Special Issue

Preface: Sight Restoration Through Stem Cell Therapy

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This publication presents chapters based on a meeting entitled "Sight Restoration Through Stem Cell Therapy" held on June 13, 2015, in Santa Monica, CA, sponsored by the Ocular Research Symposia Foundation (ORSF). It was chaired by Michael Young, PhD, Harvard Medical School, and Gerald Chader, PhD, University of Southern California. The mission of this publication and of the ORSF in general is to focus attention on unmet medical needs and current research opportunities in eye research with the objective of accelerating translation of research findings to effective clinical care. In the meeting, new research advances on stem cells and opportunities for their clinical application were highlighted and are recounted in the following chapters of this publication. By identifying "low-hanging fruit" (i.e., the best opportunities for successful transition of laboratory research to prevention and new treatments and cures for ocular diseases), we seek to spur funding at both the basic research and clinical levels, resulting in sight-saving and sight-restoration measures in the near future.

Keywords: ORSF, sight restoration, stem cell therapy

The ORSF's Unique Approach to Promoting Movement Toward Effective Treatments

Government agencies certainly provide the bulk of funding for preclinical medical research on eye diseases in the United States and around the world. Along with this, there are numerous private foundations that fund research on specific ocular diseases. Instead of this type of direct funding, the Drabkin Foundation focuses its resources on increasing awareness of the best treatment opportunities available for eye diseases and, through this, increasing the possibility of funding for treatment development. It does this through "education," essentially by spotlighting research opportunities that are ripe for translation into clinical therapies. First, experts in a particular area of basic vision research and in clinical ophthalmology are convened to review the most current scientific advances and identify those avenues that could directly lead to a viable disease treatment. A summary document is then written and distributed to stakeholders in the funding arena: members of Congress, government agencies, foundations, and companies, as well as to academic departments and individual investigators. This document then can be used as a "roadmap to a cure" (i.e., a clear, logical path to a treatment for a major blinding condition). Funding, therefore, can best be focused on the projects with the highest payoff. The ORSF thus occupies a unique position in eye research. It not only identifies the greatest needs in ophthalmic disease treatment but it highlights and promotes the best and most practicable opportunities for treatments for these conditions as pin-pointed by worldwide leaders in eye research and clinical care.

Following is a short description of the aims of the meeting and of this publication, as well as bullet-point listings of key needs and opportunities for addressing sight restoration using stem cell therapy.

What Are the Needs for Finding New Treatments?

Every year, millions of Americans and countless more around the globe lose significant vision or become blind. This number is expanding dramatically as life spans increase along with changes in lifestyles that run counter to good eye health. As well as losing what Americans perceive as their most important sense, there is a huge cost to pay in the loss of an individual's quality of life and independent living. In addition, the increased financial burden on the individual and on society is enormous.

What Are the Opportunities for New Treatments?

Research on the causes of specific eye diseases has been especially productive in recent years, such that the causes of many of these diseases are now understood. Some, for example, are now know to be genetic in nature with the underlying cause a mutation in a gene that regulates the synthesis of an important protein needed for proper cell or tissue functioning. The past 2 decades have taken us from virtually no knowledge of these faulty genes to knowing the identity of a substantial number of them along with now having the gene therapy tools to replace these genes with normal copies and restore at least partial visual function. Biologically, many of the cellular pathways leading to ocular cell dysfunction and death have also been mapped. Understanding these pathways now allows us to use specific agents and drugs that block retinal cell death and prolong and even improve vision. This is not only true for young patients with limited damage but for older patients who may have already lost a substantial amount of their eyesight.

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The result of all this accumulated knowledge is a recent upsurge in our capability to move from the bench to the clinic, testing out new therapies in clinical trials. Importantly, preclinical studies have shown that we can stave off blindness and partially restore vision in many animal models of ocular disease. Although there remains a need for more basic research in understanding chronic ocular problems, the scientific barriers have now fallen in our understanding of many of the causes of ocular diseases and in the approaches we can take for their treatment. Now is the time to capitalize on these opportunities. What remains is finding the will and the financial resources to use this knowledge to move through clinical testing and provide treatments to all in need.

What Are the Major Unmet Needs and Opportunities for Treating Individual Eye Diseases?

In general, scientists and clinicians believe that the greatest "unmet needs" in eye treatment remain in diseases of the neural retina and in specific conditions of the ocular surface. In particular, diseases of the neural retina have been difficult to understand and, therefore, to treat. Successful treatment of blinding retinal diseases lags far behind conditions such as cataract, for example, which can usually be quickly remedied through relatively simple surgery. However, new information on retinal diseases such as glaucoma, AMD, retinitis pigmentosa (RP), and diabetic retinopathy (DR) now makes successful treatment of these conditions a practical reality. Major target areas are described in the following paragraphs.

Dry Eye and Other Ocular Surface Conditions

Conditions of the ocular surface are a major health problem around the world. These problems range from external insult and trauma to diseases such as dry eye syndrome that can lead to vision loss and blindness as well as great discomfort. Although dry eye can occur at any age, the National Eye Institute estimates that 5 million Americans 50 years of age or older suffer from dry eye. Being on the external surface of the eye, these conditions are particularly amenable to treatments such as stem cell therapy.

Glaucoma

Glaucoma affects more than 2 million Americans with an additional 5 to 10 million at risk. It is now recognized that glaucoma is a collection of diseases whose common endpoint is death of ganglion cells in the neural retina with resulting severe vision loss and in some cases blindness. Many factors increase a person's risk for glaucoma, increased IOP within the eye being just one of them. Our new knowledge allows us to go well beyond simply trying to lower IOP through drugs and surgical interventions, but to directly treat the ganglion cells and their extended processes, collectively called the optic nerve. Drugs called "neuron-survival agents" are now being tested to see if they can block ganglion cell death and maintain a healthy optic nerve, thus prolonging functional vision. This is called "neuroprotection." Preclinical testing of these agents delivered to the eye through sophisticated gene therapy techniques is well advanced. Stem cell therapy is a particularly attractive candidate for use in glaucoma treatment because it can be delivered to both anterior and posterior segment targets for either cell replacement or delivery of trophic agents. It was agreed at the meeting, though, that significant hurdles remain, especially reconnection to the brain by regenerated retinal ganglion cells.

Age-Related Macular Degeneration

Authorities estimate that there are well over 2 million cases of AMD in the United States and that this figure will be more than 3 million by 2020 in the absence of new, effective therapeutic measures. An additional 10 to 15 million are at risk because of factors such as population aging, bringing the count to epidemic proportions if remedies are not soon found. Age-related macular degeneration is now recognized to be a "complex disease" in that its causes have both genetic and environmental components. Recently, several genes have been identified whose mutations increase the risk of developing AMD. Environmental factors, such as oxidative damage caused by smoking, also significantly increase the risk and can interact with the genetic factors, making the combined risk extremely high in the population older than 60 years. Identification of the genetic factors as well as factors such as oxidative damage now gives us a firm scientific basis for developing new treatment modalities. Even in the situation of wet AMD where there is the growth and leakage of abnormal blood vessels, new research is leading to the possibility of effective treatments other than anti-VEGF agents. In both wet and dry AMD, stem cell therapy could supply needed neurotrophic factors as well as replacement of either or both photoreceptor and retinal pigment epithelial cells.

Diabetic Retinopathy

There are approximately 18 million Americans with diabetes. Within 10 years of onset, 75% of these will have some signs of DR. This can lead to severe vision impairment or blindness, even by midlife. In DR, abnormal growth and leakage of retinal vessels occurs, somewhat as in wet AMD. We now know many of the basic factors that, secondary to the underlying diabetic condition, lead to DR. One of these is oxidative damage, again as seen in AMD. New research demonstrates the efficacy of specific antioxidants in decreasing oxidative stress and inflammation in the retina and thus the problems in vision caused by DR or AMD. Here too, though, stem cell therapy could alleviate at least some of the many problems encountered in DR through both neuroprotection and cell replacement.

Retinitis Pigmentosa and Allied Diseases

Retinitis pigmentosa is a large family of hereditary diseases that causes degeneration and death of retinal photoreceptor neurons. The RP degenerative diseases are rare, but generally begin early in life, resulting in reduced vision in the young adult and often blindness as the degeneration relentlessly progresses in subsequent years. In 1990, the first gene mutation leading to a form of RP was reported. Now, more than 200 genes are known whose mutations lead to different types of retinal degeneration. Using gene therapy, remarkable progress has recently been made in replacing defective genes and restoring functional sight. Moreover, progress is being made in several other research areas, such as stem cell therapy, that allows for current clinical trials on sight restoration or should enable these trials to take place in the near future. Stem cell therapies could be particularly effective in restoring cellular photoreceptor and RPE layers. Besides RP, stem cell replacement also appears to be applicable to other retinal degenerative diseases, such as Leber Congenital Amaurosis, an early childhood form of blindness.

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Treatment Approaches

There are at least six different types of therapies that are currently being applied in treating ocular diseases. The type of therapy, though, depends on the condition of the target cell: yet alive (although impaired) or dead/dysfunctional. For example, gene therapy in a retina in which photoreceptor cells are all dead will not be effective in restoring vision. Thus, gene therapy, the use of neurotrophic agents, and antioxidant therapy can be applied only if enough target cells (e.g., photoreceptors, ganglion cells) are present to elicit a therapeutic effect. When target cells are dead though, there is a need to actually replace them or at least replace their function. One of the recent great therapeutic successes in sight restoration is use of the electronic prosthesis, a device that replaces function of dead/dysfunctional photoreceptor cells in advanced cases of RP. Similarly, optogenetics offers a new method for functional replacement of photoreceptor cells. On the whole though, the simplest and possibly most effective treatment could be stem cell replacement therapy, where "natural cells" could replenish the supply of lost cell types such as photoreceptor and RPE cells in AMD/RP and ganglion neurons in glaucoma.

In our ORSF symposium, we have considered the state of the art in the use of stem cells to restore functional vision in a number of currently untreatable or poorly treatable ocular conditions. Although many challenges remain that still necessitate significant preclinical research, the field has advanced to a point where well-defined opportunities are now present that could lead to restored vision in several major blinding eye conditions. In particular, there are many opportunities now for translating this "low-hanging fruit" of successful bench work on stem cells into clinical trials and sight-saving and restoring treatments.

Following is a summary of key needs and opportunities for such advances in stem cell research compiled by the panel of experts convened at the ORSF symposium on June 13, 2015.

1) **Stem Cell Sources – Valeria Canto-Soler, PhD, Wilmer Eye Institute, Johns Hopkins University, Baltimore, Maryland, United States**

Short-Term Needs and Opportunities:

a) Prioritize progress in human-induced pluripotent stem cell (hiPSC) technology, the most promising stem cell source to date:
   i) Improve methods for cell line manufacturing to reproducibly obtain cell lines with optimum differentiation efficiency and reproducibility. This includes a combination of the best primary cell source and the best reprogramming method.
   ii) Continue efforts to improve (and accelerate?) cell differentiation in vitro.
   iii) Continue efforts to establish cell line production pipelines compliant with current good manufacturing practices.
   iv) Continue efforts to establish banks for HLA-matched hiPSC universal donors.
   v) Develop methods to generate clinical grade, genetically corrected hiPSC for autologous transplantation.

b) Establish appropriate platforms for evaluating non–cell-based therapeutic strategies. This includes appropriate disease models and drug screening platforms, for example.

Long-Term Needs and Opportunities:

c) Continue efforts to "unlock" the mechanisms of endogenous regeneration in the human adult retina as a potential regenerative strategy in the future.

2) **What Can We Learn from Retinal Development? – Sui Wang, PhD, Department of Genetics, Harvard Medical School, Boston, Massachusetts, United States**

a) Dissect all genetic regulatory networks (GRNs) that regulate the genesis of rods and cones (different cone types) in different retinal progenitor cells (RPCs) and precursors.
b) Understand how these GRNs transition between different developmental stages.
c) Characterize RPC heterogeneity and label distinct RPCs.
d) Discover the GRNs that control *photoreceptor maturation* (outer segments and synaptogenesis) and *maintain photoreceptor function*, and understand how these GRNs are dysregulated under disease conditions.

3) **Mimicking Retinal Development and Disease With Stem Cells – David Gamm, MD, PhD, Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison, Wisconsin, United States**

a) Understanding and modulating the diversity of neural retinal cell types differentiated from human pluripotent stem cells over time.
b) Maximizing retinal cell maturity and function.
c) Achieving targeted photoreceptor enrichment, cone subtypes, and rods.
d) Building more complex three-dimensional retinal model systems by adding RPE, vasculature, and so forth.
e) Understanding and manipulating "aging" of cells/tissues in culture in relation to AMD, primary open-angle glaucoma, and so forth.
f) Recapitulating relevant environmental influences in vitro, considering factors such as oxidation, light, high IOP, and so on.
g) Understanding the role of epigenetics and gene modifiers in disease modeling.

4) **Strategies for Photoreceptor Cell Regeneration – Thomas Reh, PhD, Department of Biological Structure, University of Washington, Seattle, Washington, United States**

a) Transplantation of photoreceptors as dissociated cells or in small aggregates into the subretinal space of normal mice causes a small percentage of the transplanted cells to migrate into the host retina outer nuclear layer. Improve this percentage.
b) Methods to purify rods and cones from stem cells will be important for translation of these results to humans.
c) Improvements to the degree of integration between transplants of either retinal sheets or dissociated cells in late stages of retinal degeneration are needed.
d) Most studies have been done in *normal* mice, more studies need to be carried out in late-stage retinal disease.
e) Most transplant studies are done in *mice*; transplants in animals with larger eyes would be useful to predict how the cells will behave in human retinas.
f) Only two studies have analyzed the effects of transplants on the survival of the remaining host cones. *It is important to determine whether transplants early in the disease can affect the progression.*
g) The retina undergoes extensive remodeling after rods and cones have degenerated, with aberrant sprouting, glial hypertrophy, and disruptions in normal lamination of the inner retina. *No study has yet tested*

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whether this can be reversed, or even prevented, by a photoreceptor transplant.

h) The degree to which the Muller glial hypertrophy and retinal reorganization that occurs after cone degeneration contributes to the very limited visual improvement observed in the human patients who have received transplants needs further evaluation.

5) **The Application of Stem Cells to Ocular Surface Diseases – Sheffer Tseng, MD, PhD, Ocular Surface Foundation, Miami, Florida, United States**
   a) Recognize the causative relationship between inflammation and regeneration.
      i) Consider control of inflammation (focusing on M1 to M2 macrophages) to abort inflammation and to promote regeneration.
   b) Formulate a different and more effective strategy to control inflammation.
      i) Consider HC-HA/PTX3 as the novel matrix to target multiple aspects of inflammation mediated by different innate and adaptive immune cells.
   c) Reprogram into lineage-committed progenitors in lieu of iPSCs.
      i) Consider using HC-HA/PTX3 for such reprogramming.
   d) Investigate how the in vitro niche regulates limbal stem cell quiescence, self-renewal, and fate decision.
      i) Consider using in vitro niche to devise an effective ex vivo expansion protocol and other therapeutics.

6) **Stem Cell Uses in Glaucoma – Donald Zack, MD, PhD, Wilmer Eye Institute, Johns Hopkins University, Baltimore, Maryland, United States**

   Short-Term Needs and Opportunities:
   a) Further explore the use of stem cell–derived trabecular meshwork cells as a complementary approach to reduce IOP.
   b) Identify small molecules and other factors to speed up and improve the generation of human stem cell–derived retinal ganglion cells (RGCs).
   c) Develop methods to generate and characterize stem cell–derived RGCs corresponding to the various subclasses of human RGCs.
   d) Human stem cell–derived RGCs used in combination with CRISPR/Cas9 genome editing offer an unprecedented opportunity to increase our understanding of the mechanisms of RGC injury in glaucoma and other forms of optic neuropathy.
   e) Expand the use of human stem cell–derived RGCs to identify and develop safe and effective neuroprotective drugs.

   Long-Term Needs and Opportunities:
   a) The holy grail of glaucoma stem cell research is to develop the ability of transplanted human RGCs to repopulate a damaged optic nerve and make appropriate synaptic connections so as to restore lost vision.
   b) Develop methods to achieve efficient integration of transplanted human stem cell–derived RGCs.
   c) Develop increased understanding of the identity and mechanisms of axonal guidance systems that are still active, or are reactivatable, in the adult retina and optic nerve (as most patients with optic nerve disease are adults, yet normal optic nerve formation takes place during development).
   d) Identify small molecules, optimize bioengineering-based scaffolds and fibers, and develop other methods to enhance the formation of synapses of RGCs to appropriate neurons in the lateral geniculate nucleus and other target tissues.

7) **The Use of Stem Cells in Treating Retinal Vascular Diseases: Diabetic Retinopathy and Retinal Vein Occlusions – Susanna Park, MD, PhD, Department of Ophthalmology, University of California, Davis Eye Center, Sacramento, California, United States**
   a) Stem cell therapy that can potentially regenerate both the damaged retinal vasculature and retinal neurons is desired.
      i) As such, adult stem cells with paracrine trophic effects on multiple cell types in the retina show promise.
      ii) Whether endothelial precursor cells or mesenchymal stem cells derived from cord blood or pluripotent sources are more pluripotent and therapeutic than adult cells remains to be determined but should be investigated.
   b) Adult stem cell therapies are in early clinical trial but efficacy and safety results are still pending.
      i) If these studies move forward to larger clinical studies, a more standardized approach to evaluating safety and efficacy of cell therapy for treatment of retinal vascular disorders will need to be developed.
   c) Understanding the interplay between various precursor cells is important to developing the ideal cell therapy for vascular regeneration.
      i) The optimal cell therapy may involve a combination of stem cells or precursor cells.
   d) Host factors might affect the regenerative potential of stem cells for autologous cell therapy. Pharmacologic methods to overcome these potential host factors are being developed and these methods may enhance the regenerative potential of these stem cells.
   e) Understanding the molecular basis for the regenerative effect of stem cells in retinal vascular conditions should shed light on new pharmacologic approaches to treating retinal vascular disorders and new approaches to enhancing the therapeutic effects of currently available stem cell therapies.

8) **Stem Cell Therapy in the Treatment of Retinitis Pigmentosa – Michael Young, PhD, Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts, United States**

   Short-Term Needs and Opportunities:
   a) New photoreceptors can be generated from both fetal retinal and pluripotent stem cells. Better definition is needed.
   b) Transplantation of RPCs can replace lost photoreceptors in an analogous porcine allograft model. More work is needed on larger animal models.
   c) A clinical trial will examine the safety of human RPCs in treatment of advanced RP. More trials are needed for different diseases using different disease models.

   Long-Term Needs and Opportunities:
   a) The holy grail of retinal stem cells remains a combined RPE/cone composite grafts that restores high-acuity vision. Work should be focused to achieve this end.

9) **The Treatment of Rare Retinal Degenerative Diseases by Stem Cell Therapy – Anand Swaroop, PhD, Neurobiology, Neurodegeneration, and Repair Laboratory, National Eye Institute, National Institutes of Health, Bethesda, Maryland, United States**
   a) Further investigate the importance of reporter pluripotent stem cell lines for studying retinal biology and disease.
   b) Further develop fluorescent reporters driven by specific promoters to help in the identification of

cell types or developmental stages in three-dimensional retinal cultures.
c) Purification and use of reporter-tagged cells for next-generation sequencing analyses or for transplantation in animal models.
d) Use of three-dimensional retina derived from reporter stem cell lines for developing disease models and for designing targeted drug screens for retinal and macular diseases.
e) To expedite scientific discovery by sharing of resources to avoid duplication of effort through the formation of a Retinal Stem Cell Consortium that can maximize the vast potential of stem cells in developing therapies for retinal and macular diseases.

10) **Stem Cell Use in AMD – Amir Kashani, MD, PhD, Department of Ophthalmology, USC School of Medicine, Los Angeles, California, United States**
a) There is a great need to develop novel, noninvasive diagnostic tests to assay RPE and retinal function at the molecular and cellular levels.
b) Development of novel transplantation tools and surgical methods for optimal delivery of RPE to the subretinal space is needed.
c) Expansion and advancement of stem cell science is important for the purpose of understanding host immune response in the subretinal space.
d) Developing clinical grade methods to genetically modify stem cell–derived RPE is needed.

11) **Stem Cell Clinical Planning: Patients, Trials, and Expectations – Samuel Jacobson, MD, PhD, Scheie Eye Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, United States**
a) Develop a workup that will include the types of data (beyond the clinical examination) needed to decide whether a patient is or is not a future candidate for clinical trials of stem cell–based therapies. There should be specific workups for widespread retinal degenerations as well as maculopathies.
b) Segmented optical coherence tomography images over a wide expanse of fundus should be part of all such workups, as well as appropriate perceptual testing to quantify the severity of visual loss.
c) Develop robust outcome measures so that these future trials are not mired in controversy and without a definitive answer about efficacy as well as safety.
d) Perform further noninvasive human research to understand the relationship between inner retinal abnormalities associated with photoreceptor loss and retinal remodeling so as to clarify if there will be any impediments to the goals of stem cell therapy or other forms of photoreceptor-based treatments before rather than after the trials are initiated.

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