge PPV was performed in all cases using a Constellation (Alcon Inc.) vitrectomy system and standard cannula placement 3.5 mm posterior to the limbus. Intraocular visualization was achieved using the OPMI Lumera 700 surgical microscope with RESIGHT viewing system (Carl Zeiss Meditec Inc.). Chandelier illumination (Synergetics Inc.) was used to facilitate bimanual surgical procedures. To create a space for the CPCB-RPE1 implant, a subretinal pocket was created in the following fashion: A 41-gauge subretinal infusion cannula (Accutome Inc.) was used to flatten the retina overlying the implant. The retinal pocket was created in the following fashion: A 41-gauge subretinal infusion cannula (MedOne Surgical Inc.) was used to elevate the retinal space, as illustrated in fig. S1.

Air-fluid exchange was performed, and PFC was completely removed. Retinotomy was sealed with light application of laser retinopexy. An insertion forceps and delivery into the eye and facilitate delivery through small scleral and retinal incisions (~1 mm versus unfolded implant that would require ~4-mm incision). The introduction of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration: A 5-year, randomised, placebo-controlled, double-blind, primary prevention clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration: A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch. Ophthalmol.* **119**, 1417–1436 (2001).


Acknowledgments: We sincerely thank the participants and their families for their altruism and their inspiration. We would like to thank the subinvestigators, physicians, and support staff at participating sites for their support of the ongoing study, including the Retina Vitreous Associates of Beverly Hills (CA) and California Retina Consultants of Santa Barbara (CA). We would also like to thank the support staff at California Institute for Regenerative Medicine, University of Southern California (USC) Roski Eye Institute, USC Institute for Biomedical Therapeutics, Camtek LLC, California Institute of Technology (Caltech), Regenerative Patch Technologies, the Center for Biomedicine and Genetics at the Beckman Research Institute of City of Hope, and University of California, Santa Barbara Center for Stem Cell Biology and Engineering. Funding: This study received funding from California Institute for Regenerative Medicine and Regenerative Patch Technologies; gifts from the Lori Mars Foundation, the William K. Bowes Jr. Foundation, the Vermont Community Foundation, the Breaux Foundation, and the Wilcox Family Foundation; and unrestricted departmental support to the USC Roski Eye Institute from Research to Prevent Blindness. Author contributions: A.H.K. is a principal investigator of the clinical trial at USC, performed the surgeries, and was involved in the study design, data collection and analysis, and manuscript preparation. M.S.H. was involved in the design of the delivery tool and membrane, surgeries, study design, oversight and execution of pivotal proof-of-concept, IND-enabling studies, and manuscript preparation. J.S.L. was involved in study design, execution of trial, oversight of implants, injector tool production, providing regulatory guidance, overall program coordination, data collection and analysis, and manuscript preparation. D.R.H. was involved in the design of the membrane, the development of methods for cell production and characterization, oversight and execution of pivotal proof-of-concept, IND-enabling studies, data collection and analysis, and manuscript preparation. D.O.C. was involved in the development of methods for cell production and characterization, oversight and execution of pivotal proof-of-concept, IND-enabling studies, data collection and analysis, and manuscript preparation. H.S.-H. was involved in the data collection, data analysis, and manuscript editing. L.V.J., B.B.T., W.D., C.-M.L., S.T.H., D.M., and B.O.P. were involved in product assay and manufacturing process development, project oversight, data analysis, and manuscript editing. F.M.R and R.A. are principal investigators of participating clinical sites and were involved in the data collection, data analysis, and manuscript review. Competing interests: The US, D.O.C., D.R.H., M.S.H., L.V.J., and J.S.L. have financial interests in the subject matter of this study. D.O.C., D.R.H., M.S.H., L.V.J., and J.S.L. have an equity interest in and are consultants for Regenerative Patch Technologies. B.O.P. is a contractor for Regenerative Patch Technologies. A.H.K. receives speaking fees, grants, and honoraria from Carl Zeiss Meditec Inc. and is on an Advisory Board for Alimera Sciences Inc., both of which are unrelated to the subject matter of this study. The technology described in this publication is covered by issued U.S. patents related to the parylene membrane and implant (US 8,808,687, submitted by the USC, the California Institute of Technology, and the Regents of the University of California with inventors including authors M.S.H., L.V.J., D.O.C., S.T.H., and DRH, and US 8,877,489, submitted by the California Institute of Technology and the USC with inventors that include author M.S.H.) and the RPE cells (US 9,850,463, submitted by the California Institute of Technology and the USC, the California Institute of Technology, and the University of California with inventors including authors D.O.C. and B.O.P.). Regenerative Patch Technologies holds an exclusive license to these patents. The other authors declare that they have no competing interests. Data and materials availability: Requests for the CPCB-RPE1 implants should be directed to Regenerative Patch Technologies (j.lebkowski@regenerativedrug.com) and will be supplied upon completion of a material transfer agreement, which will contain a description of the proposed research using the materials.

Submitted 26 July 2017
Resubmitted 28 September 2017
Accepted 23 March 2018
Published 4 April 2018
10.1126/scitranslmed.aao4097

A bioengineered retinal pigment epithelial monolayer for advanced, dry age-related macular degeneration

Amir H. Kashani, Jane S. Lebkowski, Firas M. Rahhal, Robert L. Avery, Hani Salehi-Had, Wei Dang, Chih-Min Lin, Debbie Mitra, Danhong Zhu, Biju B. Thomas, Sherry T. Hikita, Britney O. Pennington, Lincoln V. Johnson, Dennis O. Clegg, David R. Hinton and Mark S. Humayun

Sci Transl Med 10, eaao4097.
DOI: 10.1126/scitranslmed.aao4097

Treating vision loss, a goal within sight

Non-neovascular age-related macular degeneration (NNAMD) is a progressive blinding disease primarily due to loss of the retinal pigment epithelium (RPE) of the eye. Currently, there is no effective treatment for NNAMD. Now, Kashani and colleagues have developed a clinical-grade retinal implant made of human embryonic stem cell (hESC) - derived RPE grown on a synthetic substrate. In a first-in-human phase 1 clinical trial in five patients with advanced NNAMD, the implant was shown to be safe and well tolerated. Preliminary results reported potential therapeutic effects on visual acuity, suggesting that this approach might be useful for treating retinal disorders involving RPE loss.