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Targeting collective behaviors of transplanted retinal cells as a strategy to improve cellular integration

Miles Markey, Maribel Vazquez*

Introduction: The rapidly growing field of regenerative medicine incorporates fundamental principles of stem cell biology and biomedical engineering to repair tissues damaged by genetic disorder, degeneration, or traumatic injury. The global market for stem cell therapies is expanding at an accelerating rate and projected to triple to over 100 Billion USD by the end of the decade (No author listed, 2019), as per **Figure 1A**. However, the full market and health potential of regenerative therapies depends upon successful clinical translation of contemporary treatments, such as cell replacement therapy. Replacement strategies offer newfound promise to treat vision loss caused by degeneration of the retina, a photosensitive tissue that lines the back of the human eye to convert light into bioelectrical signals for vision. Retinal disorders, such as macular degeneration and diabetic retinopathy, are leading causes of irreversible blindness in adults and are projected to increase in prevalence in the coming decades (GBD 2019 Blindness and Vision Impairment Collaborators, 2021). Emerging cell replacement strategies (No author listed, 2019; GBD 2019 Blindness and Vision Impairment Collaborators and Vision Loss Expert Group of the Global Burden of Disease Study, 2021) showcase innovative treatments for vision loss that will dramatically increase the current market share for retinal therapeutics.

Cell replacement therapy: Regenerative medicine seeks to restore vision by replacing damaged retinal neurons with healthy, functional stem cells. Previous studies have demonstrated the ability of precursor cells, progenitor cells, and adult mesenchymal stem cells to be safely transplanted, *in vivo*, without tumorigenic capacity (Oswald and Baranov, 2018). The human retina is a highly cellular structure with millions of cells positioned across a maximum thickness of 250 µm. The outer, non-neural tissue is called the retinal pigment epithelium (RPE), a single cell layer that helps regulate the flow of nutrients, transport waste, and relieve oxidative stress (Baden et al., 2020). The neural retina contains millions of neurons and glia synaptically interconnected across three nuclear layers, as shown in **Figure 1B**. Vision occurs when incident light is absorbed and transduced into electrical signals by rod and cone photoreceptors of the outer nuclear layer. Rods/cones then synapse with secondary neurons of the inner nuclear layer, such as bipolar, horizontal and amacrine cells, which in turn network with cells in the ganglion layer to transmit signals along the optic nerve to the visual cortex. Damage to neurons in any portion of this sophisticated network leads to progressive vision loss, which is incurable using current treatment options.

Contemporary cell replacement therapies have transplanted stem-like cells directly into damaged retina, where cells must then migrate to sites of injury, differentiate appropriately into specialized neurons, and synapse within native cellular networks to restore vision (Oswald and Baranov, 2018). While numerous projects have independently illustrated that stem-like cells can survive, migrate, and differentiate when transplanted into adult retina, the integration needed to restore vision has yet to be achieved (Warre-Cornish et al., 2014). A primary challenge is the inability of replacement cells to migrate appropriately within retinal tissue to achieve desired cellular positioning, a process that occurs reliably during retinal development, *in vivo*. Surprisingly, while developmental neurobiology has enabled transformative production of transplantable cells at various stages of de/differentiation (Oswald and Baranov, 2018), few projects have relied upon developmental models to evaluate the migratory responses of these replacement cells to adult retinal cues.

Developmental models to guide transplantation: Fundamental principles of retinogenesis have been greatly elucidated using invertebrate models, such as Drosophila Melanogaster, or fruit fly, a model genetic organism used to illustrate how signaling cues of eye development are remarkably well-conserved across species (Kumar, 2018). Drosophila is an excellent model for study of complex behaviors of retinal stem cells, as numerous groups have used Drosophila to identify how different subtypes of retinal neurons are produced, collectively differentiate, and inter-communicate to develop the adult retinal architecture. Proteins such as fibroblast-growth factor (FGF) have been shown to play a number of important roles in Drosophila retinogenesis, including determining cell fate, controlling synapse formation, and serving as chemoattractants to induce stem cell migration and positioning prior to terminal differentiation. Our lab has recently reported that retinal stem cells are able to migrate collectively in response to FGF concentration gradients, *in vitro*, by migrating as cohesive cell groups rather than as individual cells (Pena et al., 2019; Zhang et al., 2020). While this behavior is consistent with what is observed during *in vivo* retinal development, collective responses of replacement cells have been poorly explored as targets to promote or direct the cellular positioning needed for integration. Surprisingly, transplantation studies continue to examine and/or invoke individual cell behaviors rather than collective cell responses.

Cell-cell junctions for collective migration: FGFR1, the cognate receptor for FGF, is also significantly implicated in expression of the cohesion molecule N-cadherin, a transmembrane protein that enables calcium-dependent, homophilic binding to promote collective behaviors. N-cadherin molecules bind to cytoskeletal actin filaments, which allow N-cadherin to transmit forces between adjacent migratory cells to promote collective migration via actin cytoskeletal coupling. Recent studies have further shown that N-cadherin and FGFR1 are co-stabilized at cell-cell junctions (Kon et al., 2019), allowing cells to maintain sensitivity to FGF concentration gradients over longer periods of time for prolonged migration. These independent findings suggest that formation of cell-cell junctions is a significant factor in promoting collective chemotaxis among groups of transplantable cells. Study of the concurrent effects of cell-cell cohesion and chemotactic receptors thereby opens a new strategy to better understand, and eventually regulate, the migratory behaviors of transplanted cells. Among the variety of platforms used to examine single cell adhesion, cohesion, and motility (Vazquez, 2020), microfluidic systems can uniquely facilitate precise and controlled study of, both, individual and collective cell processes in response to a variety of external stimuli and conditions (Vazquez, 2020).

Microfluidic models of collective behaviors: Microfluidic systems are highly customizable, *in vitro* assays with characteristic lengths of less than 1000 microns, or 1 millimeter. The microfluidic scale is highly applicable to the study of retinal cells and the migration scales desired during cell replacement therapy. Previous work from our group has developed microfluidic systems to examine the collective and individual behaviors of replacement cells in response to numerous factors and stimuli (Zhang et al., 2020). Further, contemporary fabrication readily facilitates the custom design and manufacture of microfluidics with nanometer structures able to model complex retinal anatomy during retinogenesis and in adulthood. Our laboratory recently developed a microsystem to model the *in vivo* geometry of the optic stalk (Zhang et al., 2020), a precursor to the optic nerve where retinal neuroblasts migrate from the developing brain to form the retina. As shown in **Figure 1C** and **D**, the full device is smaller than a coin and can be readily fabricated using elastomeric molding to characteristic lengths of less than 100 µm. The device can be seeded with cells and matrix on one side and used to observe migration across the central channel array under precise conditions. Our experiments have demonstrated that neuroblasts migrated collectively in clusters of cells with

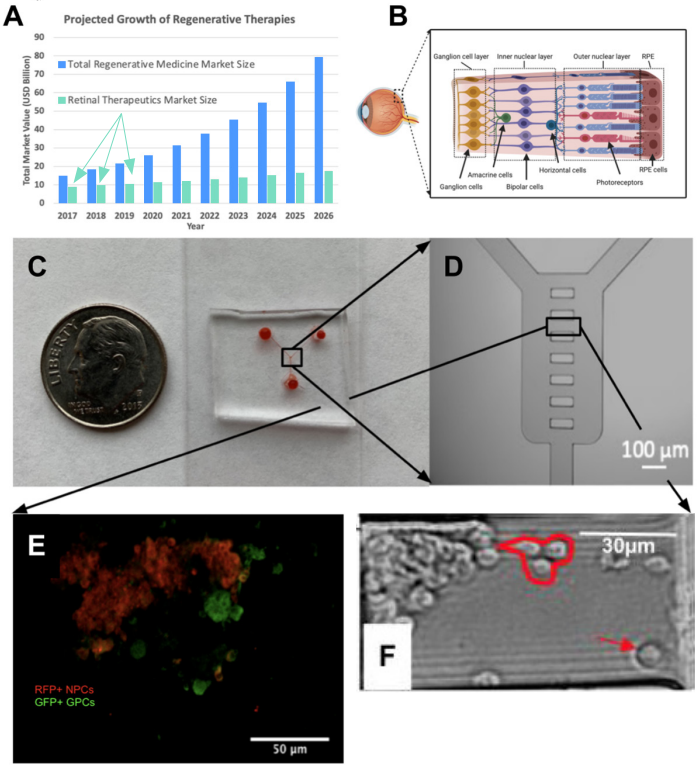

Figure 1 | **Cell replacement therapy shows promise as an emerging retinal therapeutic that can help the growth of global market for retinal therapeutics.**
(A) Global market value of regenerative medicine versus retinal therapeutics projected until 2026 (Data gathered from (No author listed, 2019)). (B) Schematic of the mature retina consisting of (from right to left): the retinal pigment epithelium (RPE), outer nuclear layer (ONL) containing photoreceptor cells, inner nuclear layer (INL) containing horizontal, bipolar and amacrine cells, and ganglion cell layer (GCL) containing ganglion cells. (C) Micro-optic stalk (µOS) device bonded to a glass microscope slide with dime for scale (Diameter = 17.91 mm), reproduced with permission from Zhang et al. (2020). (D) Brightfield image of the device design to illustrate micrometer scale and compartmentalized geometry (scale bar: 100 µm). (E) Confocal image illustrating the heterogeneous composition of clusters of retinal neuroblasts using RFP^+ to identify neuronal precursor cells (NPCs) and GFP^+ to denote glial precursor cells (GCPs) (scale bar: 50 µm). Borrowed with permission from Pena et al. (2019). (F) Brightfield image of motile retinal neuroblasts migrating collectively within the µOS device, arrow indicates single, non-motile retinal neuroblast (scale bar: 30 µm), reproduced with permission from Zhang et al. (2020).

both neuronal and glial lineage in response to FGF concentration gradients (Pena et al., 2019; Zhang et al., 2020), as per **Figure 1E**. Moreover, smaller cohesive clusters (typically of 3–5 cells) were able to detach from larger cell groups to chemotax longer distances in the same gradient field, as shown in **Figure 1F**. These exciting results illustrate that collective behaviors observed in development can be modeled within microfluidic models to enable quantitative study of the role of cohesion in the migration of transplanted cells. These data further suggest an optimal cluster size for migration of de/differentiated retinal cells that may be correlated with N-cadherin activation and require further investigation. Taken together, these studies highlight how microfluidic study of inter-dependent collective chemotaxis and cohesion can produce novel insights to inform emerging strategies in retinal cell replacement (Zhang et al., 2020).

Conclusion: Cell replacement therapy has transformative potential to treat degenerative retinal diseases that are rising rapidly, worldwide, among mature adults. The limitations of current treatments point to the significance of cellular strategies to enrich regenerative therapies and highlights vision loss as an important global health challenge. Our research suggests that transplantation strategies will be greatly enriched by promoting cell-cell cohesion among replacement cells to enhance cellular integration. These strategies can include the use of soluble factors, such as growth factors that promote cell-cell cohesion, adoption of specific extracellular matrix molecules that encourage cell-cell interaction, such as laminin and hyaluronan, and/or the application of progenitor cells derived to exhibit cell-cell behaviors. In addition, underexplored opportunities using microfluidic tools will help examine the collective behaviors of replacement cells as targets to improve the cellular positioning needed for regeneration.

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