Multiscale Modeling of Wound Healing

Krithika Mohan[1], Jamie Nosbisch[2], Callie Miller[3], Sreeja Asokan[4,5], James Bear[4,5,6],Timothy Elston[3], and Jason Haugh[1]

[1]Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh,NC, [2]Biomathematics Graduate Program, North Carolina State University, Raleigh, NC,
[3]Department of Pharmacology, University of North Carolina, Chapel Hill, NC, [4]UNC Lineberger Cancer Center, University of North Carolina, Chapel Hill, NC,
[5]Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC, [6]Howard Hughes Medical Institute, University of North Carolina, Chapel Hill, NC.

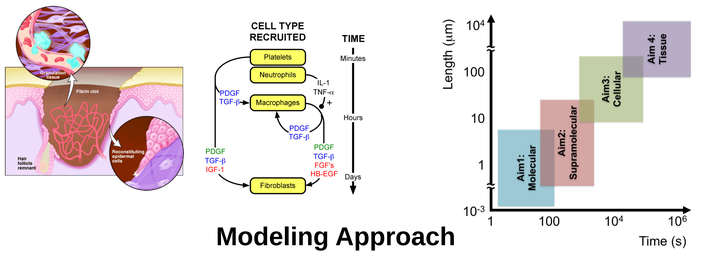
Abstract

Chronic wounds are a major threat to public health and the economy and present as a comorbid complication with major diseases with humans. Although the proper healing of cutaneous wounds requires collective and coordinated behaviors of multiple cell types, the rate-determining step is the recruitment and function of dermal fibroblasts, which are directed to invade the wound by a gradient in the concentration of platelet-derived growth factor (PDGF).

A great deal is known about the signal transduction pathways activated by PDGF receptors and other receptor tyrosine kinases; yet mechanistic insights about how those pathways are spatially organized to bias the dynamics of the actin cytoskeleton and the directionality of cell migration are still emerging. Furthermore, a larger fundamental gap lies in the integration of molecular, supramolecular, cellular, and tissue- level dynamics of wound healing, which then spans disparate time (seconds to weeks) and spatial (nm to cm) scales.

To advance this field, novel approaches are needed to fuse experimental and observational scales that are relatively data-rich (signaling, cytoskeletal dynamics) and data-poor (in vivo dynamics). To that end, we propose to develop a predictive, multiscale model of the proliferative phase of wound healing, incorporating 1) receptor-mediated signal transduction (molecular scale), 2) self-assembly of contractile actomyosin structures (supramolecular scale), 3) morphodynamics and statistics of cell migration (cellular scale) and 4) collective cell behavior in vivo (tissue scale). By combining our expertise in experimental cell biology and biophysical modeling, the proposed model development will be guided by new quantitative measurements at every scale of biological abstraction.

Wound healing is a multiscale phenomenon

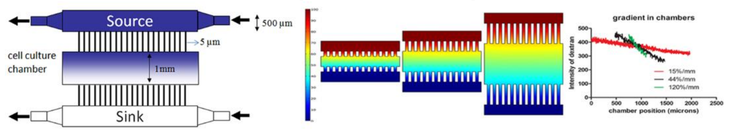


Modeling Approach

PDGF gradient —Aim 1→ Receptor activation, DAG patterns (Reaction-diffusion (PDEs)) —Aim 2→ Myosin RLC pattern (Physico-chemical (stochastic)) —Aim 3→ Fibroblast morphology, translocation (Generalized random walks (stochastic)) —Aim 4→ Fibroblast invasion *in vivo* (Multi-agent (stochastic/hybrid))

Evidence: phospholipase C/protein kinase C signaling is critical for PDGF chemotaxis

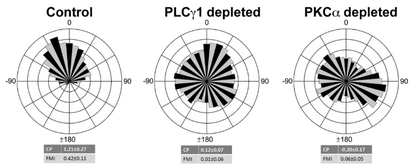
Experimental Setup



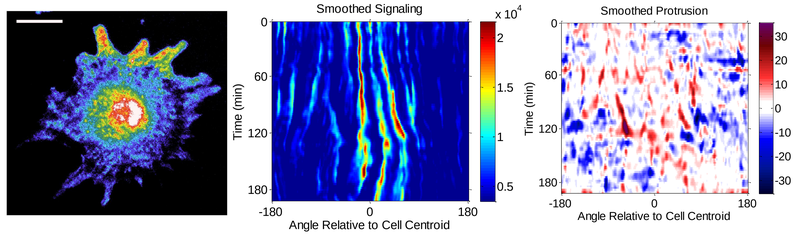
Putative chemotaxis inhibitors

	Control	PI3-kinase inhibitor	Pan-Tor inhibitor	PDGF-R inhibitor
CP	1.35±0.15	1.11±0.1	1.2±0.2	-0.28±0.22
FMI	0.33±0.09	0.24±0.1	0.21±0.1	-0.07±0.07
N	274	63	74	68
V (μm/hr)	18.20±2.2	11.4±1.8	19.2±2.4	36.78±3.30

Underappreciated PLC/PKC pathway

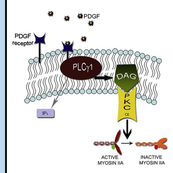


Chemotaxing cells maintain an internal gradient of diacylglycerol

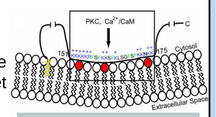


What are the feedback mechanisms leading to amplification of this internal gradient ?

Aim 1 (molecular scale): reaction-diffusion model to characterize amplification mechanisms in PDGF gradient sensing

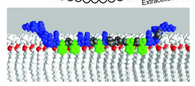


PLC/PKC pathway: Activated PDGF receptors engage PLC, which hydrolyzes PIP_2 to produce DAG and inositol triphosphate (IP_3). PKC is activated by binding to DAG at the membrane, and active PKC regulates non-muscle myosin II contractility by phosphorylating the regulatory light chain (RLC).

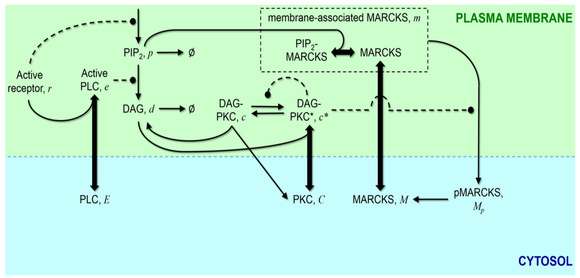


MARCKS: Myristoylated alanine-rich C kinase substrate (MARCKS) binds to the plasma membrane by the hydrophobic insertion of myristate into the inner leaflet and the electrostatic interactions of its effector region with acidic phospholipids.

- One MARCKS sequesters up to three PIP_2 molecules.
- MARCKS phosphorylation by PKC liberates PIP_2.
- Thus, the pool of free PIP_2 available for hydrolysis is controlled.



Gambhir et al., BJ 2004



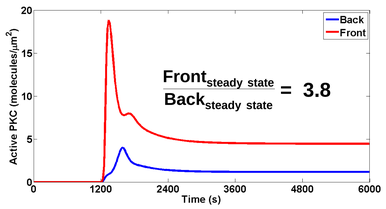
Model schematic and implementation

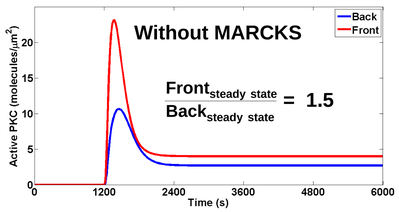
A predictive reaction-diffusion model was developed to link the external PDGF concentration field to the dynamics of PKC activation.

A shareable version of the model, with 2D and 3D geometries, was implemented in the Virtual Cell software environment.

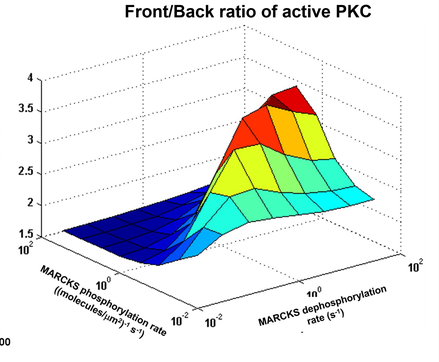
MARCKS dynamics are sufficient to amplify the PLC/PKC pathway

Simulated time courses of the active PKC concentrations show amplification of the PLC/PKC pathway across a cell following stimulation by a gradient of PDGF at 1200s.

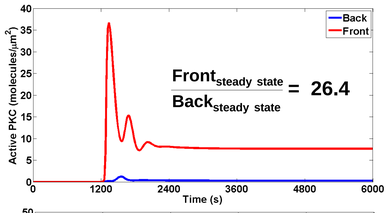


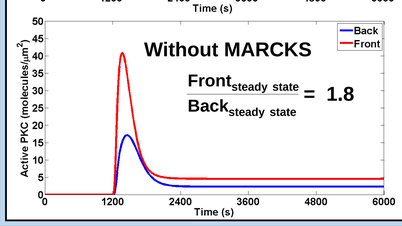


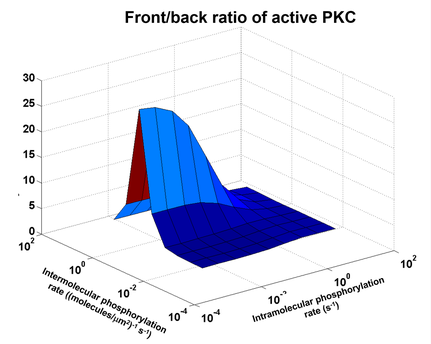
[PDGF] at front of cell = 0.13 nM
[PDGF] at back of cell = 0.067 nM



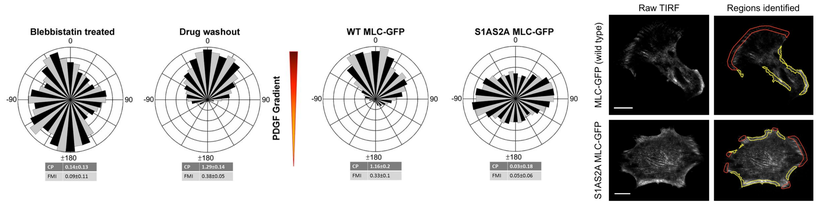
MARCKS synergizes with PKC feedback to enhance amplification of the pathway



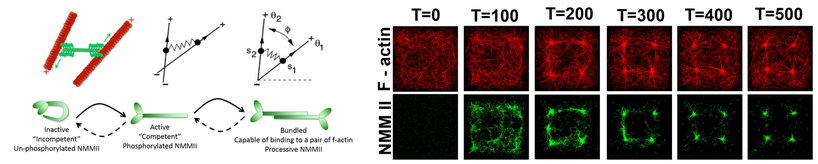




Evidence: regulation of myosin II by PKC is required for PDGF chemotaxis

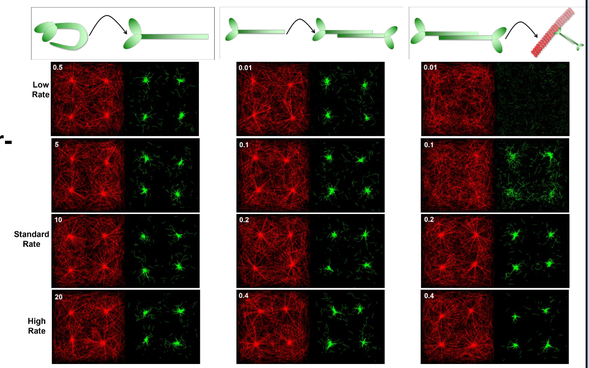


Aim 2 (supramolecular scale): physicochemical model of actomyosin self-organization

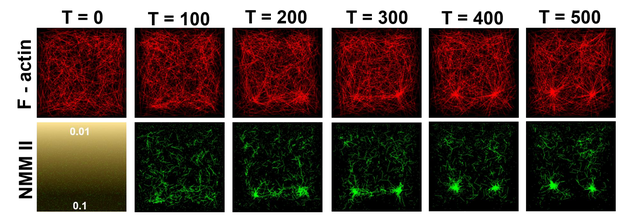


Model description: Filaments polymerize/depolymerize, and they rotate and translate in response to myosin contractile force. Motors attach to/detach from filaments, and they bundle to form mini-filaments. While bound to filaments, motors walk to plus ends and then fall off.

Regulation of motor-filament binding explains the effect of myosin II deactivation



Organization of actomyosin with graded binding rate



Future Work

- Assess robustness of PKC amplification with varying PDGF gradient conditions.
- Characterize the mechanism by which MARCKS amplifies the PLC/PKC pathway.
- Assess the effect of boundary conditions on actomyosin structures.
- Parametric analysis of myosin II activation and inactivation rates.
- Couple the molecular and supramolecular scales and relate to cell motility/migration bias.

References

Asokan, SB, Johnson, HE, Rahman, A, King, SJ, Rotty, JD, Lebedeva, IP, Haugh, JM, Bear, JE. Mesencymal chemotaxis requires selective inactivation of Myosin II at the leading edge via a noncanonical PLCγ/PKCα pathway. *Developmental Cell*, 31: 747-760 (2014).

Miller CJ, Ermentrout GB, Davidson LA. Rotational model for actin filament alignment by myosin. *Journal of Theoretical Biology*, 300: 344-359 (2012).

Acknowledgments

We are grateful to the NIH (U01EB018816) for support, and we thank members of the Bear, Elston, and Haugh labs for fruitful discussions.