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New Technique Allows Researchers to Visualize Local Outflow Enhancement in an Affordable Pig Eye Glaucoma Model



by **Nils A. Loewen, MD, PhD**

We have tackled two paramount challenges in glaucoma outflow research: 1) the unavailability of an affordable gene delivery model and 2) the inability to see and measure focal outflow enhancement.

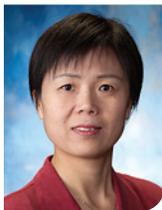
The reduced availability and high cost of human donor eyes are significant problems in glaucoma research. This challenge is becoming more urgent as glaucoma continues to increase in prevalence, making it the leading cause of irreversible vision loss with a disproportional impact on productivity and quality of life in the prime of an individual's life.^{1,2} Humans have a unique aqueous humor drainage system that is difficult and expensive to study. Because of the shortage of human donor eyes, my laboratory was initially not able to proceed with our outflow engineering studies, in which we deploy bioengineering methods from stem cell research and gene therapy to unravel and treat the cause of increased pressure in glaucoma.

When assessing alternative model systems, we found that pig eyes, obtained from a local butcher, can be used as a reliable and high quality source for our studies. Pig eyes have human biochemical glaucoma markers³ and microphysiological properties, such as giant vacuole formation along Schlemm's canal.⁴ Interestingly, the recently sequenced pig genome also shows that pigs and humans have a closer match than, for instance, mice and humans.⁵⁻⁷ We were able to genetically modify the structure that guards outflow in the eye, the trabecular meshwork, and follow expression of a fluorescent marker gene over time.

Bioengineering of the outflow tract requires that improved outflow be visualized and measured, because the effect likely affects only a part of the drainage system. Methods that estimate outflow by global pressure changes are not sufficient.

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Stem Cells for Lowering Intraocular Pressure and Preventing Glaucomatous Blindness



Yiqin Du, MD, PhD, an assistant professor in the Department of Ophthalmology with a secondary appointment in the Department of Developmental Biology at UPMC, has been working for many years in the fields of

stem cell biology and stem cell applications on curing ocular diseases. Based on her years of experience as an ophthalmologist in China, Dr. Du sees what is needed in ophthalmologic translational research and what research will be beneficial to patients. Over the past several years, her research has focused on lowering intraocular pressure (IOP) and preventing glaucomatous blindness with stem cells.

Glaucoma is a progressive optic neuropathy with loss of retinal ganglion cells and optic nerve axons,

resulting in visual field loss. Elevated IOP is the primary risk factor for most forms of glaucoma. The conventional aqueous outflow pathway of the eye is the main route in humans and consists of a series of tissues at the angle of the anterior chamber comprising the trabecular

meshwork (TM), including the uveal and corneoscleral meshworks and juxtacanalicular connective tissue (JCT), the endothelial lining of Schlemm's canal, the collector channels, and the episcleral venous system. The JCT and the Schlemm's canal endothelial cells are generally believed to be the major site of resistance to aqueous outflow, and thus are central to control of IOP. TM cells lining the beams of the meshwork may also have a major role in regulation of aqueous outflow via ligand release to modulate

permeability of Schlemm's canal endothelial cells, extracellular matrix (ECM) turnover, and phagocytic activities to remove debris and foreign bodies. Stem cell-based therapy to regenerate the TM, restore the function of the outflow pathway, and reduce IOP is a new direction for glaucoma treatment.

The research team in the Stem Cell Biology and Glaucoma Laboratory led by Dr. Du has isolated and characterized stem cells from the trabecular meshwork and discovered that these stem cells have the ability to selectively home to the TM tissue, repair the damaged tissue structure, restore the function of the outflow pathway, and reduce IOP in a mouse glaucoma-like model. These stem cells are capable of suppressing inflammation and fibrosis locally and free of tumorigenesis *in vivo*.

Furthermore, Dr. Du and her team are working on discovering other sources of stem cells, which will make autologous transplantation feasible. The cells they are working on include induced pluripotent stem cells, adipose-derived stem cells, and corneal stromal stem cells, which are readily isolated from the patients themselves.

They will test the cells on nonhuman primates for safety and effectivity. Dr. Du predicts that applying stem cells into glaucoma patients to lower IOP and prevent optic nerve damage will become reality in a few years. This might be a onetime treatment since stem cells will stay quiescent *in vivo* and differentiate into functional cells when needed.

Trabecular meshwork stem cells are capable of suppressing inflammation and fibrosis locally and free of tumorigenesis *in vivo*.

Corneal Stem Cell Research



Approximately 8 million people worldwide have potentially blinding corneal scars. Although corneal transplantation can restore vision to these eyes, in many cases it is not available or suitable. Success of these corneal transplantations can be limited by a number of factors, including graft failure, immune rejection, and limited access to donor tissue. Additionally, countries with the highest rates of corneal blindness often lack the infrastructure needed to treat those with corneal disease.

For more than a decade, **James Funderburgh, PhD**, and his colleagues in the Corneal Cell Biology Laboratory at the UPMC Eye Center have worked at developing a stem cell-based approach to therapies that can cure corneal blindness without the need for surgery.

Early in the research process, Dr. Funderburgh and his team derived stem cells from human corneal stromal tissue and demonstrated that they were capable of differentiating into keratocytes. The team showed that these cells can be greatly expanded in culture and under the proper conditions, generating a cornea-like tissue that may be suitable for bioengineering off-the-shelf grafts to replace scarred regions of corneas.

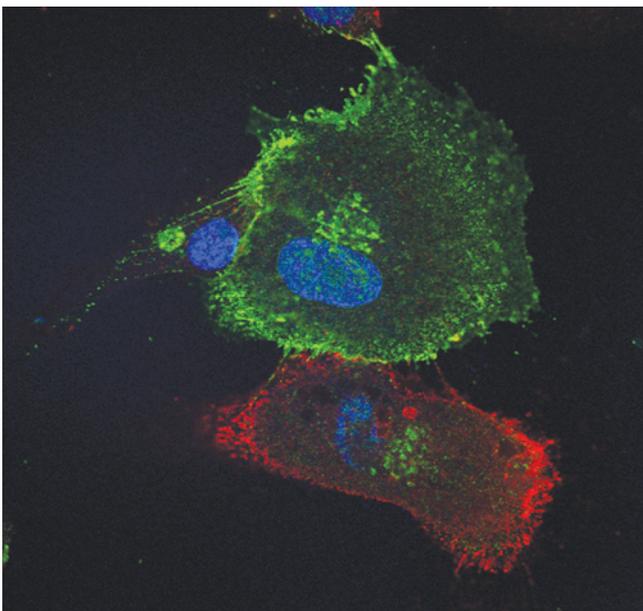
In more recent years, Sayan Basu, MBBS, MS, a physician-scientist from L.V. Prasad Eye Institute in Hyderabad, India, has spent time working in the Cell Biology Lab and found that stem cells suppress scarring in animal models by simply layering cells on the surface

of the cornea. This suggests the potential that scarred human corneas might be treated without penetrating surgery.

Since that time, Dr. Basu has returned to India and obtained approval to assess the technique in patients. In an initial trial, results were encouraging and no adverse reactions were observed. Dr. Basu has now obtained permission to carry out a more involved clinical trial, to test the idea that a patient's own stem cells might cure corneal scarring without the need for donor tissue or surgery. Dr. Funderburgh is directing work toward obtaining FDA approval for similar studies in the United States.

"It not always be possible to derive stem cells from corneal tissue, especially if the cornea is diseased," says Dr. Funderburgh. So during the past year, the team demonstrated that stem cells derived from wisdom teeth can differentiate to corneal cells and produce transparent corneal tissues. There is currently a movement to "bank" baby teeth when they fall out, providing children with a potential supply of their own stem cells. Dr. Funderburgh believes that these cells will also be useful in restoring vision.

As the U.S. population ages, demand for corneal transplants likely will increase, notes Joel S. Schuman, MD, FACS, of the UPMC Eye Center. However, the current popularity of laser vision correction surgery may substantially reduce the availability of donor corneal tissue, because corneas treated in this way are unsuitable for transplantation. "Stem cell transplantation holds out the promise of one day providing a permanent solution to corneal blindness without surgery," says Dr. Schuman.



What Is This?

"Dancing with Stem Cells"

The Corneal Cell Biology Lab is at the forefront of research to advance stem cell-based technologies into clinical therapies that can cure corneal blindness without the need for surgery.

Image by: James Funderburgh, PhD

Local Outflow Enhancement *(Continued from Page 1)*

A tool that allows us to see focal outflow blockage or to demonstrate segmental improvement of drainage would be very desirable. This is a problem well known in interventional cardiology, where stents are inserted into coronary arteries only after carefully mapping the perfusion after which a specific blockage is opened.

We have now used fluorescein, a dye first synthesized in 1871 by Adolf von Baeyer,⁸ to develop a canalography technique (Figure 1) with extensive application in ophthalmology, for instance to measure eye pressure and to examine the ocular surface.⁸ Our automated qualitative and quantitative image analysis can describe regionally different outflow patterns and filling times using standard fluorescence equipment and open source image analysis software. We found that whole eyes have faster nasal filling times. This is consistent with the presence of a larger Schlemm's canal and larger aqueous veins on the nasal compared to the temporal circumference.⁹ It also matches the location commonly used to enhance outflow in minimally invasive glaucoma surgery.¹⁰

We found that our refined pig eye glaucoma model has such a high tissue quality that it can be used to practice trabectome (ab interno trabeculectomy)¹⁰ and iStent¹¹ surgery. When we used the fluorescein canalography method in this model, we saw that nasal ablation of trabecular meshwork increased outflow not only in this location but circumferentially.

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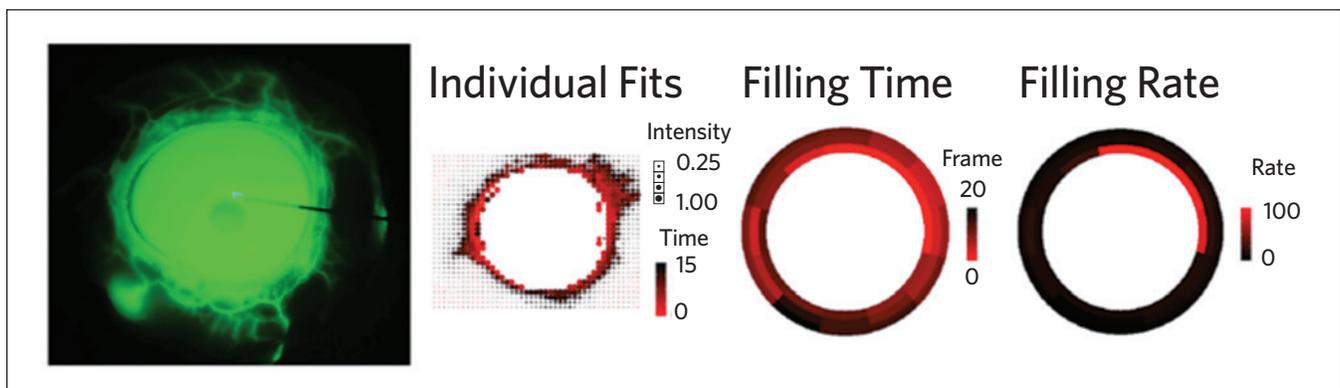


Figure 1: Quantitative canalography with dot plots using individual fits (center), and modeled perilimbal aqueous filling time and filling rate (right)

Department Briefs

Research Grants

Yiqin Du, MD, PhD, received her first RO1 grant, funded for the full five years and at the amount requested. NIH/NEI \$250,000 DC

Mechanisms of Trabecular Meshwork Regeneration by Stem Cells

The proposed studies will unveil the mechanisms of stem cell homing and regeneration for trabecular meshwork, which will lead to stem cell-based therapies for controlling intraocular pressure and prevent glaucomatous vision loss.

Gadi Wollstein, MD, Ian Conner, MD, PhD, and Matt Smith, PhD, collaborative RO1 funded for four years NIH/NEI \$250,000

Interplay Between IOP and Intracranial Pressure Effects on the Optic Nerve Head

The long-term goal of this project is to determine the effects of IOP and CSFP on the ONH and their interactions, and to identify the characteristics that are best predictors of individual sensitivity to these pressures. In an in vivo monkey model, we will control IOP and CSFP independently and simultaneously while imaging the ONH and LC with optical coherence tomography (OCT). Image-tracking techniques will be used to analyze the OCT images and measure with very high detail biomechanical effects of the pressures, such as LC displacement, scleral canal expansion, and local tissue stretch, compression and shear. Histomorphometry and modeling will be used to ascertain eye-specific anatomy and morphology to determine the best predictors of individual-specific sensitivity to the pressures, and to validate in vivo OCT findings.

Paul Kinchington, PhD, received bridge funding for the full amount asked (one year) NIH/NINDS \$307,895

Varicella-Zoster Virus-Induced Pain in a Rat Model of Post-Herpetic Neuralgia

This project studies a new model of pain induced by the herpesvirus varicella-zoster virus that is reflective of a common and highly debilitating human disease of the elderly, post-herpetic neuralgia (PHN). The examination of the model may identify new targets for the development of anti-pain strategies and may lead to the identification of improved vaccine candidates who are unable to induce pain. The project may also identify new methods to alleviate PHN using gene therapy approaches.

Jeff Gross, PhD NIH/NEI \$245,000

Genetic Regulation of Eye Development

This proposal focuses on the cellular and molecular underpinnings of choroid fissure (CF) closure. CF closure is critical for the containment of the retina and RPE within the optic cup. Defects in CF closure result in colobomas, a congenital defect in the formation of the eye. Experiments in Aim 1 test the hypothesis that podosome/invasosome-like degradative complexes mediate BM breakdown during CF closure. Experiments in Aim 2 test the

hypothesis that Par3/Par6/aPKC complex activity is required in CF cells for the formation of p190RhoGAP and Rac1-dependent nascent adhesion complexes, which spread and mature to facilitate fusion of the lateral edges of the CF.

Jeff Gross, PhD NIH/NEI \$125,000 DC

Functional Analysis of MAB21L2 Mutations in MAC Spectrum Disorders

Research in this proposal utilizes zebrafish MAB21L2 mutants as a translational model system through which we can identify the molecular underpinnings of human MAB21L2 mutations and determine the cellular function of MAB21L2 during normal eye development. We test the hypothesis that MAB21L2 activity is required for normal proliferation within the optic cup and that MAB21L2 deficiencies result in microphthalmia, which prevents apposition of the lateral edges of the choroid fissure, thereby contributing to colobomata. We combine RNA-Seq, chromatin-association assays, and LC-MS/MS proteomics to determine the in vivo function of MAB21L2 and elucidate the MAB21L2-dependent gene regulatory network and protein interactome underlying normal eye formation.

(Continued on Page 6)

Recent Publications

Ho, LC; **Wang, B; Conner IP; van der Merwe Y; Bilonick RA; Kim SG; Wu EX; Sigal IA; Wollstein G; Schuman, JS; Chan KC.** In Vivo Evaluation of White Matter Integrity and Anterograde Transport in Visual System After Excitotoxic Retinal Injury with Multimodal MRI and OCT. *Invest Ophthalmol Vis Sci.* 2015 June; 56(6):3788-800.

Hertsenberg AJ; **Funderburgh JL.** Stem Cells in the Cornea. *Prog Mol Biol Transl Sci.* 2015; 134:25-41.

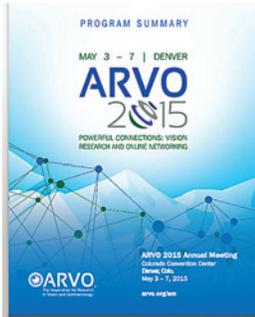
Chen CL; **Ishikawa H; Wollstein G; Bilonick RA; Sigal IA; Kagemann L; Schuman JS.** Histogram Matching Extends Acceptable Signal Strength Range on Optical Coherence Tomography Images. *Invest Ophthalmol Vis Sci.* 2015 June; 56(6):3810-09.

Kagemann L; Wang B; Wollstein G; Ishikawa H; Mentley B; Sigal IA; Bilonick RA; Schuman JS. Trabecular Meshwork Response to Pressure Elevation in the Living Human Eye. *J Vis Exp.* 2015 June 20; (100):e52611.

Delp EE; **Swamynathan S; Kao WW; Swamynathan SK.** Spatiotemporally Regulated Ablation of Klf4 in Adult Mouse Corneal Epithelial Cells Results in Altered Epithelial Cell Identity and Disrupted Homeostasis. *Invest Ophthalmol Vis Sci.* 2015 June; 56(6):3549-58.

Department Briefs *(Continued from Page 5)*

Recent Conferences



The Association for Research in Vision and Ophthalmology (ARVO)

Powerful Connections: Vision Research and Online Networking

May 2-7, 2015 • Denver, Colo.

The UPMC Eye Center had a strong presence at ARVO, being involved in more than 70 poster, paper, workshop, moderator, or symposium presentations.

Highlights

- **Robert Hendricks, PhD**, moderator “Corneal Immunology and Infections”
- **Joel Schuman, MD**, moderator “Glaucoma Imaging”
- **Joseph Martel, MD**, poster “Predictive Value of Perioperative Circumpapillary Retinal Nerve Fiber Layer Thickness in Primary Rhegmatogenous Retinal Detachment Macular Anatomic Outcomes”
- **Thomas Friberg, MD**, poster “The Effect of Scleral Buckling Selection and Corneal Thinning on Axial Length of the Eye: A Biomechanical Model”

For a full listing of involvement by UPMC Eye Center faculty members, please visit UPMCPHYSICIANRESOURCES.COM.

Nantucket Glaucoma 2015

June 27-28, 2015 • Nantucket, Mass.

This meeting brought together leaders within the field of glaucoma, each with their own unique area of clinical and/or basic glaucoma research. The format was an open conversation after brief presentations by each attendee, designed to guide discussion rather than impart knowledge. The end result was to leverage new crowdsourced knowledge to reach novel insights and open new roads for collaboration and discovery.

Attendees included Jeffrey Goldberg, MD, PhD (Professor and Chairman of Ophthalmology, Stanford University); Malik Kahook, MD (Slater Family Endowed Chair in Ophthalmology, University of Colorado); Elke Lutjen-Drecoll, MD, PhD (Emeritus Chair of the Department of Anatomy II, University of Erlangen-Nurnberg); John Berdahl, MD (Vance Thompson Vision); and Yvonne Ou, MD (Assistant Professor of Ophthalmology, University of California San Francisco).

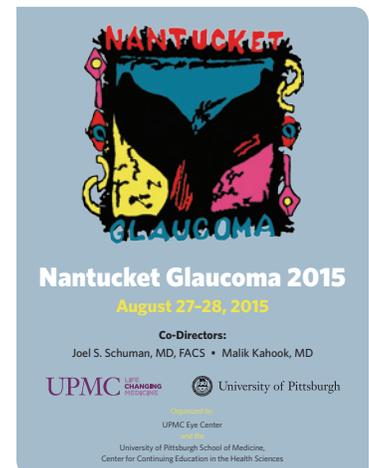
The 5th Annual International Conference Vision Restoration: Regenerative Medicine in Ophthalmology

June 25-26, 2015 • Pittsburgh, Pa.

This conference brought together world-renowned experts in the fields of ophthalmology and regenerative medicine. Medical advances in the field of regenerative ophthalmology are accelerating at an unprecedented rate. Technologies such as cellular therapies, bioscaffolds, and assistive technologies are now being used or tested in clinical trials across the country. This conference created a forum for collaborative communication that will lead to cross-disciplinary work and concrete results.

Dr. Sheila Nirenberg (Professor of Physiology and Biophysics, Cornell University) presented the keynote address.

Speakers included Colonel Jeffrey Cleland (Director, Ocular Trauma Division US Army), Jeffrey Goldberg, MD, PhD (Professor and Chairman of Ophthalmology, Stanford University), Larry Benowitz, PhD (Professor of Neurosurgery, Ophthalmology, and Neurobiology, Harvard Medical School), and Robert Gulberg, PhD (Executive Director, Institute for Bioengineering and Bioscience, Georgia Institute of Technology).



VIDEO ROUNDS AND CME

Video Rounds is a series of short, informative, and educational videos created for physicians and covering a variety of medical and surgical disciplines.

Evolution of Corrective Eye Surgery

Presented by Deepinder Dhaliwal, MD, LAc



Beginning with the development of LASIK, ophthalmologists have had a wide array of resources at their disposal, including both new technologies and improvements to existing tools. Two particular devices are now available with the potential to revolutionize the field of vision correction.

Deepinder Dhaliwal, MD, LAc, director of Refractive and Laser Surgery at the UPMC Eye Center, talks about the evolution of vision correction surgery and how new devices show great potential in improving vision and quality of life.

To view this video or others in our Video Rounds library, visit UPMCPhysicianResources.com/VideoRounds

UPMC Physician Resources brings free educational **CME** opportunities to your computer and iPad®.

Transorbital Approaches: An Oculoplastic Perspective



In this free online CME, **Tonya Stefko, MD**, reviews orbital anatomy, reviews transorbital and lateral orbital approaches, and provides some case examples.

To view this video or to view more resources, visit UPMCPhysicianResources.com/Ophthalmology



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Call for Nominations

2016 Albert C. Muse Prize for Excellence in Ophthalmology

Nominations are now being accepted for the 2016 Albert C. Muse Prize for Excellence in Ophthalmology.

The Eye & Ear Foundation of Pittsburgh and the UPMC Eye Center invite you to submit a nomination for the 2016 Albert C. Muse Prize in Ophthalmology.

The Albert C. Muse Prize was established in 2001 by the Eye & Ear Foundation of Pittsburgh to honor world leaders in the fields of ophthalmology and otolaryngology. The Prize, which alternates annually between the two fields, carries

a cash award of \$5,000, and recognizes individuals who have made outstanding contributions to science and medicine in these specialties.

The 2016 Albert C. Muse Prize in Ophthalmology is open to individuals responsible for extraordinary advances in ophthalmology and vision science. For more information, including nomination form and submission information, visit EyeandEar.org.

The deadline for nominations is January 16, 2016.

ABOUT THE UPMC EYE CENTER

The UPMC Eye Center is a leader in the diagnosis and treatment of eye diseases and disorders, and is helping to advance the field through extensive research.

- Seeing more than 80,000 patients annually, the UPMC Eye Center provides state-of-the-art testing, including electrophysiology and psychophysics services, to diagnose eye, retinal, and optic nerve disorders.
- The Department of Ophthalmology has one of the top basic and clinical research programs in the country. As a leader in National Eye Institute funding, the department's research focuses on ocular immunology, infectious disease, molecular genetics and molecular biology of retinal disease, glaucoma, regenerative ophthalmology, and advanced diagnostic imaging technology development.
- The Louis J. Fox Center for Vision Restoration is the world's first comprehensive program dedicated to ocular regenerative medicine. Researchers at the Fox Center and the University of Pittsburgh continue to develop innovative solutions, such as bioengineering of ocular tissue, stem cell and gene therapy approaches, and novel drugs or drug delivery systems.



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UPMC Eye Center's mission is to improve the quality of life through the preservation and restoration of vision.

As part of the commitment to delivering high-quality clinical and research information to physicians, the UPMC Eye Center partnered with *Ocular Surgery News* for a monthly column. More information and links to the articles can be found on UPMCPPhysicianResources.com/Ocular.

Related Links:

Louis J. Fox Center for Vision Restoration
FoxCenter.Pitt.edu

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A world-renowned health care provider and insurer, Pittsburgh-based UPMC is inventing new models of accountable, cost-effective, patient-centered care. It provides more than \$888 million a year in benefits to its communities, including more care to the region's most vulnerable citizens than any other health care institution. The largest nongovernmental employer in Pennsylvania, UPMC integrates 60,000 employees, more than 20 hospitals, more than 500 doctors' offices and outpatient sites, a 2.8-million-member health insurance division, and international and commercial operations. Affiliated with the University of Pittsburgh Schools of the Health Sciences, UPMC ranks No. 13 in the prestigious *U.S. News & World Report* annual Honor Roll of America's Best Hospitals. For more information, go to UPMC.com.