Toward a Virtual Cornea:

An Agent-Based Model to Study Interactions between the Cells and Layers of the Cornea under Homeostasis and following Chemical Exposure

Joel Vanin[1], James A. Glazier[1], Thomas B. Knudsen[2] & Catherine Mahony[3]

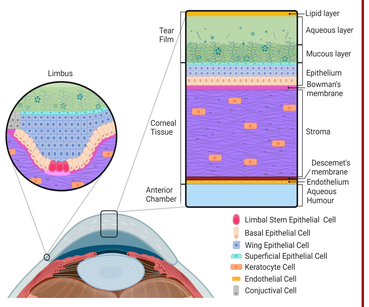
[1]Department of Intelligent Systems Engineering and Biocomplexity Institute, Indiana University, Bloomington, IN;
[2]Center for Computational Toxicology and Exposure, Office of Research and Development, United States Environmental Protection Agency, Research Triangle Park, NC;
[3]Procter & Gamble, Technical Centre, Reading, United Kingdom.

jvanin@iu.edu
mahony.c@pg.com

Introduction

Corneal biology and Homeostasis

- The Cornea, overlaid by tear film is composed of Corneal Epithelium, Bowman's layer, Stroma, Descemet's membrane, Corneal Endothelium, and underling Aqueous Humour.
- The corneal epithelium is a self-renewing stratified layer including superficial squamous cells, central wing cells, and a single layer of columnar basal cells.
- Over 7–10 days[1], the entire epithelium regenerates through proliferation of stem cells contained in the peripheral limbal niche, to produce progenitor and amplifying cells, which divide, migrate towards the central cornea, terminally differentiate, and slough off.



Corneal Injury and Wound Healing

- Chemical or physical injury invokes complex autocrine or paracrine interactions relating biophysical, electrophysiological, and physiological cues.
- Heterogeneous cytokines, chemokines and growth factors derived from regions of the cornea play a role in the resultant 'healing' response, influencing the time to recovery and/or severity of the adverse outcome.
- Epithelial healing relies on limbal stem cells and remodeling of the Bowman's membrane. Pain, swelling and sloughing of epithelial tissues accompany a transient loss of visual acuity in cases of minor injuries.
- More invasive injuries require several months to heal, particularly if the stroma is breached. Stromal healing occurs via transformation of keratocytes to fibroblasts and myofibroblasts, which may reorganize the extracellular matrix (ECM) and cause opacity/scaring.
- Progressive inflammatory response in moderate to severe injuries, supported by cells in the limbal niche and the autonomic nerve system, promotes neovascularization.
- Deeper stromal and endothelial damage causes permanent visual impairment since corneal endothelial cells do not regenerate. This allows excessive passage of fluid from the aqueous humour, swelling the cornea and permanently reducing transparency.

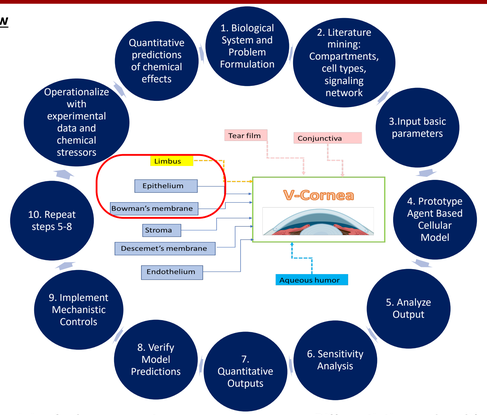
Virtual tissue

- The dynamic and spatial nature of corneal wound healing lends itself to mechanistic multiscale, multicellular computer simulation, known as Virtual Tissue modelling.
- We have developed a prototype virtual corneal model of homeostasis and recovery after damage using CompuCell3D[2], with the goal of not only obtaining emergent features, but to understand the mechanisms involved in corneal wound healing.

https://www.compucell3d.org/

Methods

Workflow



Literature mining for key parameters

Inputs

Cornea compartments
Tear
Conjunctiva
Epithelium*
Limbal niche (stem*, vascular, immune)
Bowmans membrane
Stroma
Descemet's membrane
Endothelium
Aqueous humour

Cells types & Structural Proteins
Limbal Stem Cells (epithelial*, mesenchymal)
Epithelial (Wing*, Basal*, TDC*)
Extracellular Matrix (laminin, collagen, fibronectin, proteoglycans)
Endothelial (vascular, lymphatic)
Immune (M1 & M2 macrophage, monocyte, neutrophil, dendritic, T cells)
Melanocyte
Keratocyte
Fibroblasts
Myofibroblasts

Signals/Receptors
TGFbeta, BMP
TNF-alpha
IL-1
IL-10
KGF (also known as FGF-7)/FGFR2
HGF/c-Met
EGF/EGFR
IGF/IGFR
VEGFA/VEGFR2
PDGF/PDGF-R
MMP
N/E Cadherin
Estrogen/ER

Transducers
FZD (Wnt signalling)
Ptc (Shh signalling)
PI3K-Akt
Notch

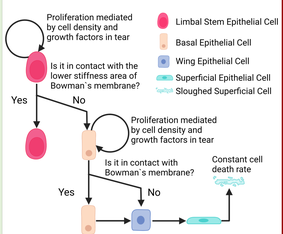
Barriergenesis
Tear Flow/Blinking shear force
Tight junctions
Membrane transporters
Basement membrane adhesion

Outputs

Emergent features
Epithelial cell mass maintenance*
Epithelium structure*
Persistent myofibroblast presence
Decline in macrophage population
Stromal vascularisation
Stromal haze
Stromal fibrosis
Endothelial cell bridging

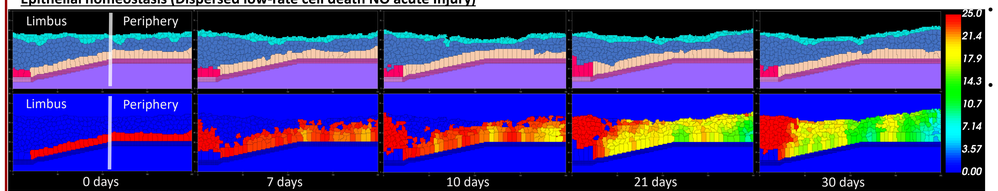
Items with * are already implemented in current model

Differentiation and proliferation rules



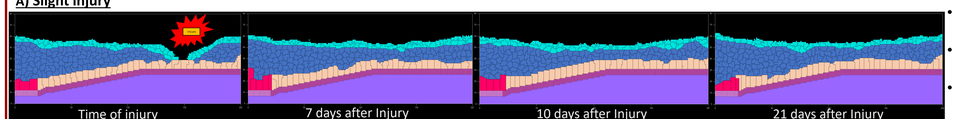
Results

Epithelial homeostasis (Dispersed low-rate cell death NO acute Injury)



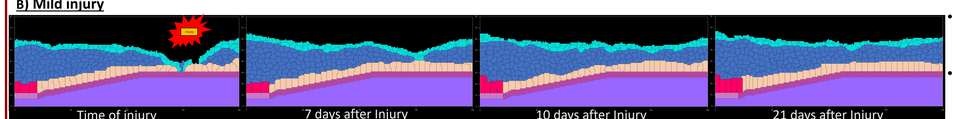
- Top strip presents the microanatomy being simulated over a month and shows regenerative growth.
- Bottom strip shows the number of cell divisions remaining in basal epithelial cells before losing proliferative properties and the state prior to differentiation to wing cell.
- By day 7, the corneal periphery was partially substituted, evident by the coloured cells in the superficial layer. On day 10 almost all cells were replaced in the corneal periphery.
- Day 21 cells in the limbus have been replaced.

A) Slight injury



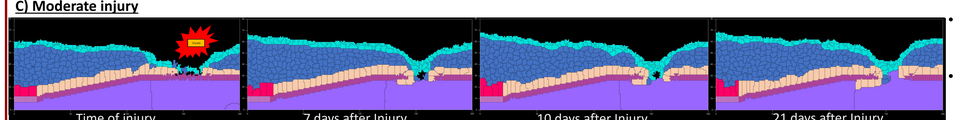
- Showing damage at a slight depth, killing only wing and superficial cells.
- After 7 days the tissue recovers its original structure.
- Homeostasis is returned with no overgrowth observed on day 10 to 21.

B) Mild injury



- Showing damage at a mild depth, disrupting **all** epithelial cell types but not Bowman`s membrane.
- After 10 days, the tissue recovers its original structure with no overgrowth as shown on day 21.

C) Moderate injury

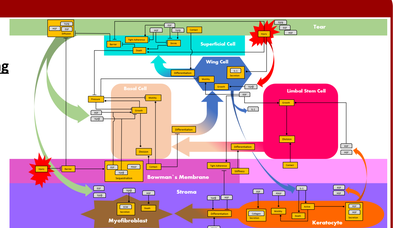


- Showing moderate damage, disrupting Bowman`s membrane and part of the stroma.
- Up to 7 days the simulation resembles experimental data re-establishing the superficial protective layer.[3]
- The simulated tissue does not recover from moderate injury, diverging from expected *in vivo* recovery forward of day 8[3]. This is due to the lack of necessary cell types in the current model, e.g. Keratocytes which, after interactions with molecules released following injury, would trigger restoration of the Bowman's membrane and stromal structure.

Conclusions and Next Steps

- Simulating biological processes as virtual tissues, like V-Cornea, are essential for accelerating our understanding of complex biological systems, which would be difficult or impossible using only traditional experimental methods.
- Virtual tissues allows us to make predictions and test hypotheses in a controlled environment, while being cost-effective, and allowing predictions at the individual level.
- The current model has the corneal epithelial structure as an emergent feature and recapitulates the time of recovery for slight and mild injuries. Our goal is to integrate bioactivity data from *in vitro* models of corneal toxicity and predict human-relevant adverse outcomes, including loss of structural integrity, time to recovery, area and density of opacification.
- New components like Descemet's membrane, endothelium, nerve cells, blood vessels and immune cells and function like active pumping of fluid can be integrated to improve the predictivity of the model.

Biological Signaling Network



References

[1] Sridhar M. S. (2018). Anatomy of cornea and ocular surface. Indian journal of ophthalmology, 66(2), 190–194. https://doi.org/10.4103/ijo.IJO_646_17
[2] Swat, M. H., Thomas, G. L., Belmonte, J. M., Shirinifard, A., Hmeljak, D., & Glazier, J. A.. (2012). Multi-Scale Modeling of Tissues Using CompuCell3D. In Methods in Cell Biology (pp. 325–366). Methods in Cell Biology. https://doi.org/10.1016/b978-0-12-388403-9.00013-8
[3] Singh, P., Tyagi, M., Kumar, Y., Gupta, K. K., & Sharma, P. D. (2013). Ocular chemical injuries and their management. Oman journal of ophthalmology, 6(2), 83–86. https://doi.org/10.4103/0974-620X.116624
[4] Vanin, J, Glazier, J. A, Knudsen, T. B., & Mahony, C. (2023). Towards a Virtual Cornea. Manuscript in preparation

Disclaimer: This poster does not represent EPA policy

Videos and Supplemental Materials