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ABSTRACT

Ocular trauma or laser injury can result in loss of sight due to damage of photoreceptors. Similarly, retinal degeneration leads to a gradual loss of photoreceptors and associated visual impairment. In these conditions, the inner retinal neurons are preserved to a large extent, and visual perception can be restored by electrical stimulation of the remaining retinal network. We developed photovoltaic replacement of photoreceptors, which directly converts light into pulsed electric current in each pixel, stimulating the nearby inner retinal neurons. Clinical trial with such implants having 100 μm pixels confirmed feasibility of such approach and demonstrated that spatial resolution closely matches the pixel pitch (20/420). For a wide-spread acceptance of this technology, prosthetic visual acuity should exceed 20/100, which requires pixels smaller than 25μm. We are developing and testing a novel 3-dimensional electro-neural interface to significantly decrease the stimulation threshold and reduce the pixel size down to 10-20μm. During the current reporting period, we demonstrated that the inner retinal cells migrating into the 3-D implants retain healthy appearance, and that tertiary neurons remain outside the wells. We also show that retinal migration does not negatively affect its electrical excitability, proving feasibility of the 3-D implants for high resolution retinal prosthetics.

Introduction

Ocular trauma can result in traumatic retinopathy associated with the loss of sight due to damage of photoreceptors. Similarly, retinal degenerative diseases result in a gradual loss of photoreceptors and associated visual impairment. In these conditions, the inner retinal neurons are preserved to a large extent, and therefore visual perception can be restored by electrical stimulation of the remaining retinal network.

Photovoltaic subretinal prosthesis directly converts light into pulsed electric current in each pixel, stimulating the nearby neurons. Visual information is projected onto the retina from video glasses using pulsed near-infrared light. Wireless design of these arrays allows scalability to thousands of pixels, and combined with the ease of implantation, offers a promising approach to restoration of sight in patients blinded by retinal trauma or degeneration. Clinical trial with such implants (PRIMA) having 100μm pixels confirmed that patients perceive visual patterns, including letters and numbers, with spatial resolution very close to the theoretical limit for this pixel size (20/420).

To further advance this remarkably successful technology toward highly functional restoration of sight, the pixel size should be decreased. For a wide-spread acceptance in patients with the loss of central vision, acuity of prosthetic vision should exceed 20/100, which requires pixels smaller than 25μm. We are developing novel 3-dimensional electrodes to significantly decrease the stimulation threshold and enable reducing the pixel size down to 10-20, which geometrically correspond to visual acuity of 20/40 - 20/80, respectively. If successful, this technology can be rapidly transferred to our industrial partner Pixium Vision for clinical testing.

Keywords

Retinal prosthesis, photovoltaic, retinal degeneration, traumatic retinopathy, restoration of sight.

Major goals of the project

Specific aims, subtasks and milestones	Timeline, quarters	% complete
Specific Aim 1 **Develop mock-up honeycomb arrays with pixels of 10, 20, 30 and 40μm in width to study retinal migration into the implants in-vivo.**		
Subtask 1 Submit documents to ACURO.	Q4, 2019	100
Milestone: Obtain ACURO approval.		
Subtask 2 a) Develop inactive 3-D arrays with honeycomb-shaped walls surrounding each pixel. b) Study retinal migration into such cavities, including the optimal height of the walls for targeting neurons in the inner nuclear layer. We expect to use up to10 animals per group, so with 4 implant heights about 20-40 animals will be required in this set of experiments.	Q4 2019-Q1 2020 Q2 2020 – Q3 2021	100 90
Milestone: Established optimal height of the honeycomb walls and minimum width of the pixels.		
Subtask 3 Using immunohistochemistry, analyze distribution of the neural and glial cells in the honeycombs of various dimensions. We expect to use 5-10 animals per group, so with 4 implant heights about 20-40 animals will be required in this set of experiments.	Q2 2020 – Q2 2021	90
Subtask 4 Based on initial results, optimize the pixel size and height for a single-cell resolution using smaller size increments (2μm).	Q3 2020 - Q4 2021	80
Milestone: Established optimal geometry of the honeycomb arrays for single-cell resolution. We expect to use about 20-40 animals (5-10 animals per group, two embedding methods, two heights of the honeycomb wells.		
Specific Aim 2 **Model, design and manufacture photovoltaic arrays with optimal geometry of the honeycombs and electrodes.**		
Subtask 1 Add polymer honeycomb walls to our current flat photovoltaic arrays with 40 μm pixels, using multiphoton polymerization (Nanoscribe).	Q3 2019 – Q3 2020	100

Subtask 2 a) Map electric fields in front of these photovoltaic arrays to validate the computational model of electric field in COMSOL. b) Optimize the computational model of the network-mediated retinal stimulation by comparing to in-vivo stimulation thresholds measured with flat implants. c) Using computational modeling of electric field and retinal stimulation, optimize the sizes of the active and return electrodes for lowest stimulation threshold.	Q2 2020 – Q2 2021 Q2 2020 – Q2 2021 Q2 2020 – Q2 2021	100 80 80
Milestone: Design of the honeycomb array is optimized, and electrical requirements for stimulation specified.		
Subtask 3 c) Manufacture photovoltaic arrays with optimal electrodes configuration.	Q3 2020 – Q3 2021	70
Milestone: Active photovoltaic arrays are manufactured.		
Specific Aim 3 **Test stimulation thresholds, spatial resolution and dynamic range of prosthetic vision using VEP measurements in-vivo.**		
Subtask 1 Implant photovoltaic arrays with honeycombs of various pixel sizes as well as transcranial screws for VEP recordings. Measure full-field stimulation thresholds and dynamic range of VEP response as a function of light intensity and pulse duration.	Q2 2021 – Q4 2021	90
Subtask 2 Using alternating gratings, measure spatial resolution of the retinal response to honeycomb implants with various pixel sizes. With 4 pixel sizes (40, 30, 20 and ~10μm), we expect about 40 animals being used in these measurements. The same animals will be used for the stimulation thresholds, dynamic range and acuity measurements.	Q2 2021 – Q4 2021	50
Milestone: Simulation thresholds and spatial resolution of prosthetic vision in rats based on honeycomb arrays is measured.		

Accomplishments

Photovoltaic subretinal prosthesis converts the incident light into electric current to stimulate neurons in the inner nuclear layer (INL). Pixels on the implant functionally replace the natural photoreceptors, with many critical signal-processing features of the retina preserved [1]. To provide sufficient light intensity for photovoltaic stimulation while avoiding visual perception by the remaining photoreceptors, images captured by a camera are projected onto the retina from augmented-reality glasses using pulsed near-infrared (NIR, 880nm) light [2], and the thickness of the implant - 30μm, is chosen to balance the absorption of NIR light and the integrity of the retinal anatomy (Figure 1).

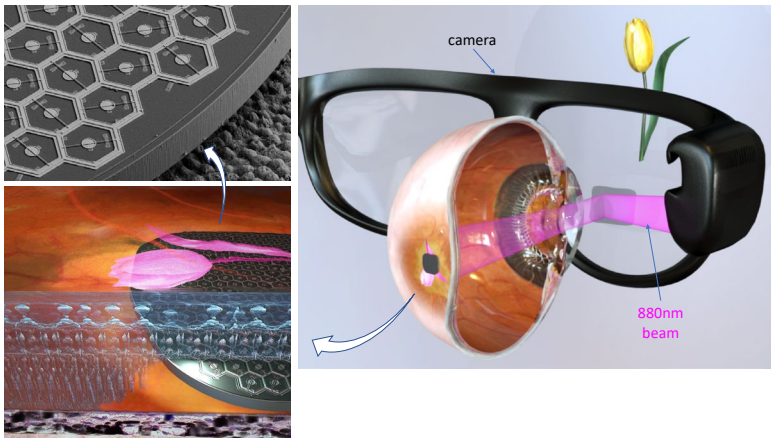

Figure 1. System diagram: photovoltaic subretinal prosthesis activated by the augmented reality glasses.

The biggest challenge with scaling the pixel size down to cellular dimensions is the efficacy of neural stimulation with spatial selectivity, which requires (1) vertical penetration of the electric field elicited by the implant into the INL, and (2) minimal lateral crosstalk between neighboring pixels. With planar configuration of electrodes, these two goals cannot be achieved simultaneously for pixel sizes smaller than the desired penetration depth of electric field, because lateral confinement also limits the vertical span in any conservative and solenoidal vector field. As a result of shallow penetration of electric field, with planar pixels smaller than 40μm, the stimulation threshold exceeds the capacity of even the sputtered iridium oxide film – one of the best electrode materials for neural stimulation to date [3].

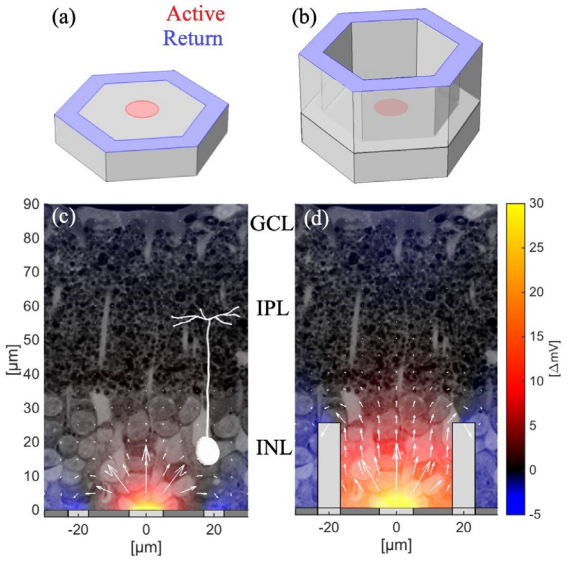

Figure 3. Honeycomb walls vertically align the electric field, reducing the stimulation threshold and decoupling the field penetration depth from the pixel size.

To address this problem, we developed a novel honeycomb configuration of the electrode array with vertical walls surrounding each pixel, and the cavities between walls are filled with neurons in the INL due to retinal migration [3]. Such geometry decouples the penetration depth of electric field from the pixel size by vertically aligning the electric current along the axons of bipolar cells, thereby maximizing the efficiency of electrical stimulation, and blocking the crosstalk by the insulating walls. Simulation demonstrated that honeycomb structures drastically reduce the stimulation threshold compared with planar

configurations (up to 33 folds for 20µm pixels), while the spatial contrast was fully retained (Figure 3).

We built honeycomb-shaped implants and studied the retinal migration into these 3D structures (Aim 1, subtasks 2, 3). As can be seen in Figure 4, cone bipolar cells retain structural integrity after migration and their density and vertical distribution across the inner nuclear layer is similar to that in the intact RCS retina (control). Staining for amacrine cells (Figure 5) demonstrated that these cells remain above the wells, which is very important to avoid the direct stimulation of the tertiary neurons: amacrine and ganglion cells in the retina. It is also very encouraging that stratification of the amacrine cells in the inner plexiform layer (IPL) above the honeycomb appears similar to that in the control RCS retina (B), indicating that retinal cellular migration does not affect the network wiring.

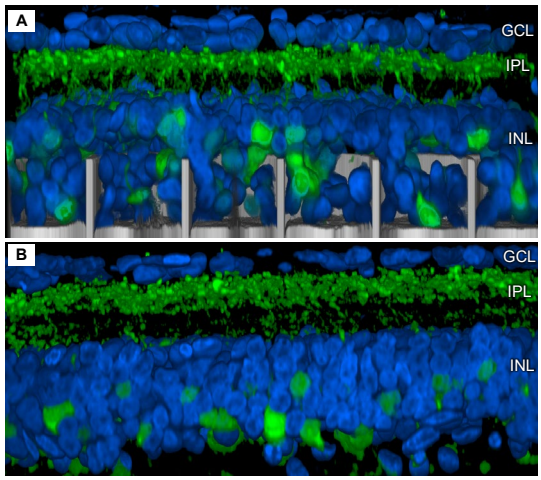

Figure 4. Cone bipolar cells labelled with secretagogin (green). All cell nuclei are also stained with DAPI (blue). A. Cell migrated into the 25µm deep wells of 40µm in width. B. Intact RCS retina in a fellow eye (control).

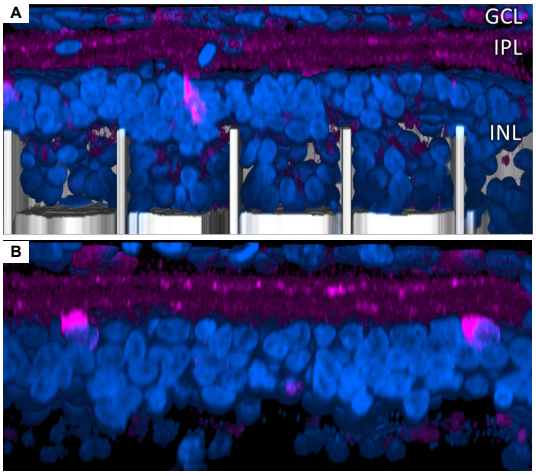

Figure 5. Cholinergic amacrine cells labelled with Choline Acetyltransferase (CHAT, magenta). All cell nuclei are also stained with DAPI (blue). A) No amacrine cells are observed inside 40µm wells - they remain above the honeycomb walls. Amacrine cells and the IPL stratification above the honeycomb appear similar to that in the control RCS retina (B).

To assess the effect of the honeycomb structures on retinal excitability, we manufactured the prototype implants with polymerized walls of 25 µm in height around photovoltaic pixels of 20 and 40 µm in width (Aim 2, subtask 1). These arrays were implanted into RCS rats, and the visually evoked potentials were measured 6-8 weeks later (Aim 3, subtask 1). As shown in Figure 6, stimulation threshold with

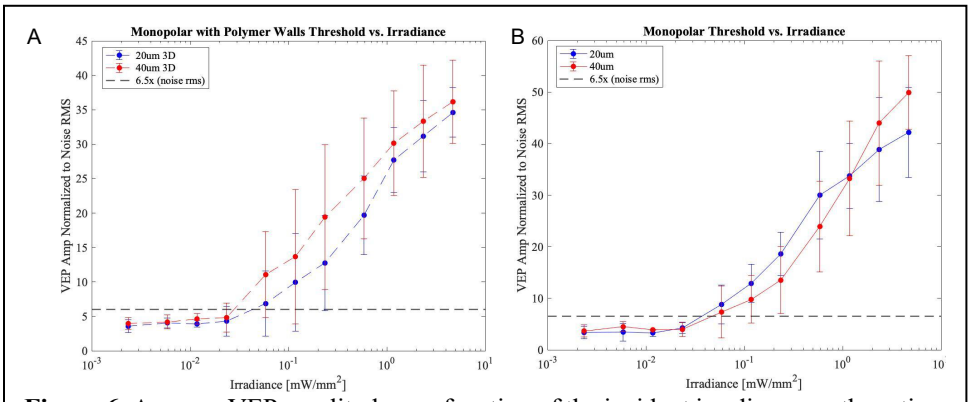

Figure 6. Average VEP amplitude as a function of the incident irradiance on the retina, at 10 ms, 2Hz, normalized to RMS of noise in each animal. **A)** Honeycomb and **B)** flat devices have similar full-field thresholds of 0.057 mW/mm^2 for 10ms pulses. N= 4 for each pixel size; error bars represent standard deviation.

monopolar flat and honeycomb implants are similar: 0.06 mW/mm^2 with 10 ms pulses for both, 20 and 40 µm pixels. The signal-to-noise ratio is also very similar among all the implants. These measurements confirm that migration of the INL cells into the honeycomb wells does not adversely affect the retinal excitability.

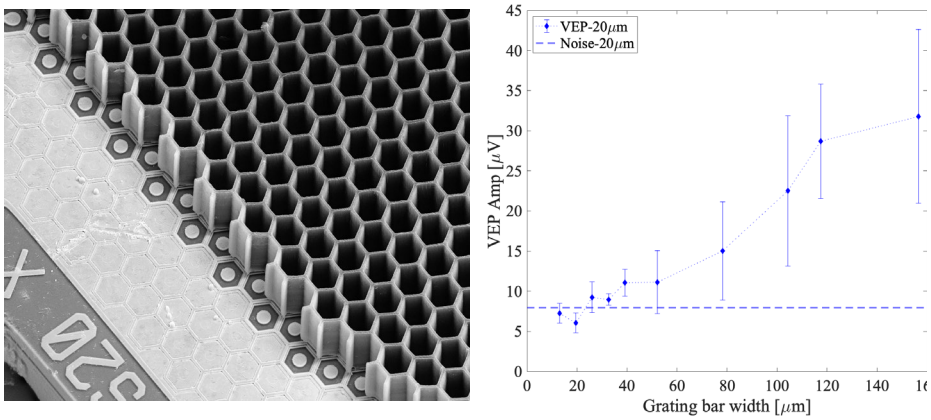

Figure 7. A) Monopolar photovoltaic array with 20 µm pixels having 25µm tall walls polymerized on top of it. B) VEP amplitude as a function of the grating bar width. The smallest measurable response was with a grating width of 26 µm, similar to the natural acuity limit in RCS rats.

Using the same implants, we started measuring the visual acuity with alternating gratings (Aim 3, subtask 2). The initial results with 20µm pixels (Figure 7) show that the smallest measurable response is 26µm, similar to the natural acuity limit in rats[4].

In summary for the current research period:

- Wells are populated primarily by bipolar cells, glial cells and a few horizontal cells.
- Cells inside the wells retain healthy appearance, suggesting that oxygenation from the vessels remaining above the wells is sufficient.
- The glial response is comparable between honeycomb and flat implants.
- Tertiary neurons, such as amacrine and ganglion cells, remain outside (above) the wells.
- Retinal migration into the honeycombs does not negatively affect its electrical excitability.

Products

Dissemination of the Results

Peer-reviewed publications

1. Electronic Retinal Prostheses, Chapter 1.39 in The Senses: A Comprehensive Reference, 2nd edition, Bernd Fritzsch (Ed), Elsevier 2021.
2. Real-Time Optimization of the Current Steering for Visual Prosthesis. Z.C. Chen, B.Y. Wang, D. Palanker. Proceedings of the 10th International IEEE/EMBS Conference on Neural Engineering (NER) 2020. DOI: 10.1109/NER49283.2021.9441400
3. Vertical-junction Photodiodes for Smaller Pixels in Retinal Prostheses. T.W Huang, T.I Kamins, Z.C. Chen, B. Wang, M. Bhuckory, L. Galambos, E. Ho, T. Ling, S. Afshar, A. Shin, V. Zuckerman, J.S Harris, K. Mathieson, D. Palanker. *J. Neural Eng*. 18 036015 (2021).
4. Decoding Network-mediated Retinal Response to Electrical Stimulation: Implications for Fidelity of Prosthetic Vision. E. Ho, A. Shmakov, D. Palanker. *J. Neural Eng*. 17 066018 (2020).

Papers in peer-review

5. Simultaneous Perception of Prosthetic and Natural Vision in AMD Patients. Daniel Palanker, Yannick Le Mer, Saddek Mohand-Said, José-Alain Sahel. (March 26, 2021).
6. Electronic "Photoreceptors" Enable Prosthetic Vision with Acuity Matching the Natural Resolution in Rats. Bing-Yi Wang, Zhijie Charles Chen, Mohajeet Bhuckory, Tiffany W Huang, Andrew Shin, Valentina Zuckerman, Elton Ho, Ethan Rosenfeld, Ludwig Galambos, Theodore Kamins, Keith Mathieson, Daniel Palanker (June 12, 2021).

Conference presentations

Five abstracts have been submitted to the international congress on artificial vision: "The Eye and the Chip", which will take place in October 2021.

- Toward high-acuity prosthetic vision based on optically configurable confinement of electric field with photovoltaic pixels
- A real-time image optimization algorithm for PRIMA patients
- Subretinal planar and 3D photovoltaic arrays enable prosthetic vision matching the natural acuity of 27μm in rats
- On anatomy and physiology of the retinal integration with a subretinal honeycomb-shaped prosthesis
- Electronic "photoreceptors" enable prosthetic vision with acuity matching the natural resolution in rats

Patent filed:

- Photovoltaic retinal prosthesis with optically configurable confinement of electric field.

Daniel V. Palanker, Zhijie C. Chen, Bing-Yi Wang. Application number: 63/168786. Filing date: 3/31/2021

Impact

Shear forces inflicted by explosion or head impact may result in traumatic retinopathy due to damage of the photoreceptors, leading to irreversible loss of sight. Similarly, retinal degeneration leads to gradual loss of photoreceptors and associated visual impairment. In these conditions, the inner retinal neurons that process the visual signals and relay them to the brain are relatively well preserved. Photovoltaic replacement of the lost photoreceptors offers a very promising approach to restoration of sight due to its high resolution, wireless nature of the implants, small size, modularity and ease of implantation. We continue advancing this technology according to the SOW. If successful, we expect the current implants to provide visual acuity exceeding 20/100.

References

1. Werginz, P., et al., *On optimal coupling of the 'electronic photoreceptors' into the degenerate retina.* Journal of Neural Engineering, 2020. **17**(4): p. 045008.
2. Lorach, H., et al., *Photovoltaic restoration of sight with high visual acuity.* Nature Medicine, 2015. **21**(5): p. 476-82.
3. Flores, T., et al., *Honeycomb-shaped electro-neural interface enables cellular-scale pixels in subretinal prosthesis.* Sci Rep, 2019. **9**(1): p. 10657.

4. Wang, B.-Y., et al., *Electronic "photoreceptors" enable prosthetic vision with acuity matching the natural resolution in rats.* bioRxiv, 2021: p. 2021.07.12.452093.

Participants & other Collaborating Organizations

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Location of Organization: Hansen Experimental Physics Laboratory,
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Name:	Daniel Palanker
Project Role:	PI
Nearest person month worked:	1.8 CM
Contribution to Project:	Directs the project, evaluates the results, and writes the reports and publications.

2. Ms. Valentina Zuckerman

Name:	Valentina Zuckerman
Project Role:	Sr. Research Associate
Nearest person month worked:	2.65 CM
Contribution to Project:	Animal protocols, sub-retinal implantations, transcranial electrodes, and on in-vivo imaging.

3. Mr. Tong Ling

Name:	Tong Ling
Project Role:	Postdoc
Nearest person month worked:	1.0 CM
Contribution to Project:	Works on optical and electronic system for in-vivo evaluation of the photovoltaic arrays (left Stanford at Oct 2020.)

4. Mr. Ludwig Galambos

Name:	Ludwig Ga;ambos
Project Role:	Sr. Research Engineer
Nearest person month worked:	1.38 CM
Contribution to Project:	Support of the implant fabrication at the Stanford Nanofabrication Facility

5. Mr. Zhijie Chen

Name:	Zhijie (Charles) Chen
Project Role:	Graduate Student
Nearest person month worked:	2.25 CM
Contribution to Project:	Works on design and fabrication of the photovoltaic arrays.

6. Mr. Andrew Shin

Name:	Andrew Shin
Project Role:	Graduate Student
Nearest person month worked:	5.0 CM
Contribution to Project:	Implant fabrication development, including the novel design of the shunt resistor and electroplating procedures for 3-D implants (Replaced RA-Shayan Afshar).

7. Mr. Shayan Afshar

Name:	Shayan Afshar
Project Role:	Graduate Student
Nearest person month worked:	2.5 CM
Contribution to Project:	Implant fabrication development, including the novel design of the shunt resistor and electroplating procedures for 3-D implants.