Competitive resource allocation to metabolic pathways contributes to overflow metabolisms and emergent properties in cross feeding microbial consortia

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1. ABSTRACT

Biofilms are ubiquitous in medical, environmental, and engineered microbial systems. The majority of naturally occurring microbes grow as mixed species biofilms. These complicated consortia are comprised of a large number of cell phenotypes with complex interactions and self-organize into three-dimensional structures. While foundational to the vast majority of microbial life on the planet, the basic design principles including resource allocation strategies of consortia biofilms are still poorly understood.

Multiscale, spatiotemporal models were developed to investigate the intersection of resource gradients, resource competition and metabolism in a multispecies biofilm comprised of two common chronic wound isolates: the aerobe *Pseudomonas aeruginosa* and the facultative anaerobe *Staphylococcus aureus*. By combining genome-scale metabolic reconstructions with partial differential equations for metabolite diffusion, the models provided both temporal and spatial predictions with genome-scale, metabolic resolution. The models analyzed the phenotypic differences between monoculture and coculture biofilms and demonstrated the tendency of the two bacteria to spatially partition in the multispecies biofilm, along resource gradients, as observed experimentally.

Resource scarcity is a common stress in nature and has a major impact on microbial physiology in medical wounds. This poster highlights microbial acclimations to resource scarcity, focusing on resource investment strategies for chemoheterotrophs, including chronic wound isolates *Staphylococcus aureus* and *Pseudomonas aeruginosa,* from the molecular level to the pathway level. Competitive resource allocation strategies often lead to a phenotype known as overflow metabolism; the resulting overflow byproducts can stabilize cooperative interactions in microbial communities and can lead to cross feeding consortia. These consortia can exhibit emergent properties such as enhanced resource usage and biomass productivity which are both detrimental to patient health. The data presented here connects *in silico* analysis of temporally and spatially resolved consortia physiology with laboratory studies and ties the data together with ecological theories to better understand microbial stress responses and mutualistic consortia functioning

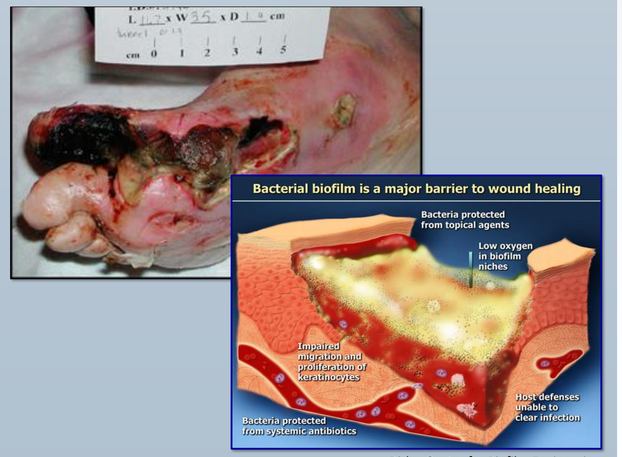
2. Chronic Wounds & Biofilms

Majority of microorganisms live as biofilm consortia

Microbial biofilms are polymer-encapsulated communities often associated with interfaces and are critically important in medical, environmental and engineered biological systems. Spatial heterogeneity is a key hallmark of biofilms due to diffusion constraints. Microbes residing in biofilms exhibit a phenotype distinct from planktonic growth, making antibiotic-based treatment a major challenge. For instance, bacteria in biofilms can tolerate antimicrobial agent concentrations 10,000 times higher than the same microbes grown planktonically[1].

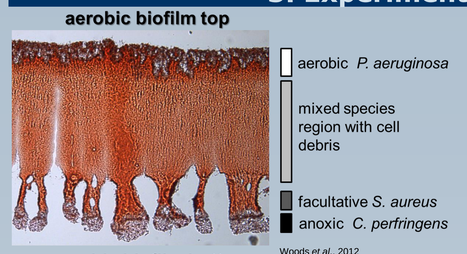
Chronic wounds are defined as a host-pathogen environment that has failed to proceed through a timely healing process. Chronic wound biofilms are a major and worsening health care challenge. An estimated 2% of the US population (6 million people) have a non-healing chronic wound with treatment costing more than $25 billion per year[3,4]. The occurrence of chronic wounds and the associated costs are anticipated to grow rapidly based on the aging US population and the dramatic rise in diabetes and obesity[5]. Chronic wounds are often colonized by microorganisms growing as biofilms, cells encapsulated in a self-produced extracellular polymer matrix, on a complex mixture of wound exudate[3-6].

Chronic wounds are typically colonized by consortia comprised of different microbial species including aerobes and anaerobes. A recent study measured an average of 5.4 species in each clinical wound[7]. A growing literature documents the emergent properties of polymicrobial systems, further highlighting limitations to Koch's postulate of 'one microbe, one disease'. For instance, polymicrobial infections have been reported to have elevated mortality rates relative to monocultures[8], and *in vivo* rabbit model systems demonstrated that consortia of *Pseudomonas aeruginosa* and *Staphylococcus aureus* prevented wound healing compared to the respective monocultures[9].

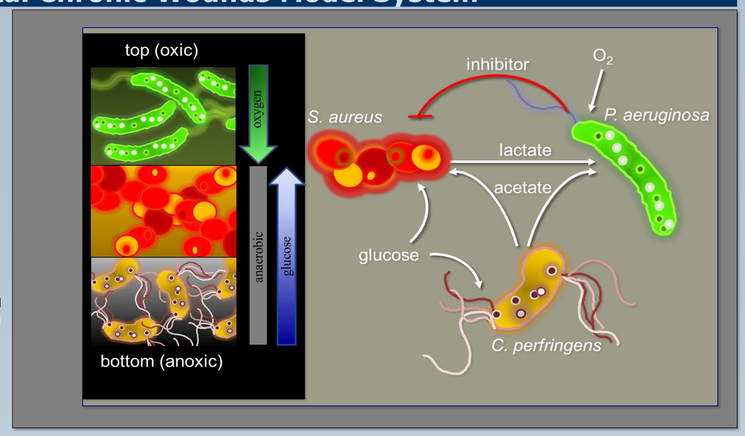


Dirkx, Center for Biofilm Engineering

3. Experimental Chronic Wounds Model System



Woods *et al.*, 2012

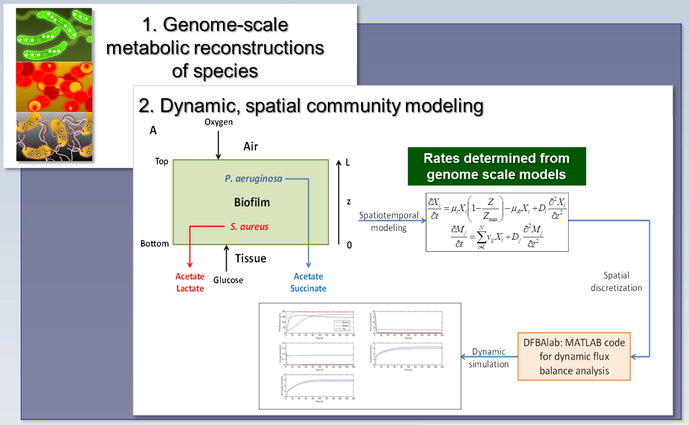


Biofilm polymeric matrix results in diffusion limitation and concentration gradients and the partitioning of microorganisms along those gradients. Understanding the multiscale organization of biofilm consortia requires explicit consideration of reaction-diffusion and cellular phenotypes.

While foundational to the vast majority of microbial life on the planet, the basic design principles of consortia biofilms are still poorly understood due largely to the complexity of many naturally occurring systems[2].

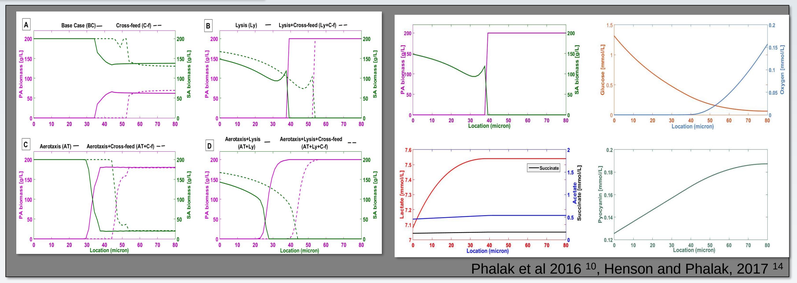
4. *In Silico* Multiscale Dynamic-Spatial Consortia Model

genes → enzymes → metabolites → cellular phenotype (z,t) → consortium phenotype (z,t)



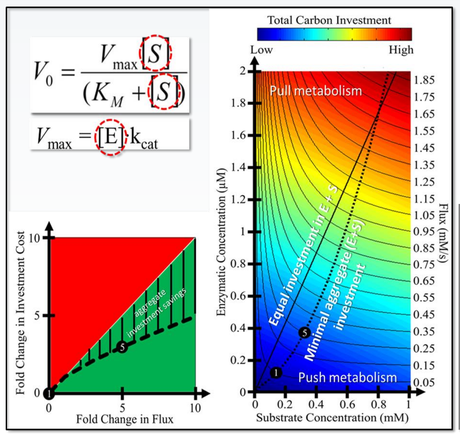
We developed **spatiotemporal models** to investigate the multispecies metabolism of a biofilm consortium comprised of two common chronic wound isolates: ***Pseudomonas aeruginosa*** and ***Staphylococcus aureus***. By combining genome-scale metabolic reconstructions with partial differential equations for metabolite diffusion, the models were able to provide both temporal and spatial predictions with genome-scale resolution. The models were used to analyze the metabolic differences between single species and two species biofilms and to demonstrate the tendency of the two bacteria to spatially partition in the multispecies biofilm as observed experimentally. Nutrient gradients imposed by supplying glucose at the bottom and oxygen at the top of the biofilm induced spatial partitioning of the two species, with *S. aureus* most concentrated in the anaerobic region and *P. aeruginosa* present only in the aerobic region. When each species was allowed to enhance its growth through consumption of secreted metabolic byproducts assuming identical uptake kinetics, the competitiveness of *P. aeruginosa* was further reduced due primarily to the more efficient lactate metabolism of *S. aureus*. Lysis of *S. aureus* by a small molecule inhibitor secreted from *P. aeruginosa* and/or *P. aeruginosa* aerotaxis were predicted to substantially increase *P. aeruginosa* competitiveness in the aerobic region, consistent with *in vitro* experimental studies.

The multiscale chronic wound biofilm simulations enabled an investigation of system properties that induce the observed *S. aureus* and *P. aeruginosa* spatial partitioning along concentration gradients. Predictions for two species biofilms of thickness W = 80 um with different species interaction mechanisms. Base case (BC): competition for the nutrients glucose and oxygen. Cross-feed (C-f): nutrient competition plus cross feeding of lactate, succinate and acetate. Lysis (Ly): nutrient competition plus *P. aeruginosa* mediated lysis of *S. aureus*. Aerotaxis (AT): nutrient competition plus *P. aeruginosa* chemotaxis towards oxygen. Predicted resource concentration gradients and resource acquisition rates.



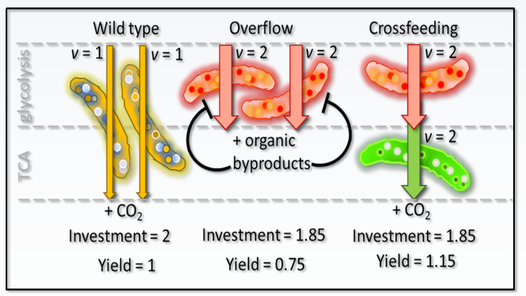
Phalak et al 2016 [10], Henson and Phalak, 2017 [14]

5. Consortia, Cell Interactions and Enhanced Resource Usage



Cellular function (flux) is driven by resource investments into a combinations of enzymes and substrate pools. The **resource allocation problem** can be quantified for a desired flux (light, solid line, left). There is an optimal investment for each desired flux (left)

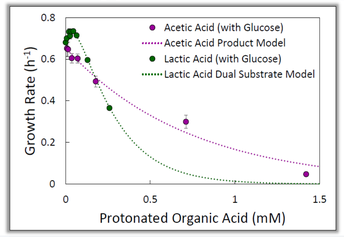
- Small fluxes are better driven by [substrate], large fluxes are better driven by [enzyme] (push vs. pull mechanisms)
- Higher fluxes are a better return on investment
- A cross feeding consortium can support a more biomass from a limiting resource based on enhanced functional return



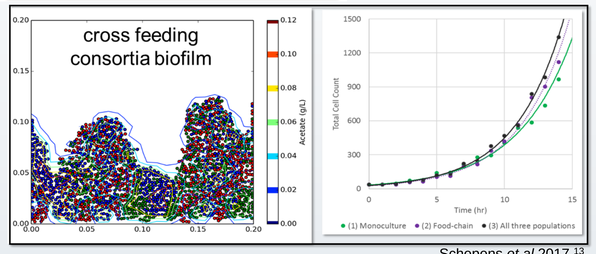
Beck *et al.*, 2016 [11] , Hunt *et al.*, 2016 [12], Carlson *et al.*, 2018

Division of labor can create highly efficient food webs with enhanced functional return on resource investment. The example illustrates a wildtype population performing a complete metabolism compared to a two member consortium where each population specializes in half the metabolism (*e.g.* glycolysis, respiration).

6. Byproduct Inhibition and Biofilms



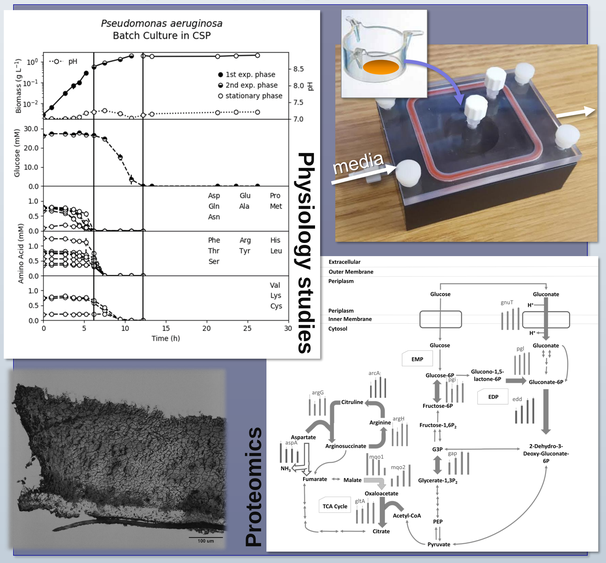
Division of labor and cross feeding metabolic species can enhance growth in biofilms by removing inhibitory products of metabolism. An agent based methodology was used to examine biomass productivity as a function of three consortia configurations. Kinetic parameters are based on experimental measurements. Model files available: github.com/jeffheys/biofilmSegregation



Schepens *et al* 2017 [13]

7. Experimental Analysis

Computational resource allocation research is supported by comprehensive experimental analysis including physiological and proteomics studies.



Despite their large impact on societal health, there are no systems-based computational models of microbial consortia responsible for biofilm formation in chronic wounds. We believe our model will enable the predictive testing of emergent consortia properties including enhanced nutrient accessibility, nutrient utilization and antibiotic tolerance and will provide the necessary knowledge for devising rational biofilm control strategies.

8. REFERENCES

1. Zuroff, T.R., et al., BMC Microbiol, 2010. **10**: p. 185.
2. Torsvik, V. and L. Øvreås,. Current Opinion in Microbiology, 2002. **5**(3): p. 240-245.
3. Gottrup, F., Am J Surg, 2004. **187**(5A): p. 38S-43S.
4. Sen, C.K., et al., Wound Repair Regen, 2009. **17**(6): p. 763-71.
5. Woods, J., et al., J Appl Microbiol, 2012. **112**(5): p. 998-1006.
6. Dowd, S.E., et al., BMC Microbiol, 2008. **8**: p. 43.
7. Thomsen, T.R., et al., Wound Repair Regen, 2010. **18**(1): p. 38-49.
8. Pulimood, S., et al., Diagnostic Microbiology and Infectious Disease, 2002. **44**(4): p. 353-357.
9. Seth, A.K., et al., PLoS One, 2012. **7**(8): p. e42897.
10. Phalak, Chen, Carlson, Henson., *BMC Sys Biol*, 2016. 10:90.
11. Beck, Hunt, Bernstein, Carlson, *Biotechnologies for Biofuel Production and Optimization*. 2016. 1: 407-432.
12. Hunt, Jennings, Inskeep, Carlson, *Envion Microbiol*. 2016. 18: 4946-4960
13. Schepens, Beck, Heys, Gedeon, Carlson, 2017, *Advances in Systems and Synthetic Biology,* pp 137-148. ISBN 978-2-7598-2116-7 .
14. Henson and Phalak, 2017, *in press*.
15. Carlson et al. 2018, *Biochemical Transactions, in press*.

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