Opportunities for agent based modeling of retinal stem cell transplantation

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Late stage blindness and visual impairment (BVI) affects over 400 million adults worldwide. These disabilities severely impact the ability of adults to function independently, reduce their quality of life, and worsen socio-economic burdens on health care systems. Importantly, the World Health Organization projects worldwide BVI from degenerated retina to more than double by the year 2050 (Bourne et al., 2021). To understand the clinical problem, consider **Figure 1A** depicting the retina's seven neuronal cell types that interconnect across three nuclear layers. Retinal photoreceptors of the outer nuclear layer (ONL) are light sensitive neurons that absorb and convert photons into bioelectrical signals. Photoreceptors synapse with neurons in the inner nuclear layer, which in turn synapse with neurons of the retinal ganglion layer to transduce photonic signals through the optic nerve to the brain. Degeneration or dysfunction in any of these neuronal components can disrupt the visual circuity and result in BVI.

BVI lacks an effective cure since retinal neurons cannot self-repair. Moreover, BVI is often progressive, as synaptic dysfunction can propagate along the retina's interconnected networks. Recent advances in regenerative medicine offer new promise to restore vision through cell replacement therapy, in which cells are transplanted into adult tissue to replace damaged or dysfunctional neurons (Gasparini et al., 2019). During transplantation, stem or progenitor cells are inserted into the host retina, where in order to restore vision, they must navigate to areas of damage, achieve functional positioning within the host laminae, and create synaptic connections with healthy, native cells. Photoreceptors are attractive targets for replacement therapy, both because of their proximity to the subretinal space (**Figure 1A**) used for surgery and because their functionality requires a single synaptic connection with adjacent bipolar cells. However, contemporary transplantation studies have demonstrated variable success, as the biological mechanisms underlying each of the neuronal processes required for regeneration are complex and incompletely understood (Gasparini et al., 2019). While there have been a number of *in vivo* and *in vitro* studies conducted to understand these mechanisms (Mishra et al., 2019), few groups have utilized the power of *in silico* approaches for the advancement of vision restoration.

Opportunities for computational modeling: Computational modeling provides the potential to evaluate both individual and collective roles of replacement cells in regeneration by mechanistically and quantitatively simulating key cellular processes. Agent based modeling (ABM) in particular defines individual cells as autonomous "agents" that are programmed to follow a set of interaction "rules," which themselves mimic either established biological mechanisms or behaviors newly obtained from *in vivo* experiments. In this way, complex, multi-component processes and interactions can be analyzed to improve both structure and function of replacement cells.

The ABM approach simulates both individual and collective behaviors in sufficient detail to predict both local behaviors and global scale tissue or organ phenomena. As shown schematically in **Figure 1B**, the biological structure of organisms can be deconstructed into hierarchical components: An organism is comprised of organs, the organ of tissues, the tissues of cells, and the cells are regulated by molecular mechanisms. The architecture of ABM mirrors this hierarchy, but from bottom up, rather than from top down. Agents, representing cells, operate according to rules, producing population, aggregate, and ultimately tissue- or organ- level behaviors (Glen et al., 2019). The resulting simulations can then be validated in a variety of ways including using *in vitro* microfluidic platforms or *in vivo*/*ex vivo* methods such cyrosectioning, imaging, or live cell tracking.

In order to meaningfully predict cell replacement behaviors, a modeling approach must veridically describe migration of injected cells into an adult tissue environment. Several well-known methods have been used to model cellular migration; these include Monte Carlo simulations, Finite Element and other discretized models as reviewed in (Masuzzo et al., 2016). Each of these approaches has an important place: Monte Carlo models obtain ultimate states (e.g., cell positions) by randomly altering variables to identify the lowest energy or most likely state. Finite Element models mechanically simulate each part of a cell in response to external stresses. Other discretized models (e.g., Potts, or Cellular Automata) apply *ad hoc* rules to mimic cell behaviors. These methods, however, do not easily lend themselves to comprehensibly modeling cells as individual entities, whose behaviors change depending on environmental cues. Thus migration occurs in response to mechanical, chemical and electrical cues, which are not easily (if at all) incorporated into other modeling methods – yet by defining each cell to be an agent, its shape, directionality, metabolic activity and other features essential to migration can easily be individually specified and modified according to external cues.

Moreover, each cell in an ABM model is specified by a small number of parameters (defining shape, mechanical properties etc.), and so large-scale simulations can be developed in an efficient manner. Other models (e.g., Finite Element or Cellular Automata), by contrast, either require large numbers of components for every cell, and so tend to be slow and cumbersome, or provide only qualitative, coarse scale data. Either approach significantly limits the ability of simulations to capture either individual or collective changes in cell shape, velocity, and force interactions with other cells (Rajagopal et al., 2018).

By contrast, ABM can provide a comprehensive model of migration – as well as differentiation, deformation, chemical gradient response, etc. – over a heterogeneous range of scales and architectures (Glen et al., 2019). In particular, ABMs can include important details such as progenitor and native receptor expressions, cytoskeletal trafficking and alignment, and both

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local (cadherins) and longer range (integrin-extracellular matrix) adhesive interactions (reviewed in (Bodor et al., 2020)). Effects of extrinsic guidance cues that stimulate biochemical pathways controlling cell shape, polarity, and movement can also be included in ABMs, and importantly ABMs readily model a large range of spatial distances, from microns to millimeters (e.g. filopodial extension and retraction), and temporal scales, from nanoseconds to days (e.g. extracellular matrix formation and remodeling). Agent Based Modeling of biological systems has only recently gained traction. Several models have been developed to describe cancer cell invasion or host-pathogen immune system responses. Notwithstanding the potential of ABMs, their use in the area of the nervous system has been substantially under-exploited, and has never been applied to the important problem of retinal regeneration.

ABM advantages–spatial architecture: To illustrate how ABMs achieve the flexibility and verisimilitude needed to model cell replacement in the retina, consider **Figure 1C**, where we provide a visual representation of a retinal photoreceptor that is modeled as a column of overlapping spherical agents. By specifying agent placement, separation, compressive and bending stiffness, etc., we can model any shape and mechanical response desired. In the ONL of the retina, columnar photoreceptors have lengths 20–30 µm and diameters 1–2 µm and are configured in a tightly packed arrangement with center-to-center distances as little as 2 µm. This leaves nanometer spaces for replacement cells to migrate (Wells-Gray et al., 2016), yet transplanted cells are introduced into the subretinal space as 10 µm-diameter cells. These spatial restrictions require replacement cells to infiltrate a columnar network of cells with limited spacing, a process that involves migration in response to chemotactic gradients and adhesive interactions as well as shape changes to propel the replacement cell into the network. **Figure 1D** shows a preliminary simulation of a single replacement cell infiltrating between two tightly packed photoreceptor columns at three successive time points. Notice that an ABM is uniquely suited to this kind of analysis: the photoreceptor agents are linked together into columns, while the infiltrating cell can change shape, for example by extending the leading, smaller, red agent, by changing

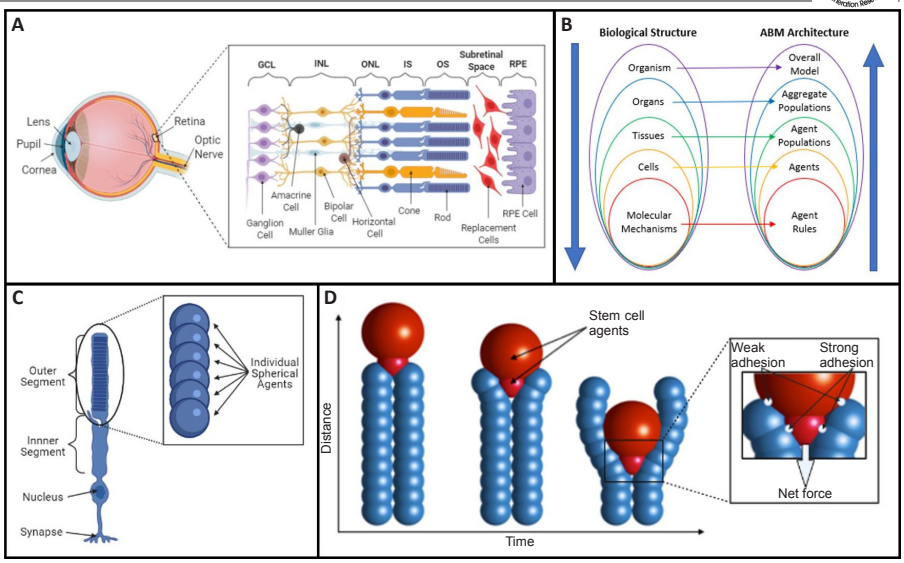

Figure 1 | Agent based modeling (ABM) provides underexplored opportunities to enrich cell replacement therapies in the adult retina.
(A) Schematic of adult retinal structure that lines the back of the human eye. Photoreceptor cells comprised of outer segments (OS) and inner segments (IS) are located in the outer nuclear layer (ONL) where they synapse directly across the outer plexiform layer with neurons of the inner nuclear layer (INL), which in turn synapse across the inner plexiform layer with cells in the ganglion cell layer (GCL) to transmit photonic signals to the brain through the optic nerve. The subretinal space, located between the retinal pigmented epithelial (RPE) layer and the ONL, is a well-established transplantation site for retinal progenitor cells in cell replacement therapies. (B) Direct comparison between the biological structure of organisms and ABM programming structure. Organisms can be decomposed into smaller building blocks from organs to molecular mechanisms. ABM follows the biological structure from the bottom up, starting with rules that define molecular mechanisms up to aggregated populations of agents that represent organs to simulate a whole organism. (C) Schematic of a photoreceptor comprised of 4 components: the outer segment, inner segment, nucleus, and synaptic region. A photoreceptor cell can be represented by ABM using an N:1 agent to cell ratio, as shown by the blue spheres. (D) ABM simulation of a transplanted stem cell (represented by the red spheres) infiltrated within a native photoreceptor columnar network (represented by the blue spheres). A complex migration mechanism that includes differential adhesion results in a net force propelling the stem cell into the photoreceptor network.

relative sizes of agents, or by spawning new agents as the cell shape evolves. Moreover as shown in the inset to **Figure 1D**, force balances required for forward migration can be accurately modeled, with stronger adhesion ahead and weaker behind, as mediated by cadherin trafficking within the cell and stimulated by chemotactic gradients (Lauffenburger and Horwitz, 1996). Additionally, ABMs more naturally represent these force balances than more abstract numerical discretization of differential equations whose values change discontinuously across material interfaces. This facilitates analysis of changes in retinal tissue structure, such as the bending stiffness of the photoreceptor cells, and the cellular morphological changes that result from interactions between transplanted and native cells. All these advantages provide a clear cut framework for understanding the biomechanics involved in key processes and inform future research and clinical directions in a transparent way.

ABM advantages–heterogeneity: ABM models readily incorporate heterogeneity, allowing simulations to include and differentiate between replacement stem cells and the retina's highly specialized native cells, each of which have different mechanical, migratory and chemical response behaviors. This is especially important for the complex retinal network comprised of multiple neuronal types and subtypes (Masland, 2001), and stem cell derived retinal neurons could result in mixed cell populations. Such phenotypic differences may impact transplantation outcomes based on their communication with other cells, how they form synaptic connections, or their adhesion properties. Unlike a differential equation, which describes a whole group of cells to generally behave similarly based on a set of desired parameters, ABMs simulate and track individual cells, which are dependent on local cell-cell interactions and cell-matrix interactions. This individualized tracking of agent experiences and factors that impact how a cell behaves over time enables simulations with a level of detail that would often get lost in continuum equations. This tracking ability facilitates the prediction of useful migration

parameters related to transplantation including the net distance and path distance of transplanted stem cells as well as the cellular displacement of native cells needed to accommodate replacements. Additionally, the heterogeneity of agents increases the flexibility of how agents might be defined. For example, the computational agent to biological cell ratio is most commonly 1:1. However, this ratio can be changed to an N:1 ratio, where N number of agents are used to define a single cell, such as a photoreceptor, shown in **Figure 1C**. Modeling photoreceptors and stem cells in this way facilitates unique exploration of the viscoelastic properties and bending stiffness of photoreceptors as well as the morphological changes of stem cells as they undergo migration within such limited spacing (see **Figure 1D**) (Wells-Gray et al., 2016).

ABM advantages–stochasticity: The processes involved in replacement cell evolution, including differentiation, proliferation and chemotactic migration are all intrinsically stochastic. Indeed, adhesion-mediated migration as illustrated in **Figure 1D** would never occur without bonds being formed and broken, and cellular dynamics have long been observed to be highly intermittent in studies ranging from neurogenesis to wound healing. Stochastic elements are arguably essential to developing systems as cells must explore their host environment for the optimization of tissue structure and functionality. Agent based models readily incorporate stochastic behaviors of this kind, for example by varying adhesive strengths and adding random walks to agent motions. Chemotactic migration is similarly modeled in ABMs, by probabilistically steering cells toward a normally distributed chemical gradient (Glen et al., 2019). Finally, the locations of photoreceptors and the initial injection of numerous replacement cells into a subretinal space are themselves stochastic, and ABM cells can easily be placed randomly to assess effects of this unavoidable variability.

Conclusion: Late onset blindness and visual impairment is a debilitating and currently incurable disability that affects millions of people worldwide. Replacement of dysfunctional neurons with stem cells offers newfound promise to restore vision but this promise is currently unrealized, in large part by a lack of understanding of precisely how transplanted cells behave in a host retinal architecture. ABM is advantageous for the study of retinal transplantation because it reduces the expense and technical expertise required to conduct animal studies, cryosectioning, and imaging within enucleated specimens and tissues. Further, ABM enables analyses of cell behaviors and morphological changes at time points in between sectioned specimens as well as cells and/or tissues that provide critical information for time dependent processes. While ABM allows modelers to incorporate a great number of agents, types, and parameters for exploration, a shortcoming as a result is that the simulation becomes computationally expensive as the model complexity increases, and too many inputs can result in incomprehensible or trivial results. As Robert Millikan has put it, scientific progress walks forward on two feet: theory and experiment. Advancement of cell replacement therapies depends on better theories, and we propose that agent based modeling is a valuable tool to visually and transparently develop these theories and so improve experimental outcomes.

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References

Bodor DL, Pönisch W, Endres RG, Paluch EK (2020) Of cell shapes and motion: the physical basis of animal cell migration. Dev Cell 52:550-562.

Bourne R, Steinmetz JD, Flaxman S, Briant PS, Taylor HR, Resnikoff S, Casson RJ, Abdoli A, Abu-Gharbieh E, Afshin A (2021) Trends in prevalence of blindness and distance and near vision impairment over 30 years: an analysis for the global burden of disease study. Lancet Glob Health 9:e130-143.

Gasparini SJ, Llonch S, Borsch O, Ader M (2019) Transplantation of photoreceptors into the degenerative retina: Current state and future perspectives. Prog Retin Eye Res 69:1-37.

Glen CM, Kemp ML, Voit EO (2019) Agent-based modeling of morphogenetic systems: Advantages and challenges. PLoS Comput Biol 15:e1006577.

Lauffenburger DA, Horwitz AF (1996) Cell migration: a physically integrated molecular process. Cell 84:359-369.

Masland RH (2001) The fundamental plan of the retina. Nat Neurosci 4:877-886.

Masuzzo P, Van Troys M, Ampe C, Martens L (2016) Taking aim at moving targets in computational cell migration. Trends Cell Biol 26:88-110.

Mishra S, Peña JS, Redenti S, Vazquez M (2019) A novel electro-chemotactic approach to impact the directional migration of transplantable retinal progenitor cells. Exp Eye Res 185:107688.

Rajagopal V, Holmes WR, Lee PVS (2018) Computational modeling of single-cell mechanics and cytoskeletal mechanobiology. Wiley Interdiscip Rev Syst Biol Med 10:e1407.

Wells-Gray E, Choi S, Bries A, Doble N (2016) Variation in rod and cone density from the fovea to the mid-periphery in healthy human retinas using adaptive optics scanning laser ophthalmoscopy. Eye 30:1135-1143.

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