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Naomi Balaban
Editor

Control of Biofilm Infections by Signal Manipulation

Foreword by J. William Costerton

With 53 Figures, 13 in color

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Naomi Balaban
Tufts University
Cummings School of Veterinary Medicine
Department of Biomedical Sciences
200 Westboro Rd
North Grafton, MA 01536
USA

Series Editor:
J. William Costerton
Director, Center for Biofilms, School of Dentistry
University of Southern California
925 West 34th Street
Los Angeles, CA 90089
USA

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Foreword

In the well-watered groves of academe, most of us are content to gather worshipful students and technicians in a shady nook to contemplate the eternal verities and to plan extravagant feasts to celebrate our contributions to "knowledge" and to the gradual improvement of the human condition. As one convocation follows another, and as our funding agencies pump billions of dollars into incremental research that fills every possible pigeon-hole in which a gene makes a protein, a small number of intellectual athletes seize a pivotal concept and plunge into the real world. It is this small band of nimble and impossibly brave intellectual halfbacks who win games in the real world, and this book is the result of the drive and intellectual athleticism of its editor and several of her contributors.

Bacteria affect humans more than any other life forms with which we share the blue planet, but our understanding of these invisible companions has developed in a staggering pattern, crippled by our panic and consequent shifts of emphasis. When our race was threatened by epidemic diseases, we visualized bacteria as swarms of potentially lethal planktonic cells from which we must remain isolated by sanitation and which we had to kill by immunization and chemical antibacterial compounds. By the time this overriding threat had been obviated, we began to examine natural and pathogenic ecosystems by direct methods, and we were surprised to find that planktonic bacteria are comparatively rare and that most prokaryotes grow in matrix-enclosed biofilms. This discovery helped explain the failure of immunization and antibiotic therapy in controlling the device-related and other chronic bacterial diseases that replaced epidemic diseases, but it provided no new weapons to the clinical armamentarium.

The academic team that assembled to study these fascinating multicellular bacterial communities was well funded; a network of research centers sprang up around the world; and a clear picture of the structure and function of biofilms began to emerge. The academic biofilm community penetrated the societies that present microbiology to the world, and the American Society for Microbiology (ASM) soon incorporated biofilm symposia and (most important) biofilm poster sessions into its annual meetings. The "slime people" assembled at weeklong ASM-sponsored symposia and workshops (1996, 2000, 2003, and 2007), and selected gurus were invited to meetings of the whole gamut of medical and dental specialties impacted by biofilms. But no biofilm-based therapies emerged.

Intellectual athletes look at the facts and theories in a field, as Wayne Gretzky would survey the scared thugs of Broad Street, and they design an experiment that

asks a well-defined question. Pete Greenberg surveyed the facts and theories of the biofilm field, listened to my enthusiastic ramblings about the complexity of biofilms, and assembled Jim Pearson's acyl homoserine lactone (AHL) minus mutants to see whether they could form proper biofilms. They couldn't, and the rest is history. We had published hundreds of papers and dozens of reviews suggesting that some kind of "communication" must be involved in the development of architecturally complex biofilms, but one simple experimental design by Pete nailed down the fact that AHL signals enable biofilm formation by gram-negative bacteria. In the meantime, Naomi Balaban had explored the control of biofilm formation in gram-positive bacteria by polypeptide signals and had designed and synthesized an effective inhibitor of the TRAP and *agr* system in *Staphylococci*. Also in the meantime, Mike Givskov had joined Staffan Kjelleberg's team in the bucolic reaches of Coogee Bay in Sydney and had participated in the demonstration that natural and synthetic brominated furanones inhibit biofilm formation by a wide variety of bacteria. More recently, David Davies has focused hard on the phenomenon of natural detachment and has identified a signal that triggers this release of planktonic cells from established biofilms.

The original notion that chemical signals must be involved in the complexity of biofilms gradually emerged from our speculations concerning how the familiar "water channel/microcolony" pattern could be initiated and maintained in biofilms. We did not anticipate that certain signals could switch biofilm formation on and off, as the AHL signals and the polypeptide and furanone inhibitors clearly do, and we are still searching for less draconian signals with fewer far-reaching effects. But the burgeoning biofilm research community is moving inexorably towards a consensus that the most important discovery in modern microbiology is that the activities of both planktonic and sessile bacteria are controlled by chemical signals. When we saw bacteria as unicellular entities, each responding to its environment according to the dictates of its individual genome and associated mechanisms, our only option in controlling these minute creatures was either to kill them or to feed them and hope for their gratitude and cooperation. Now we see bacteria primarily as sentient members of complex communities, within which they communicate with each other to set up mutually beneficial associations and coordinate measures that protect the whole community from stress and attack. Paradoxically, their use of a chemical language in their pursuit of safety may prove to be their Achilles' heel. The academics amongst us have conjured up visions of "grow slow" signals that could be very effective in saving the lives of septic patients with overwhelming bacteremia, in which antibiotic-mediated killing and lysis of the planktonic bacteria are clearly contraindicated. Similarly, we suggest that the bacteria that cause device-related infections may be "locked" in the planktonic state by biofilm signal inhibitors, and thus rendered susceptible to antibiotics and host defense factors. More recently we have suggested, in the preambles of our health-related grants, that species-specific or general signals may be useful in causing the detachment of planktonic cells from established biofilms, with the resultant resolution of chronic biofilm infections.

The intellectual athletes amongst us have taken specific concepts from this swelling mass of biofilm data and speculation, and have asked and answered very specific

and very incisive questions. Mike Givskov has used cells of *E. coli* that turn on green fluorescent protein in response to AHL signals produced by *P. aeruginosa* to show that these organisms actually produce AHL signals when growing in biofilms. Then he showed that certain brominated furanones repress this formation of AHL signals by cells of *P. aeruginosa* in biofilms growing in vitro in flow cells and in vivo in the mouse. Then this consummate intellectual athlete established model pulmonary infections in mice, treated the mice with the most effective brominated furanone, and showed the first resolution of a biofilm infection in an animal.

When I present Mike's data to academic audiences, the most common question is "Yes, but aren't the brominated furanones toxic?" We all tend to be underwhelmed by data once they are published, but we are obliged by basic scientific morality to admire the hardnosed plunge straight at the basic questions that the less athletic amongst us dance around for decades. Bacteria in biofilms do actually produce AHL signals. These signals diffuse throughout the communities; their production is affected by signal inhibitors, and this inhibition of signal production affects the course of at least one biofilm disease. Why don't all of us do these kinds of incisive experiments? Why do very few people play nose tackle for the Pittsburgh Steelers?

Intellectual bravery is not the exclusive property of pugnacious Danish males, and certainly our mild-mannered friend Bob McLean cut straight to the chase when he found biofilm signals in the mixed-species biofilms in the river in San Marcos. But perhaps the toughest of our intellectual athletes is Naomi Balaban. She developed her RIP inhibitor of the RAP signal in the crowded milieu of the early days of polypeptide signaling, paused only briefly to confirm its activity in vitro, and proceeded directly to animal models in which she has shown its efficacy in preventing both colonization and infection by staphylococci. She has enlisted dozens of collaborators in the march towards commercialization of this biofilm inhibiting signal blocker, and she has approached any anomalies in the team data by offering to repeat the critical experiments using her own strains and conditions. She has bulldozed the entire field in the most charming and disarming manner, and she could actually play nose tackle for the Steelers if she were more muscular and less beautiful. Naomi has acted as the highly visible bellwether of signal inhibition, while frankly commercial corporate labs have operated in secret, and the eventual success of signal inhibitors in the prevention of biofilm infections by staphylococci must certainly be laid at her feet.

Every practical implementation of a scientific discovery in clinical medicine requires a partnership between intellectually athletic scientists and perceptive clinicians, and the biofilm community is very fortunate to have "converted" Randy Wolcott to our cause. Randy runs a chronic wound center in Lubbock, Texas, and he is passionately devoted to the welfare of his many patients, who come to him for the resolution of wounds that have been complicated by biofilm infections for months or years. Randy has acquired impressive skills in biofilm microbiology: He is a scientific partner in a National Institutes of Health wound center based at Montana State University (CBE) and the University of Washington (UWEB), and he uses modern molecular techniques to analyze bacterial wound populations. In this book, Randy reports on his use of the RIP inhibitor identified by Naomi and the lactoferrin identified by Pete Greenberg and Pradeep Singh as being pivotal in the control of

biofilm infections. But the full importance of Randy's involvement in this widening team of incisive intellectual athletes is that he is just as brave and just as committed as they are, and he is completely prepared to leap for any pass they can throw to help his patients.

Our policy in the production of the Springer Series on Biofilm books will be to offer the basic *Biofilm Primer* (Costerton), which was published in March of 2007, followed by a series of books on various aspects of these multicellular communities. We chose the topic of signal manipulation for the second book, with Naomi Balaban as author/editor, because we wish to capture and spotlight the area within the biofilm field that shows the greatest potential for the translation of biofilm science to medical, dental, and veterinary applications.

We will keep this book updated because this field is very active, and we will gradually add new books on specific biofilm infections and on the structure and function of these sessile communities that dominate the prokaryotic component of the biosphere.

Los Angeles, June 2007 Bill Costerton

Preface

The number of patients affected by and dying from what can be considered as a "biofilm diseases" is higher than that for heart disease and cancer combined, making medical biofilms the biggest single disease that the health care system is facing today. It is thus immensely important to better understand biofilms. When I first started studying bacterial pathogenesis, I was struck by the extent that bacteria need to communicate with one another in order to achieve functions necessary for their survival in the host. One can imagine that in a close-knit community such as that found in a biofilm, the bacteria can more effectively protect themselves as well as exploit the environment for their benefit. This book describes the molecular mechanisms of bacterial cell-to-cell communication, the development of anti-biofilm inhibitors, and the use of such inhibitors to suppress bacterial infections in animals and humans.

This book is the product of Dr. Bill Costerton's determination and inspiration to so many of us and to biofilm researchers throughout the world. I am extremely grateful to my co-authors who are carrying out such incredible work with dedication and often with great personal sacrifice, resulting in numerous contributions to science and humanity. Some of us work under the radar to make sure that scientific truth and medical advancement do not get confused with politics, fame, or monetary compensation. We hope this book will benefit all those who care to learn.

North Grafton, November 2007 Naomi Balaban

Contents

Contributors

Balaban, Naomi
Tufts University, Cummings School of Veterinary Medicine, Department of Biomedical Sciences, 200 Westboro Rd, North Grafton, MA 01536, USA
Email: naomi.balaban@tufts.edu

Banin, Ehud
Bar-Ilan University, The Mina and Everard Goodman~Facility of Life Sciences, Ramat Gan 52900, Israel
Email: banine@mail.biu.ac.il

Bryant, Sandra A.
Department of Biology, Texas State University-San Marcos, San Marcos, TX, USA
Department of Microbiology and Immunology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Cirioni, Oscar
Institute of Infectious Diseases and Public Health, Universita Politecnica delle Marche, Clinica delle Malattie Infettive, c/o Ospedale Regionale, Via Conca, 71, 60200 Ancona AN, Italy
Email: anconacmi@interfree.it

Cvitkovitch, Dennis
University of Toronto, Dental Research Institute, 124 Edward Street, Toronto, Ontario, Canada M5G 1G6
Email: d.cvitkovitch@dentistry.utoronto.ca

Giacometti, Andrea
Institute of Infectious Diseases and Public Health, Universita Politecnica delle Marche, Clinica delle Malattie Infettive, c/o Ospedale Regionale, Via Conca, 71, 60200 Ancona AN, Italy
Email: anconacmi@interfree.it

Givskov, Michael
Technical University of Denmark, BioScience and Technology, BioCentrum, Bldg 227, DK-2800 Lyngby, Denmark
Email: mgi@biocentrum.dtu.dk

Greenberg, E. Peter
University of Washington, School of Medicine, Department of Microbiology, Seattle, WA 98195, USA
Email: epgreen@u.washington.edu

McLean Robert J.C.
Texas State University, Department of Biology, 601 University Drive, San Marcos, TX 78666, USA
Email: McLean@txstate.edu

Qi, Felicia
University of Oklahoma Health Sciences Center, College of Medicine, Oral Biology, College of Dentistry and Microbiology and Immunology, BRC366, 975 NE 10th St. Oklahoma City, Ok 73104
Email: felicia-qi@ouhsc.edu

Rasmussen Thomas B.
Christian Hansen A/S, Innovation, Cultures & Enzymes Division, Bøge Allé 10-12, DK-2970, Hørsholm
Denmark

Ren, Dacheng
Syracuse University, Department of Biomedical and~Chemical~Engineering, 121 Link Hall, Syracuse, NY 13244, USA
Email: dren@syr.edu

Senadheera, M. Dilani
University of Toronto, Dental Research Institute, 124 Edward Street, Toronto, Ontario, Canada M5G 1G6

Vattem, Dhiraj A.
Texas State University, Nutritional Biomedicine~& Biotechnology, 601 University Drive, San Marcos, TX 78666, USA
Email: dv11@txstate.edu

Ward, John
School of Mathematics, Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK
Email: John.Ward@lboro.ac.uk

Wolcott, Randall D.
Southwest Regional Wound Care Center, 2002 Oxford Avenue, Lubbock, TX 79410
Email: randy@randallwolcott.com

List of Abbreviations

aa	amino acid
ABC	ATP-binding cassette
ADI	Urease and arginine deiminase
AHL	*N*-acyl homoserine lactone
AIP	Agr autoinducing peptide
CF	Cystic fibrosis
CFU	Colony forming unit
CSP	Competence stimulating peptide
CVC	Central venous catheter
EM	Electron microscopy
EPS	Extrapolymeric substances
GFP	Green fluorescent protein
HK	Histidine kinase
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSE	Methicillin-resistant *Staphylococcus epidermidis*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
MSSE	Methicillin-sensitive *Staphylococcus epidermidis*
OHHL	N-3-(oxohexanoyl)-L-homoserine lactone
OD	Optical density
PBP2a	Penicillin-binding protein 2a
PBS	Phosphate buffered saline
PQS	*Pseudomonas* quinolone signal
QS	Quorum sensing
QSA	QS autoinducers
QSI	Quorum sensing inhibitor
QSIS	QSI selector
RAP	RNAIII activating protein
RIP	RNAIII inhibiting peptide
RPB	RAP-binding protein
SAM	S-adenosylmethionine
SEM	Scanning electron microscopy
SQS	Staphylococcus quorum sensing
SRB	Sulfate-reducing bacteria
TRAP	Target of RAP

TNF	Tumor necrosis factor
VISA	Vancomycin-intermediate *S. aureus*
VISE	Vancomycin-intermediate *S. epidermidis*
VRSA	Vancomycin-resistant *S. aureus*
VRSE	Vancomycin-resistant *S. epidermidis*