COMPUTATIONAL MODEL OF THE NEUROVASCULAR UNIT (cNVU) FOR PREDICTIVE TOXICOLOGY OF BLOOD-BRAIN BARRIER DEVELOPMENT

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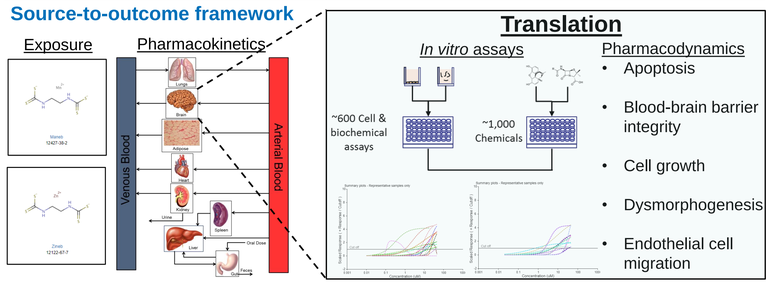
Background

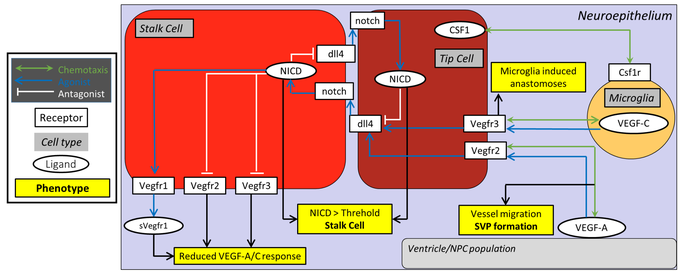
Source-to-outcome framework



Neurovascular unit development: Morphogenesis of the blood-brain barrier (BBB) is a complex process potentially disrupted by chemical exposure. Microglial-endothelial interactions are hypothesized to play a role in early BBB patterning via cytokine/chemokine signaling [1]. A cell-agent based model was built to simulate vascularization of the embryonic neuroepithelium. Here, we use the model to translate *in vitro* profiling data from ToxCast into a quantitative prediction of altered phenotype.

Model Development

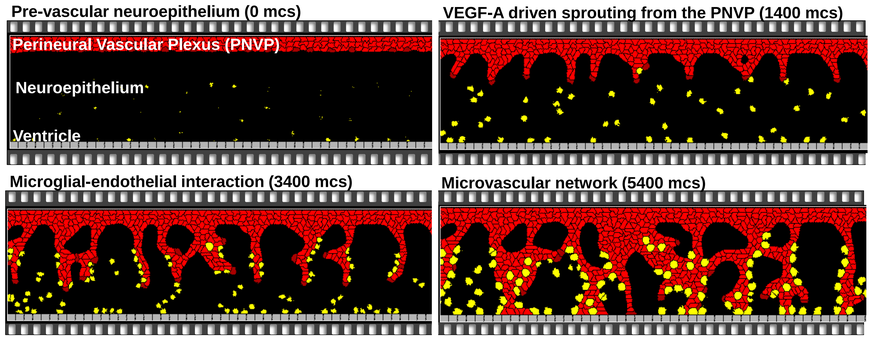
Control Network: To model the interactions between microglial cells and angiogenic sprouts invading the embryonic neuroepithelium, we extracted key signals and cellular interactions from a broader control network of BBB development [2].

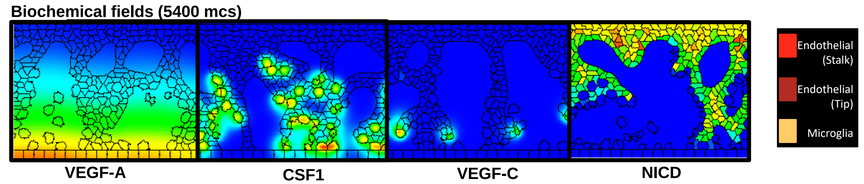


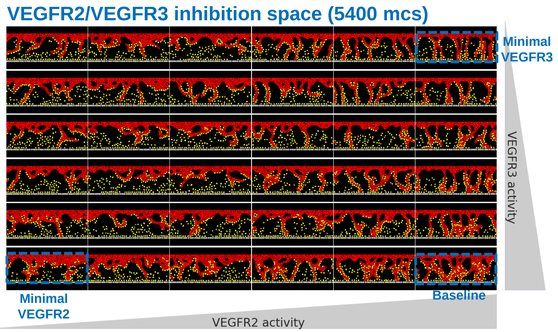
Agent-Based-Model: Cellular rules were coded into *Compucell3d* (v. 3.7.5) with Python scripting (v. 2.7). *Angiogenesis Analyzer* plugin of ImageJ [3] provided vascular network quantitation. Endothelial stalk cell / tip cell phenotype selection was driven by lateral signaling (delta-notch pathway); angiogenic sprouting was promoted by VEGFR2 signaling via VEGF-A from neuroprogenitor cells (abstracted); and branching morphogenesis was promoted by VEGFR3 signaling via VEGF-C from microglia.

Cell Agent-Based Modeling and Simulation

Stochastic interactions between multiple cell types (agents) are enabled by the control network and biochemical gradients are implemented in stepwise time-series Monte-Carlo steps (MCS). The visual readout predicts emergent behaviors not directly coded into the model in this case neuroepithelial vascularization.







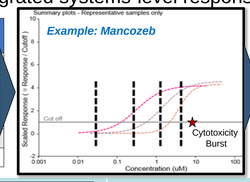
Above: Endothelial network formation and biochemical fields localized as extracellular (CSF, VEGF-A, sVegfr1) and intracellular (NICD) signals (blue → red scale).

Left: Simulation space for VEGFR2 and VEGFR3 response. Inhibiting VEGFR3 activity decreased branching while inhibiting VEGFR2 activity impaired microvascular invasion.

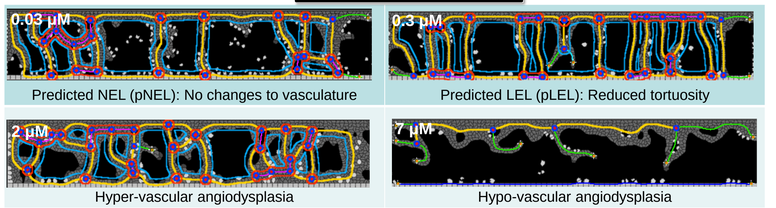
Concentration-Response: Quantitative Prediction

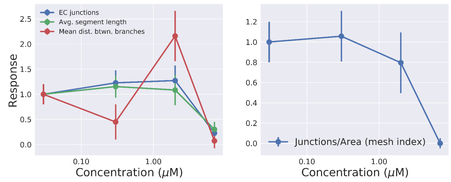
Molecular elements were linked to critical receptor targets through EPA's ToxCast high throughput screening dataset (INPUT) in order to translate bioactivity results into quantitative prediction of an integrated systems-level response (OUTPUT).

ToxCast Assay	# of active chemicals (1065 total)
NVS_ENZ_hCSF1R	18
NVS_ENZ_hCSF1R_Activator	2
NVS_ENZ_hVEGFR3	19
NVS_ENZ_hVEGFR3_Activator	0
NVS_ENZ_hVEGFR2	27
NVS_ENZ_hVEGFR2_Activator	1



Mancozeb Conc.	Resulting receptor activity
0.03 µM	No significant reduction in any receptor
0.3 µM	50% reduction in CSF1R
2 µM	50% reduction in VEGFR3 80% reduction in CSF1R
7 µM	50% reduction in VEGFR2 85% reduction in VEGFR3 95% reduction in CSF1R





Simulated results for cell network features (left) and integrated systems-level response (right). The latter reflects the number of junctions forming divided by total integrated endothelial surface area (normalized to baseline).

Summary and Conclusions

- A cell-agent based model of brain angiogenesis that executed a complex network of microglial-endothelial signaling was used to translate ToxCast *in vitro* data into a quantitative simulation of neovascularization of the embryonic neuroepithelium.
- Using Mancozeb data as an *in silico* input, computational dynamics identified a critical point (pLEL) predicting disruption of nascent BBB morphogenesis.
- This computational tool will inform hypothesis-driven testing and organ-specific prediction of neurovascular developmental toxicity.
- Future efforts will expand this model to interactions with the neuroprogenitor niche.

References

[1] Ginhoux F., Greter M., Lebouf M., Nandi S., See P., Gokhan S., Mehler M., Conway S., Ng L., Stanley E., Samokhvalov I., Merad M. (2010) *Science*
[2] Saili K., Zurlinden T., Schwab A., Silvin A., Baker N., Hunter E., Ginhoux F., Knudsen T. (2017) *BDR*
[3] Carpentier. ImageJ contribution. Angiogenesis Analyzer. (2012) *ImageJ News*