Grant Application Notes 2021
Research (3)

Institution	
OSU Optometry PI: Andrew Hartwick	**Efficacy of Light Therapy for Traumatic Brain Injury-Associated Photophobia** Many people develop intolerance to light after a head injury. With this condition, ambient light levels can cause discomfort, resulting in these individuals often having to wear dark sunglasses even while indoors. We do not fully understand why this occurs and how it should be treated. A growing number of clinicians have reported success in treating this condition through daily exposure of these individuals' eyes to blue light emitted from a light box. However, the effectiveness of this treatment has yet to be assessed in a well-controlled clinical study. In this work, we will recruit individuals who had a recent brain injury and developed light intolerance. Participants will be divided into two groups: one that is instructed to undergo at-home daily 20 minute treatments with blue light for 12 weeks; and another that will undergo a similar treatment routine with red light. Every 3 weeks, masked examiners will record pupil responses to light and collect information about participant symptoms. In a cross-over study design, the participants will then switch to the other treatment regime for another 12 weeks, with data being again collected every 3 weeks. Potential differences in pupil responses to light between the treatment groups will be assessed as an objective outcome measure and compared to subjective symptom survey information**. Relevance**: This work will test the hypothesis that daily blue light treatments can alleviate light intolerance in participants after head injury, and this improvement is associated with altered pupil responses to light. As a significant minority of eye care clinicians currently provides this treatment, this study has the potential to influence clinical practice patterns whether the light therapy is shown to be effective or not.
OSU Ophthalmology PI: Colleen Cebulla & Shigeo Tamiya	**Genetic, neurodegenerative, and proliferative mechanisms in retinal damage and ocular tumors, and potential for therapy** We have identified important inflammatory proteins that contribute to damage that occurs in retinal detachment (RD), a prevalent condition that frequently results in loss of reading vision. Visual loss results from photoreceptor degeneration as well as scarring, which alters retinal structure and function. In addition, patients may develop a form of scarring called epiretinal membrane. There are currently no effective pharmacologic treatments for these diseases. Our long-term goal is to develop neuroprotective and anti-fibrotic therapies to prevent visual loss from RD and other damaging retinal conditions, such as age-related macular degeneration. This proposal will evaluate underlying cellular, genetic, and protein changes providing the basis for neurodegeneration and retinal scarring, with a future goal to identify proteins that can be effectively targeted by therapy. This project may also lead to discoveries which benefit other retinal disorders, including diabetic retinopathy. Our work on ocular tumors like ocular melanoma is critical to identify at-risk individuals and families for cancer and hopefully allow earlier intervention and fewer cancer deaths. It will hopefully uncover mechanisms of disease that can lead to new therapeutic options. Because eye cancer is rare, drug companies typically do not support this research; groups like OLERF are critical to help advance improvements in eye cancer and other eye diseases. This work may allow detection of disease at an earlier stage and improve outcomes for patients. The results may

	improve detection and early treatment of ocular and other cancers as well as find novel mechanisms to develop new cancer treatments.
U of Cincinnati PI: Winston Kao	**Gene and Cell Therapy of Ocular Surface Disease** Lysosomal storage diseases (LSDs), a family of inherited diseases caused by a mutation in genes of lysosomal enzymes/proteins, resulting in accumulation of metabolites in situ and lack of nutrients to cells. Both of which lead to cells and tissues dysfunctions. Lysosomal enzyme/proteins are secreted almost by all cell types, i.e., epithelial, endothelial and mesenchymal cells, via EV (extracellular vesicles) into interstitial tissues, e.g., connective tissues, and interstitial fluids, e.g., blood and lymph fluid. Thus, correction of a fraction of somatic and somatic stem cells, e.g., liver cells that secret functional enzyme via EV, can ameliorate systemic symptoms. In the past few years, we have dedicated in developing novel regimens of umbilical mesenchymal stem/stromal cells (UMSCs)1-3 and gene therapy via transgenesis and CRISPR gene editing techniques to treat congenital and acquired cornea diseases in experimental mouse models. During next funding period, we will perform a series of studies in four Aims. Aim 1, Using transgenesis with self-complementary scAAVβGlu viral vector and CRISPR HMEJ (homology mediated end joining) to treat Gusb/MPS VII mice. Aim 2, Use of self-complementary scAAVCtns viral vectors for cystinosis. Aim 3, To determine the optimal culture conditions and identify biomarkers for UMSCs that have best therapeutic capacity of regenerating transparent corneas following alkali burn. Aim 4, To develop novel gene therapy strategy for glaucoma. **Relevance** The proposed studies will validate efficacy of gene therapy strategies by conventional transgenesis and novel CRISPR gene editing techniques for curing inherited LSDs, and to define the best culture conditions for preparing MSCs having the highest therapeutic capacity for regenerating transparency of traumatized and diseased corneas.
Akron Children's	
CWRU PI: Paul Park	"Generating a rhodopsin-YFP fusion knockin mouse by CRISPR/Cas9" Photoreceptor cells in the retina house the machinery for the first steps in vision. Rhodopsin is the light receptor that initiates a set of biochemical reactions called phototransduction upon photon capture. Dysfunction in rhodopsin and signaling proteins in phototransduction can cause a variety of retinal diseases including those that cause retinal degeneration. Mutations in rhodopsin are a leading cause of autosomal dominant retinitis pigmentosa, a progressive retinal degenerative disease currently without a cure or effective treatment. Questions related to the dynamics and structural properties of rhodopsin and other signaling proteins in photoreceptor cells need to be answered to better understand the mechanisms underlying retinal degeneration arising from photoreceptor cell dysfunction. Tagging endogenous proteins with fluorescent proteins is a powerful method in cell biology and will help advance studies to unravel the mechanisms underlying retinal degenerations. While this has been accomplished and used widely in cell culture systems, more work is required to achieve similar success in animal models. Expression of rhodopsin tagged with a fluorescent protein has been achieved in animals such as transgenic X. laevis and knockin mice. While these animal models have been useful in some regards, further improvements are necessary to advance studies related to photoreceptor cell biology and retinal disease. We introduce several

	improvements over previous attempts that will achieve proper expression of rhodopsin, avoid retinal degeneration, make use of an improved fluorescent protein, and generate a mouse model on a C57Bl/6J background. A knockin mouse expressing endogenously expressed rhodopsin tagged with a fluorescent protein will provide a valuable tool for a variety of applications ranging from basic biophysical and biochemical studies to the testing of therapeutics and retinal disease mechanisms. Studies utilizing this mouse model will advance our understanding of photoreceptor cell biology and retinal diseases such as retinitis pigmentosa.

Bryan grants (1)

Institution	
OSU Ophthalmology PI: Cynthia Roberts & Yanhui Ma	**Corneal Biomechanics in Diabetes with and without Diabetic Retinopathy** Diabetes is associated with high blood sugar and may lead to the development of Diabetic Retinopathy, which is associated with severe vision loss. In addition, high blood sugar is also associated with a stiffer cornea. Therefore, it is proposed to biomechanically characterize the cornea and scleral response of diabetic subjects with retinopathy and compare them to both those without retinopathy as well as normal subjects in a separate study. A clinical device which uses high-speed imaging during corneal deformation with an air puff will be used to generate a set of dynamic corneal response parameters. In addition, a device which captures the pattern of vessels in the retina will be used to compare the biomechanical data to the retinal vascular data. Once this dataset of deformation parameters and vascular parameters is generated, statistical analysis will be used to develop a biomechanical index that might be used in future studies to predict the risk of a diabetic patient developing diabetic retinopathy. Early detection will allow treatment to prevent vision loss.
CWRU None	

Fellowships (2)	
NE Ohio Medical College;	
OSU Ophthalmology Fengze Wu PI: Xiaoyi Raymond Gao (year 1)	**Automatic determination of vertical cup-to-disc ratio from fundus images using artificial intelligence** Glaucoma is a chronic, degenerative optic neuropathy and the leading cause of blindness worldwide. It is a term used for a group of disorders that share a characteristic progressive excavation of the optic nerve head with associated and irrecoverable loss of the visual field. If untreated, glaucoma ultimately results in blindness. Individuals with glaucoma typically do not show symptoms for years and their case may become advanced before they notice an extensive visual field loss in one or both eyes. Early detection and treatment are crucial for preventing vision loss from glaucoma. Glaucoma presents a significant disease burden. It affects 70-90 million people worldwide and is responsible for blindness in approximately 4.5 million people. Furthermore, about half of glaucoma cases are not aware that they have glaucoma even in developed countries. The US economic burden for glaucoma care is estimated at $2 .86 billion annually. Vertical cup-to-disc ratio (VCDR), the ratio of (vertical diameter of cup)/(vertical diameter of disc) in the optic nerve head region, is an important structural parameter for glaucoma. People with glaucoma tend to have larger VCDR. As the

	disease progresses, the optic nerve fibers begin to die and the optic cup becomes larger in comparison to the disc. VCDR greater than 0. 7 is a typical criterion for glaucoma. VCDR can be estimated by eye specialists during eye exams or from fund us images. However, this procedure is time-consuming and is subjective to personal experience. Automatic approaches deriving VCDR from fundus images not only reduce the manual burden, but also provide an objective and quantitative determination of VCDR. This study aims to design and implement an automated system for determining VCDR from fundus images using a state-of-the-art artificial intelligence approach, addressing the critical need for an automated solution for glaucoma screening.
Univ. of Cincinnati Jhuwala Venkatakrishnan (PI Winston Kao) (year 5)	Corneal diseases are one of the leading causes of blindness globally. Current treatment approaches involve surgical procedures such as full thickness corneal transplantation, partial-thickness transplantation, etc. These procedures involve the use of topical corticosteroids with or without adjuvant immunosuppressant therapy, which are often compromised by graft rejection and side-effects of immunosuppressants. Mesenchymal stem/stromal cells (MSCs) isolated many connective tissues are currently being investigated as a potential treatment approach for corneal disorders. They are widely used in experimental medicine often as the last resort for devastating health problem, e.g., pulmonary fibrosis. However, therapeutic efficacy of MSCs differs across the globe due to inability to identify and procure high quality cells owing to the lack of standardized protocol to culture MSCs and specific biomarkers associated with therapeutic capability, including umbilical mesenchymal stem/stromal cells (UMSCs) derived from umbilical cords. UMSCs are known for its immune-modulating and anti-inflammatory properties. UMSCs proliferate at a logarithmic rate. In the initial early log phase, these cells have lower cell density with high proliferation rate. As the cells enter the mid-log and late-log phase, the proliferation rate decreases. Cells in each growth phase have different cell densities and distinct secretory profiles of cytokines and chemokines. Determining the appropriate cell growth phase would improve the chances of selecting cells with higher therapeutic efficacy. In addition to the cell culture conditions, conditioning of these cells by pre-treating with certain cytokines may also enhance cells ability to produce anti-inflammatory and immune-modulating factors. Studies suggests that MSC pre-treated with Transformation Growth Factor–Beta1 (TGF-β1) can better promote wound healing capacity in experimental mouse models. Thus, it is plausible to hypothesize that selection of cells at the appropriate growth phase along with licensing these cells with TGF-β1 will provide highly efficacious UMSCs for treating ocular surface diseases.
OSU Optometry Elizabeth Day, Od, MS (PI: Andrew Hartwick)	**Relating Light-Driven Pupil Responses to Melatonin and Chronotype** Those who report sleep problems, but do not meet the criteria for diagnosis of a clinical sleep disorder, still experience functional impairment in their daily lives. Poor sleep, even at a subclinical level, increases the risk of diabetes, cardiovascular disease, obesity, and death. The current public health strategy to combat poor sleep is a collection of one-size-fits-all sleep hygiene recommendations, which produce inconclusive outcomes in controlled studies. Two major factors in sleep onset and quality are light exposure and melatonin. Light reduces melatonin release by the pineal gland. Melatonin is a molecule that slowly builds up in the bloodstream throughout the day. Once the concentration reaches a certain threshold, we feel tired enough to go to sleep. Light also worsens

	sleep quality by causing shallow sleep, more sleep interruptions, and increased brain activity. Intrinsically photosensitive retinal ganglion cells (ipRGCs) are the signalers of light to the brain, and their activity has been linked to lower melatonin levels. ipRGC activity is measured through light-induced pupil constriction, and ipRGC input creates a strong, sustained pupil constriction that lasts for multiple seconds after the light turns off (called the post-illumination pupil response, or PIPR). This phenomenon is the marker for ipRGC activity in humans. We expect that the alternating red-blue light above melanopsin threshold, the 6 Hz flickering red and blue lights, and the 6 Hz flickering checkerboard will evoke greater pupil constriction, indicative of ipRGC activity, compared to the other stimuli. These results will support our hypothesis that ipRGCs are not simple photon counters that are completely controlled by melanopsin. We also expect that those with a stronger light-induced pupil constriction, and therefore a stronger light-driven ipRGC response, will also present as later chronotypes and have a lower level of melatonin concentration at the test time compared to those with weaker light-induced pupil constriction. We can then use light-induced pupil constriction, an objective test, to complement chronotype questionnaires and gain a better understanding of each person's sleep needs and behavior. This will lead to an individualized approach to sleep intervention, "personalized sleep hygiene," adapting the current models of sleep hygiene and emphasizing some components over others to promote the best possible outcomes. For example, to those with a stronger light-induced ipRGC response, it is even more important to recommend avoiding all forms of light exposure in the hour or two before sleep. These results will also promote more research into sleep behavior and other aspects of sleep hygiene, such as cortisol levels and caffeine intake.

Grants related to AMD (1)

Institution	
Wright State University	Dr. Organesiak has retired.
OSU Optometry None	
OSU Ophthalmology PI: Nagarai Kerur, PhD	**AMD: Unraveling Inflammatory Mechanisms in Aging Eye** Geographic atrophy (GA) is an advanced form of age-related macular degeneration (AMO} characterized by central vision loss due to retinal-pigmented epithelium (RPE) cell death. Aging is the most important risk factor for AMO. Emerging data suggests that pathogenesis of GA is linked to over-activation of a tissue-damaging immune pathway. However, the connection between aging and the mechanism by which aging influences over-activation of tissue-damaging immune responses in AMO is currently not known. Here, we hypothesize that, aging-associated DNA damage in eyes with AMO is responsible for the over-activation of the tissue-damaging immune pathway.
CWRU None	

Lois Hagelberger-Huebner Young Investigator Applications (2)

Applicant	

Maryo C. Kohen MD., FICO, FEBO, FEBOS, mRCSE Physician/Research Faculty Rainbow Babies & Children's Hospital and Case Western Reserve University	NOVEL DRUG DELIVERY DEVICE FOR PIRFENODINE TO REDUCE FIBROSIS IN PEDIATRIC GLAUCOMA DRAINAGE DEVICE IMPLANTATION **The proposed bioactive material platform will set the groundwork for the first therapy that will tackle foreign body reaction issues judiciously after GDD implantation for long term. The platform holds great potential in reducing re-operation rates while enhancing low IOP to maintain good visual potential in long term for kids.** Following glaucoma drainage device (GDD) surgery it is the foreign body reaction forming a collagen-rich capsule that poses the major resistance to aqueous humor flow through GDD. This is especially important in pediatric population since they will be keeping the GDD for all their lives and need a physiological IOP not to lose vision. Our overreaching hypothesis is inhibition of foreign body reaction by pirfenidone and having a sustained anti-fibrosis effect by a slow release system via biodegradable sheath. We are proposing a biodegradable drug delivery system to address the foreign body reaction following GDD implantation by inhibiting fibrosis by a novel drug called pirfenidone. There is extensive in vivo and clinical literature demonstrating efficacy of pirfenidone for various fibrogenic conditions. Also, our preliminary data indicate that pirfenidone reduces macrophage polarization to pro-fibrogenic form, and suppress proliferation and collagen production by fibroblasts in vitro. In animal models pirfenidone has been shown to reduce the foreign body reaction after GDD implantation. However its effect has been limited by short term use. Long term effect is paramount in children as they are more prone for foreign body reaction and they need GDD to work properly for very long time not to lose vision further. For the first time in the literature we are proposing a slow release mechanism by a biodegradable sheath for the aforementioned drug to minimize the foreign body reaction thus saving the patient from another surgery to maintain the vision. Our drug delivery mechanism will be made out of biodegradable poly (lactic-co-glycolic acid) (PLGA) sheath that is loaded with pirfenidone by our patent pending computerized rotational jet spraying (CORJET) method for drug delivery. **Aim 1: Identify effective dose and material formulations for the drug delivery system.** *Refinement of biomaterial formulations:* PFD starter dose determined in in vitro studies in macrophage cell studies in 2020. We will be further testing PLGA formulation that will degrade in vivo during the first three weeks in vitro determination of a starter dose of PFD from PLGA that in different cell cultures such as fibroblasts. **Aim 2: Decrease foreign body reaction to GDD in vivo** The delivery platform formulations developed in Aim 1 will be applied in tandem in a rabbit model to determine if the foreign body reaction is reduced after GDD implantation. The biology of foreign body reaction will be assessed at early and later time-points by quantitative immunohistochemical analysis of inflammatory response and de-novo collagen deposition.