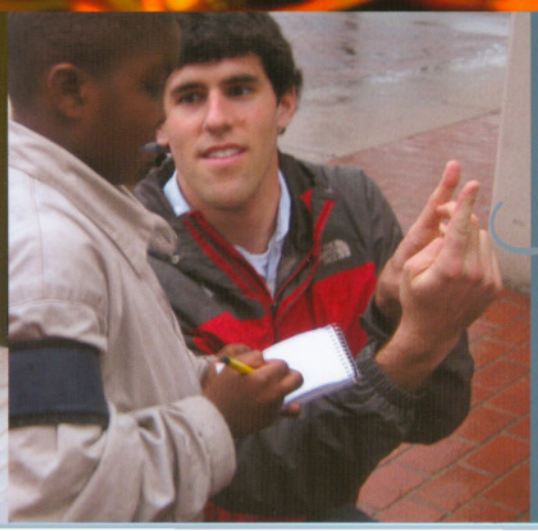
Mdental

BALTIMORE COLLEGE OF DENTAL SURGERY, UNIVERSITY OF MARYLAND
The Research and Alumni Magazine of the World's First Dental School

Spring 2007

The Power of Reinvention



Honor Roll of Donors

THE BIOFILMS THAT REALLY BUG US

Microorganisms that coexist and hang on to their hosts can lead to life-threatening consequences.

By Regina Lavette Davis

Cooperative, well-organized, and altruistic. Excellent characteristics, most would agree, for an ideal community. These "communities" can be found almost anywhere: from the tip of the tongue to pond scum. Biofilms are all around us.

Biofilms are defined as cooperating microorganisms that exist as a well-organized community. They are responsible for everything from slime on stream rocks to serious health infections. According to the Centers for Disease Control and Prevention (CDC), biofilms have great importance for public health because of their role in many infectious diseases.

These microorganisms (or superbugs, as they are known) account for millions of hospital-borne infections each year. Dental School researchers Mary Ann Rizk, PhD, and Mark Shirtliff, PhD, are leading the field in breakthrough investigations of mixed-microbial biofilms that will help prevent and fight infections that often have high mortality rates.

Microbes with a Chokehold

A biofilm consists of microbes such as bacteria and fungi that are attached to a hydrated surface and then become embedded within a polysaccharide slime. "Examples of biofilms include the slippery rocks on a mountain stream or the slime buildup on your toilet at home," says Shirtliff, assistant professor in the Department of Biomedical Sciences in the Dental School and adjunct professor in the School of Medicine's Department of Microbiology and Immunology.

Dental plaque (biofilm formed on teeth) is another example. When bacteria exist in this "attached state," antibiotics and other antimicrobial agents do not affect the bacteria, and "our own immune systems can't get rid of these microbial communities," adds Shirtliff. Brushing away the plaque is necessary, he explains, to keep cavities from forming and to prevent dental infections such as periodontitis and gingivitis.

Although plaque can be removed, a bigger concern for researchers are those biofilms that cannot be removed. What happens when pathogenic microbes adhere to surfaces and form a permanent biofilm? These biofilm-related infections can form on bone, heart valves, the lenses of your eyes, and devitalized tissue.

Indwelling medical devices also play host to biofilm infections that can cause serious illness and mortality. Examples of such biofilms, says Shirtliff, are those that form on corneal implants, cerebrospinal fluid shunts, prosthetic heart valves, facial reconstruction plastics

and metals, prosthetic vascular grafts, endocardial pacemaker leads, artificial heart valves, joints and hips, artificial lenses, dental implants, and urinary catheters. Research from the CDC states that "cystic fibrosis, native valve endocarditis, otitis media, periodontitis, and chronic prostatitis all appear to be caused by biofilm-associated microorganisms."[1]

The research in Shirtliff's laboratory is centered on understanding the dynamics of biofilms, and this is crucial to the development of novel diagnostic tools, antimicrobials that target biofilm-related infections, and possibly antibiofilm vaccines. Using two-dimensional gel electrophoresis, microarray analysis, reporter systems, and gene disruption techniques, Shirtliff and his team are able to identify biofilm-specific genes and their products in methicillin-resistant *Staphylococcus aureus* and *Proteus mirabilis*.

"The most serious, perhaps, are infections of intravenous catheters, which invariably lead to bloodstream infections with high mortality rates," he says. These infections resist antimicrobial therapy, often leading to surgical removal of the infected devices. In fact, "bacteria tend to be 50 to 500 times more resistant to antibiotics when associated with a biofilm. These types of infections are on the rise, and the CDC has estimated that up to 56 percent of all infections are now biofilm-mediated," Shirtliff says.

The CDC estimates that "nearly 2 million patients annually get an infection while being treated for another illness or injury, and nearly 88,000 die as a direct or indirect cause of their infection."[2] In terms of the economic burden, the organization also reports that these infections translate into a cost of $6.7 billion annually in the United States.[3]

Aside from bacteria-related biofilm research, other important investigations are related to fungal infections, which is Rizk's area of expertise. An assistant professor in the Dental School's Department of Diagnostic Sciences and Pathology and adjunct assistant professor in the Department of Pathology in the School of Medicine, Rizk is also an assistant director in the Clinical Microbiology Laboratories at the University of Maryland Medical System.

Her research in the Dental School focuses on the molecular characterization of virulence factors in pathogenic *Candida* species. The AIDS epidemic during the last 25 years has brought about an increase in the incidence of candidiasis. *Candida albicans* is an opportunistic pathogen capable of biofilm formation, tissue invasion, and systemic infections especially in the immunocompromised population, particularly in the HIV-infected.

"Compounding the gravity of the rise in fungal infections is the increase in the emergence of strains resistant to the commonly available antifungal drugs," Rizk says. "*C. albicans* is primarily an oral pathogen responsible for a variety of oral conditions such as pseudomembranous candidiasis, mucocutaneous candidiasis [thrush], and angular cheilitis as well as denture stomatitis."

Like the bacterial biofilms, *Candida* also is capable of adhering to the surfaces of indwelling medical devices and intravascular catheters that provide a route through the body's barrier defenses, which often leads to bloodstream infections. "In fact, *Candida* species are currently ranked as the third most

Mary Ann Rizk

Mark Shirtliff

Photographs by Robert Burke

commonly isolated bloodstream pathogens, and mortality associated with systemic *Candida* infections is approximately 50 percent," says Rizk.

According to Rizk, an estimated 80 to 90 percent of HIV-infected individuals suffer recurrent episodes of oral candidiasis during their disease progression. In fact, she adds, the onset of candidiasis in these individuals is considered "a hallmark for the initiation of AIDS and the condition is used in the staging of HIV infection, when it is observed concomitant with declining CD_4 T cell subsets."

Joining Forces

What happens when two biofilm species interact is a fertile area of research investigation. Shirtliff and Rizk's interest in microbial biofilms and their impact on antimicrobial therapy has led to the two researchers' collaboration in exploring the interaction of these microbial species in a mixed bacterial-fungal biofilm. It is an area of research not previously investigated.

"Little is presently known about the interaction between mixed species in microbial biofilms," says Shirtliff. He and Rizk are combining their efforts using imaging techniques, microarrays, and proteomics to understand this complex interaction between these two species.

Although many biofilm infections are traced to a single species, serious infections often involve multiple microbial species. Together, *C. albicans* and *S. aureus* are a powerhouse duo that can create persistent, chronic, and systemic infections. Theirs is a mutually beneficial symbiosis.

"The bacterial pathogen, *Staphylococcus aureus*, and the fungal pathogen, *Candida albicans*, are currently the leading pathogens in bloodstream and catheter-related biofilm infections in hospitalized patients," says Rizk.

The fungal *C. albicans* has two forms: a round yeast form (that can cause bloodstream and systemic infections) and an elongated filamentous (or *hyphal*) form that can invade tissues. Once *Candida* attaches to tissues, and under unfavorable conditions such as a low-nutrient environment, the invasive hyphal form predominates.

"This is the form that the bacterial species *S. aureus* attaches to and hitches a ride into the deeper tissue to invade and cause persistent infections," Shirtliff explains. "*C. albicans*, in turn, benefits from this relationship by getting the help from the virulence factors of *S. aureus* that simultaneously attack and suppress the immune system to prevent the host from eliminating the infection."

In vivo mice studies have demonstrated what the researchers refer to as "a synergistic effect" between *C. albicans* and *S. aureus* on the mortality of dually infected mice. These studies provide evidence that shows how *C. albicans* directly stimulates the growth of *S. aureus* and amplifies its virulence. The clinical significance is that this amplified virulence could have grave medical implications for people who harbor both pathogens simultaneously.

Can They Be Stopped?

Traditional antimicrobial regimens are currently too ineffective to seriously contend with the dangers posed by these pathogens. Through their joint and individual investigations, Shirtliff and Rizk hope to identify novel therapeutic strategies that will help prevent and treat biofilm-related infections that are resistant to therapy. Their collaboration has, so far, resulted in several publications and grant submissions for continued funding for their research.

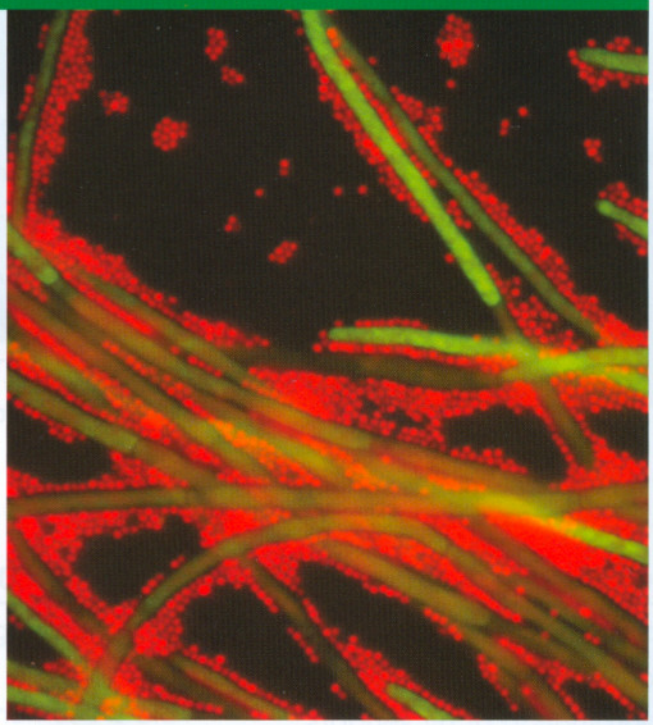
"Understanding the interactions between pathogens and devices to which they adhere, especially in mixed-species biofilm infections, is critical in relation to therapeutic strategies," says Shirtliff, who is using animal models that will lead to vaccine development and adjuvant therapeutic measures. He also is developing rapid, sensitive, and specific diagnostic assays using tagged monoclonal antibodies against biofilm-specific proteins to diagnose endocarditis, prosthetic implant infection, deep abscess, and osteomyelitis.

Additionally, a novel gene knockout system is being used to determine the relative importance of genes that exhibit increased expression in a biofilm as assessed by microarray analysis. In terms of prevention, novel indwelling medical devices, including prosthetic implants (e.g., intra-medullary bone stabilization rods), and dental implants that elute bone regenerative activators and antimicrobial agents to prevent biofilm formation are being developed.

As for Rizk, she says she is excited by her recent investigations that have identified the presence of a process of apoptosis (cell suicide) in *Candida* triggered by a "quorum-sensing molecule" known as farnesol. "Farnesol is effective at killing and inhibiting the formation of the biofilm," she says. As a biofilm matures, she explains, the aging population of cells in the biofilm altruistically "commit suicide" through apoptosis, a gene-controlled and tightly regulated process that ensures the survival of the species. "At certain concentrations, farnesol is capable of killing and inhibiting the formation of biofilm by *Candida*," she says.

Shirtliff is similarly excited by these studies because he says,

Staphylococcus aureus on catheter surface



S. aureus in red, *C. albicans* in rod-shaped hyphal forms in green

"Farnesol is produced by *Candida* in the non-invasive yeast form and this compound kills staphylococci. However, as soon as the *Candida* switch to the invasive hyphal form, farnesol production drops and *S. aureus* can attach and enter the tissues."

They have published research findings on these initial investigations (along with Timothy Meiller, DDS, PhD, a professor in the Department of Diagnostic Sciences and Pathology), which so far indicate "that modest concentrations of farnesol were sufficient to exhibit an antibacterial effect and significantly inhibit biofilm formation." [4,5]

Continued epidemiologic and laboratory research, therefore, is needed to better characterize these pathogens, allowing for improved diagnostic and therapeutic strategies. The focus, however, remains the host. "We want to take what we have learned in the lab and try to relate it to the patient and to what's actually happening in the hospital," says Rizk.

To that end, and to validate the clinical significance of their findings, Rizk and Shirtliff are collaborating with colleagues from the Department of Pathology (Clinical Microbiology Laboratory) and the Division of Infectious Diseases at the School of Medicine. Their close association with the University of Maryland Medical Center has given them access to samples from infected and critically ill patients (from blood and infected catheters and other devices). Analyzing these samples can provide the researchers a truer reflection of the dual infectious process that occurs in the host.

Reaching out for new discoveries is a challenge that both researchers welcome. Rizk sums up her approach to novel research by quoting the Nobel Prize-winning biochemist Albert Szent-Gyorgyi, who said, "Research is to see what everybody else has seen and to think what nobody else has thought."

1. Donlan R.M. "Biofilms: Microbial Life on Surfaces." Emerging Infectious Diseases [serial online] September 2002. Available from: http://www.cdc.gov/ncidod/EID/vol8no9/02-0063.htm.
2. CDC Press Release. March 2000. "Hospital Infections Cost U.S. Billions of Dollars Annually."
3. Graves N. "Economics and Preventing Hospital-Acquired Infection." Emerging Infectious Diseases [serial online] April 2004. Available from: http://www.cdc.gov/ncidod/EID/vol10no4/02-0754.htm.
4. Jabra-Rizk, M.A., Meiller, T.F., James, C.E., Shirtliff, M.E.. April 2006.. "Effect of Farnesol on Staphylococcus aureus Biofilm Formation and Antimicrobial Susceptibility." Antimicrobial Agents and Chemotherapy, pp. 1463-1469, Vol. 50, No. 4.
5. Jabra-Rizk, M.A., Shirtliff, M.E., James, C.E., Meiller, T.F.. November 2006. "Effect of Farnesol on Fluconazole Resistance in Candida dubliniensis." FEMS Yeast Research, p. 1063, Vol. 6.