STEM CELLS AND ORGANOID AS MODELS OF TISSUE DIFFERENTIATION AND EYE DISEASES

Organizers: Lisa A. Neuhold, PhD

The use of stem cell populations and organoids to generate replacement cells/tissues/organs/ to correct acquired and genetic human eye diseases as well as drug screening is proving to be crucial to the development of new therapies for eye diseases. This course will discuss the multiple aspects of cell replacement strategies including stem cell differentiation and integration into existing tissues. Regenerative therapies including morphological and molecular events underlying organ formation in vitro and in vivo, 3D stem cell culture systems, cell and tissue engineering, and transplantation methods will also be discussed.

8:30-8:45am
Stem Cell Therapy and Organoids as Potential Therapeutic Intervention
Paul A Sieving, MD, PhD, FARVO, National Eye Institute

Stem cell therapy holds the promise of repair, regeneration, and cure. Some such cell therapies are already in clinical use (e.g. hematopoietic stem cells and immunotherapies for cancer treatment), and others are currently being tested for clinical use (e.g. mesenchymal stem cells and pluripotent stem cell derived therapies). The discovery that pluripotent stem cells can self-organize themselves into a layered structure resembling the optic cup, a pouch-like structure that develops into the inner and outer layers of the retina during embryogenesis, is tantalizing. The fidelity of organoids to recapitulate in vivo development alludes to the potential of the in vitro 3D organoid system because of its ability to be manipulated to define the molecular and cellular interactions that are essential for eye development and function. These exciting areas cut across a number of disciplines including tissue engineering, tissue chips, scaffolds, biofabrication, biomimetics, cell census efforts in neuroscience, and discoveries of factors regulating retinal development and circuitry. The vision community is poised to build upon this confluence of technological capability and scientific understanding to translate discoveries to therapeutic development.

8:45-9:15am
Introduction
Thomas A. Reh, PhD, University of Washington School of Medicine

Embryonic stem cells and induced pluripotent cells are now being tested as a source for retinal pigmented epithelial (RPE) cells to replace those lost from diseases (e.g. macular degeneration). Although techniques for generating retinal neurons, including rod and cone photoreceptors, from ESCs and iPSCs were developed over 10 years ago, clinical application of ESC/iPSC-derived neural retinal cells has not advanced as rapidly as the RPE. The primary goal for neural retinal transplantation is to reconstruct the normal circuitry of the retina from (1) the physical integration of the transplanted cells with the remaining host tissue, and (2) the functional synaptic connections of the transplanted cells with existing neural circuits. Although dissociated photoreceptor cell transplants in mice appeared to provide successful integration and reconnection, it has recently been found that the transplanted cells can transfer protein to the host cells, and therefore the conclusions from these promising reports over the last ten years must now be re-evaluated. Nevertheless, there are some examples of ESC-derived retinal cell transplantation that show some degree of reintegration, though the prospects for transplantation of photoreceptors as a potential therapy for the treatment of photoreceptor degeneration will depend on developing methods to promote more effective reconnection of transplanted cells.
9:15-9:25am - Questions

9:25-9:50am
Cornea: Niche regulation of limbal stem cells
Scheffer C.G. Tseng, MD, PhD, FARVO, Ocular Surface Center, Ocular Surface Research & Education Foundation

Among all epithelial tissues, the model of the corneal epithelium is most ideal for studying and practicing regenerative medicine because of easy identification and accessibility of the stem cell (SC) niche at the limbus. In this model system, we have diagnosed corneal blindness in a number of ocular surface diseases caused by limbal SC deficiency and restored sight by transplantation of limbal SCs. To address the concern that non-resolving inflammation and progressive scarring impede the success of SC therapies, our research effort has led to discovery of HC-HA/PTX3 from human amniotic membrane and umbilical cord. HC-HA/PTX3 exerts a broad anti-inflammatory action across both innate and adaptive immune responses and a direct anti-scarring action through downregulation of TGF-b signaling. To further realize the full potential of SC-based therapies, it is important to understand how quiescence, self-renewal and fate decision of limbal SCs are regulated in the limbal niche. Toward that end, we have successfully isolated and expanded human limbal stromal niche cells and created reunion with limbal SCs through the SDF-1/CXCR4 chemokine axis to generate 3D sphere cultures. In 3D Matrigel, resultant spheres exhibit SC renewal through activation of canonical Wnt signaling. In HC-HA/PTX3, resultant spheres exhibit SC quiescence highlighted by nuclear Bm1-1 through activation of BMP signaling and non-canonical (PCP) Wnt signaling but suppression of canonical Wnt signaling. These data collectively indicate that HC-HA/PTX3 is the key matrix in amniotic membrane and umbilical cord that support SCs indirectly by restoring a non-inflamed and non-scarring microenvironment and directly by maintaining SC quiescence through limbal niche cells. A novel platform technology based on HC-HA/PTX3 as a novel regenerative matrix can be launched singly or in combination with SCs in regenerative medicine.

CONTINUING MEDICAL EDUCATION (CME) AGENDA BEGINS

9:50-10:15am
Lens: regeneration of lens from endogenous stem cells
Kang Zhang, MD, PhD, Shiley Eye Institute, University of California San Diego

The repair and regeneration of tissues using endogenous stem cells represents an ultimate goal in regenerative medicine. To our knowledge, human lens regeneration has not yet been demonstrated. Currently, the only treatment for cataracts, the leading cause of blindness worldwide, is to extract the cataractous lens and implant an artificial intraocular lens. However, this procedure poses notable risks of complications. Here we isolate lens epithelial stem/progenitor cells (LECs) in mammals and show that Pax6 and Bmi1 are required for LEC renewal. We design a surgical method of cataract removal that preserves endogenous LECs and achieves functional lens regeneration in rabbits and macaques, as well as in human infants with cataracts. Our method differs conceptually from current practice, as it preserves endogenous LECs and their natural environment maximally, and regenerates lenses with visual function. Our approach demonstrates a novel treatment strategy for cataracts and provides a new paradigm for tissue regeneration using endogenous stem cells.

10:15-10:25am – Questions
10:25-10:45am – Break

10:45-11:10am
RPE Stem Cell Transplantation and Endogenous Activation
Jeff Stern, MD, PhD, Neural Stem Cell Institute, Regenerative Research Foundation

Stem cells have the remarkable property of self-renewal, to create new progeny with the properties of the parent cell. In 2012, we discovered a retinal pigment epithelium stem cell (RPESC) poised to produce RPE progeny. RPESC are less plastic and proliferative than pluripotent stem cells, and require less differentiation prior to transplantation. This characteristic allowed us to transplant RPESC-derived RPE that were differentiated to varied extents. We found an intermediate progenitor state to be more effective than fully differentiated RPE after transplantation into an animal model of macular degeneration. I will also describe preliminary work to identify molecular pathways found in the specific stage of RPE differentiation associated with
improved transplant efficacy. In addition to transplantation, RPESC can be activated to proliferate \textit{in situ} to increase RPE cell number. Little RPE layer regeneration occurs naturally in mammals, unlike some amphibia with robust RPE self-repair. Work to awaken mammalian RPE regeneration with Fibroblast Growth Factor 2 (FGF2) has been limited by the short half-life of the growth factor. We developed controlled-release FGF2 microbeads to control intraocular FGF2 levels. When injected into the eye, these FGF2 microbeads activate RPE layer proliferation without significant off-target effect, to safely increase the number of RPE cells without subretinal surgery.

11:10-11:35am

**Photoreceptor synapse formation in vivo and in retinal organoid cultures**

*Anand Swaroop, PhD, FARVO, National Eye Institute*

Loss of photoreceptors is a major cause of blindness in retinal and macular degenerative diseases. Photoreceptor regeneration or replacement is a feasible therapy for retinal degeneration and is therefore a key mission of NEI Audacious Goals Initiative. Success of vision restoration in this approach, however, depends on appropriate functional integration of transplanted or regenerated photoreceptors in the degenerating retina. At this stage, little is known about how photoreceptors form ribbon synapses with bipolar and horizontal cells. To identify genes involved in morphogenesis of photoreceptor pre-synapse, we have performed genetic loss-of-function screens of over 70 candidate genes and identified 18 genes that seem to contribute to photoreceptor synapse formation. To investigate synaptogenesis in vitro, we have developed 3-D organoids that better mimic retinal development in vivo. Using modified culture conditions (called High Efficiency Hypoxia Induced Generation of Photoreceptors in Retinal Organoids, or HIPRO), we efficiently generated polarized and stratified neural retina from mouse pluripotent stem cells. Over 70% of the cells in organoid cultures were rod photoreceptors with elongated cilia. Transcriptome analysis demonstrated that rods in organoids at differentiation day 35 are somewhat more mature than rod photoreceptors from mouse retina at postnatal day 6. Notably, HIPRO organoids appear to show outer plexiform-like structure with synaptic vesicles. We are developing bioengineering platforms for 3-D reconstruction of outer retina. In addition to delineating basic mechanisms of synapse formation, retinal organoids are being used for high throughput screening of small molecules that can facilitate functional maturation or rescue disease phenotypes.

11:35am-12:00pm

**Retinal Ganglion Cells: Differentiation and integration of RGCs into adult retinas**

*Jeffrey L. Goldberg, MD, PhD, Byers Eye Institute, Stanford University*

Here we describe advances in our understanding of the signaling pathways that regulate retinal ganglion cell differentiation from stem and progenitor cells, and of their ability to integrate into the adult retina after transplantation. The implications for future study and for cell therapy for glaucoma will be discussed.

12:00-12:15pm – Questions

12:15-1:25pm – Lunch

1:25-1:50pm

**Bioengineering Cornea Tissues for in vitro and in vivo Utility**

*David Kaplan, PhD, Bioengineering and Biotechnology Center, Tufts University School of Engineering*

Our goal is to generate relevant human cornea tissues that can provide useful in vitro systems for the study of treatments and diseases, as well as in vivo systems for tissue regeneration as cornea replacements. Toward this goal, we have been mimicking the structure and function of cornea tissues through a series of studies that encompass biomaterial lamellar scaffold designs, cell types integrated into these biomaterials, bioreactor systems to house the tissues for in vitro studies, and animal studies in support of tissue regeneration needs.
Development of biomaterials to control the microenvironment for cell-based retinal regeneration strategies
Rebecca L. Carrier, PhD, Northeastern University School of Engineering

In this presentation, approaches for using biomaterials as "cell delivery vehicles" for cell-based treatment of retinal degeneration will be discussed. One approach to development of biomaterials for regenerative medicine, the use of decellularized tissues that provide native chemical and physical cues to cells, will be presented. Results from our own research, focused on development of biomaterials from retinal tissues, and the cell response to these materials, will be presented. We have studied the impact of retinal extracellular matrix-based materials on cell behaviors important to retinal regeneration (e.g., migration) and the cell signaling events driving these behaviors. Recent results exploring a novel concept of studying the retinal microenvironment in lower vertebrate species in which regeneration occurs, and developing materials based on lower vertebrate retina, will be reviewed.

A retinal organoid view into the mechanisms of human eye development and regeneration
Valeria Canto Soler, PhD, Retinal Degeneration Research Center, Wilmer Eye Institute

The advent of stem cell-derived retinal organoid systems has opened unprecedented opportunities for the study of human retinal development and regeneration. The cumulative knowledge of developmental mechanisms has been instrumental in informing the generation and optimization of organoid systems, and the field is now coming full circle as these organoids earn their place as essential tools that can provide new insights into the processes underlying human retinal development. Retinal organoid systems largely recapitulate the native tissue architecture and cellular interactions of the developing retina, making them powerful near-physiological models for use in both basic and translational research. This talk will provide an overview of the most recent observations on the dynamics of retinal progenitor cell differentiation in three-dimensional retinas derived from human pluripotent stem cells in vitro, and discuss the challenges and opportunities that retinal organoids present within the context of retinal regeneration.

Patient-specific 3D Engineered Ocular Tissue to Identify Mechanism of AMD Onset and Progression
Kapil Bharti, PhD, Intramural Research Program, National Eye Institute

Age-related macular degeneration (AMD) is one of the leading causes of blindness among elderly. The disease has two advanced stages, the “dry” and the “wet” stage. The dry stage is triggered by the death of retinal pigment epithelium (RPE) cells followed by photoreceptor (PR) cell death and choroidal thinning. In contrast the wet-stage is characterized by overt proliferation of choroidal capillaries. It is thought that disease processes for both these advanced stages initiate in the back of the eye around the PR/RPE/choroid complex. However, due to lacking human models, the disease initiating events that lead to functional and anatomical changes in the PR/RPE/choroid complex are not well understood. We have combined bioprinting, tissue engineering, and induced pluripotent stem (iPS) cell technology to develop a 3D <i>in vitro</i> model of RPE/“choroid”. Using a collagen-based gel for encapsulation of patient-specific iPS cell-derived endothelial cells, choroidal fibroblasts, and pericytes, we successfully bioprinted a microvascular network on one side of a biodegradable scaffold. On the other side of the scaffold, we grow a RPE monolayer differentiated from the same patient’s iPS cells. This 3D tissue mimics the anatomy and functional properties of native RPE/choroid unit. Similar to wet-AMD, the <i>in vitro</i> microvascular network also proliferates in response to VEGF. This 3D RPE/choroid construct is currently being combined with 3D retina derived from the same iPS cells to develop the entire back of the eye tissue relevant for AMD pathogenesis. This work provides a platform to discover disease initiating pathways and the possibility of identifying potential therapeutic drugs for wet-AMD.

3:15-3:25pm – Questions
3:25-3:40pm – Break
Microengineered Physiological Biomimicry: Human Organ-on-Chips
Dongeun (Dan) Huh, PhD, University of Pennsylvania

Human organs are complex living systems in which specialized cells and tissues are assembled in various patterns to carry out integrated functions essential to the survival of the entire organism. A paucity of predictive models that recapitulate structural and functional complexity of human organs and physiological systems poses major technical challenges in virtually all areas of life science and technology. This talk will present interdisciplinary research efforts focused on leveraging unique capabilities of microfluidics and microfabrication to develop microengineered biomimetic models that reconstitute complex structure, dynamic microenvironment, and physiological function of living human organs. A particular focus of this talk will be on the development and application of an organ-on-a-chip microdevice that reconstitutes the ocular surface of the human eye. Specifically, this microengineered biomimetic system provides a novel platform to replicate i) 3D curvature of the ocular surface, ii) \textit{in vivo}-like spatial distribution of corneal and conjunctival cells, iii) differentiated physiological functions of the corneal and conjunctival epithelia, and iv) ocular surface lubrication and physiological tear film dynamics enabled by blink-induced eyelid movements. The human blinking ‘eye-on-a-chip’ system will provide an innovative platform that enables simulation, visualization, and analysis of abnormal biological processes mediating ocular toxicity of various materials in the human eye. We believe that this model has paradigm-shifting potential and offers the promise to address the critical unmet need for cost-effective and more predictable alternatives to conventional animal models for screening ocular responses to drugs, chemicals, cosmetics, environmental materials, and indwelling biomedical devices.

4:05-4:15pm – Questions

4:15-4:30pm
Summary and Closing Remarks
Valeria Canto Soler, PhD

This presentation will provide a summary of the most relevant elements discussed during the course and highlight the challenges and opportunities that need to be further addressed in order to bring the field to the next stage.