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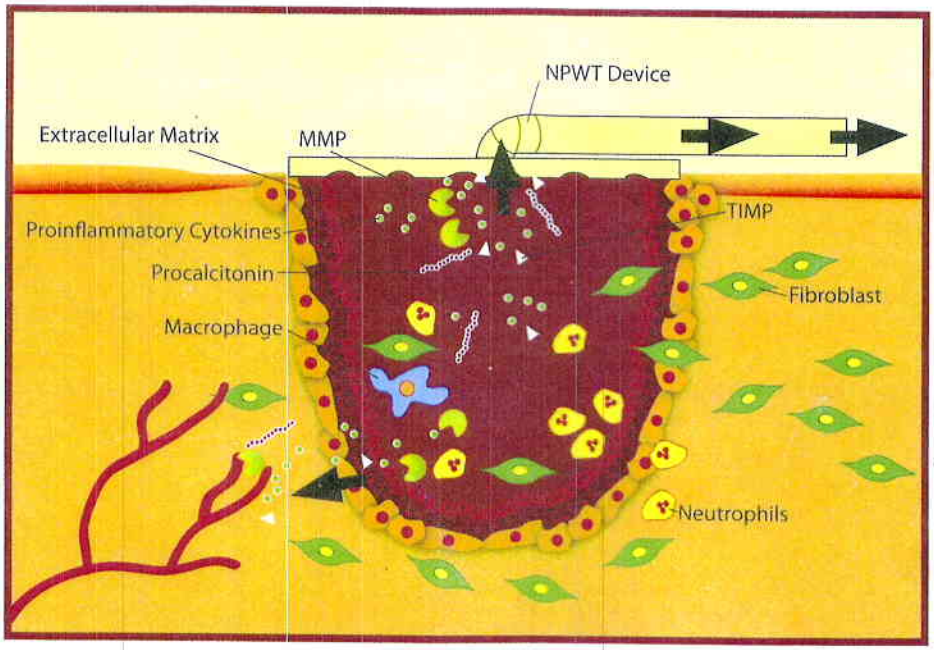

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The Role of Biofilms: Are We Hitting the Right Target?

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Background: Chronic infections affect 17 million people yearly, and approximately 550,000 people die each year from, or with, their chronic infections. Acute and chornic infection differences are well known to clinicians, but the role of bacteria in producing these clinical differences remains poorly understood.
Methods: This review relies on basic science, clinical studies, and a general review of the medical biofilm literature. The basic science studies are level A and B quality of evidence. The clinical studies are mainly retrospective cohort (level B) and case studies (level C). The biofilm literature includes reviews with varying levels of evidence. All articles have been peer reviewed and meet the standard of evidence-based medicine.
Results: Acute infections are associated with planktonic bacteria and must be diagnosed rapidly and accurately to prevent tissue damage and/or death. In contrast, biofilm behavior pursues a more parasitic course by producing sustained host hyperinflammation, with the biofilm feeding on plasma exudate. Chronic infections vacillate over long periods of time, responding only partially to antibiotics and reemerging once the antibiotics are withdrawn. Chronic wounds exhibit similar clinical behavior seen in other chronic infections and are associated with biofilm phenotype bacteria on their surface. Biofilm infections, such as chronic wounds, cannot be adequately diagnosed with current clinical cultures; therefore, molecular methods are necessary.
Conclusions: Biofilm phenotype bacteria require multiple concurrent strategies, including débridement and targeted antibiofilm agents. Biofilm phenotype bacteria predominate on the surface of wounds, and biofilm-based management improves wound healing outcomes, indicating that biofilm is the right target for managing the bioburden barrier of chronic wounds. (*Plast. Reconstr. Surg.* 127 (Suppl.): 28S, 2011.)

Biofilms are presently garnering much attention but more as a curiosity than as the seminal element of modern infectious disease. With the Centers for Disease Control and Prevention estimating that 65 percent of all human infectious disease is caused by biofilm phenotype bacteria and the National Institutes of Health suggesting that this is closer to 80 percent,[1] biofilm must be in the forefront of our thinking when considering chronic infections such as chronic wounds. A review of the literature shows that biofilm infections affect almost every tissue in the human body.[2,3] If all these chronic infections produced by medical biofilm are considered as a whole, up to 17 million new biofilm infections occur each year in the United States, with up to 550,000 people dying each year with or from these infections.[3] However, even with this immense scope of disease as evidenced by overwhelming morbidity and mortality, chronic infections are not treated with any sense of urgency. Whether the chronic infections involve tonsils, a heart valve, or a foot wound, the chronic infection is commonly

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treated episodically until the tonsils are removed, the heart valve replaced, or the leg amputated. By focusing on the right target biofilm phenotype bacteria using a different strategy to produce infections, we can obtain substantially better outcomes for healing these infections rather than removing body parts.

The main decision points for the physician in treating an infectious disease includes not only the tissue involved, the organisms, and host factors, but also the differentiation of whether the infection is acute or chronic. Physicians have a clear grasp of the vast clinical differences between an acute and a chronic infection; however, current therapeutic options do not reflect that difference. Acute infections, such as cellulitis, sepsis, and pneumonia, are generally short-duration aggressive infections associated with tissue destruction. Chronic infections, in contrast, tend to be focal infections, limited in size, that wax and wane for long durations and are only partially destructive to tissues. These differences are not necessarily a factor of time or even the type of host immune response but rather the survival strategies pursued by the infecting microorganisms. The strategies of a single-cell, mobile, free-floating bacterium (planktonic) versus those of a community of bacteria encased in a self-secreted protective matrix (biofilm) are radically different and may define two fundamentally different types of infections: "acute" and "chronic."

For the physician, there is an enormous clinical advantage in understanding the different ways in which bacteria produce infection. Chronic infections produce symptoms that are most consistent with biofilm formation by the bacteria producing the infection. Because biofilm is intrinsically resistant to host immunity, antibiotics, and biocides, different treatment strategies will be required. Physicians are more comfortable with acute infections that require only a single course of treatment (one and done) that usually eradicates the infection, never to return again. Chronic infections such as chronic wounds, surgical-site infections, and infected implants will yield only to repetitive evaluation and multiple simultaneous therapies that require much persistence from the physician.

METHODS

This review relies on the authors' basic science and clinical studies and a general review of the peer-reviewed medical biofilm literature. The basic science studies are level A and B quality of evidence. The clinical studies are mainly retrospective cohort studies (level B) and case studies (level C). The medical biofilm literature included several reviews with varying levels of evidence. For example, one study required reviewing over 2000 articles dealing with biofilm infections. All articles have been peer reviewed and meet the standard of evidence-based medicine.

DISCUSSION

Acute infections are characterized by rapid onset associated with classic signs of Celsus (rubor, tumor, dolor, calor), rapid progression, and rapid response to appropriate antibiotics.[4] At the cellular and molecular levels, acute infections appear to be the result of bacteria pursuing a planktonic phenotype growth constituted by a single species. These bacterium possess numerous molecular mechanisms to evade, subvert, and adapt to host immunity and antibiotics.[5,6]

For example, when *Pseudomonas aeruginosa* is motile (planktonic growth state), it expresses multiple virulence factors including pyocyanin (a small cidal molecule),[7] pyoverdines (a family of protein siderophores),[8] exotoxin A, phospholipases (A to C), rhamnolipids,[9] proteases (elastase, alkaline protease, LasA proteinase, protease IV),[10] and T3SS effector proteins including ExoU (phospholipase 2),[11] all of which can easily breech host epithelial or mucosal barriers.

There are almost infinitely more weapons and tactics in the arsenal of planktonic bacterium, yet most seem to be used in the general strategy of a predatory infection. That is, the bacterium upregulates virulence factors and processes designed to kill host tissue.[12,13] The bacterium then secretes proteases to digest and feed on host tissue.[14] The limitation of this infectious strategy is that the bacterium quickly kills the host or is cleared by host immunity. Either way, there is a quick resolution, and the infection is not sustained, allowing only limited time for reproduction.

Clinical culture, the most commonly used test for diagnosing infection, is a method that is over 150 years old, with multiple limitations. Clinical cultures have a growth bias, therefore selecting some organisms over others.[15] Also, many species that can infect human tissue cannot be grown in the laboratory, whereas the ones that can are identified through inexact biochemical methods. However, the most onerous feature of cultures, with regard to acute infections, is the time it takes to grow, identify, and ascertain sensitivities for a sample.

Molecular methods, however, demonstrate manageable bias for detecting different species,

identify even small amounts of up to 98 percent of known bacteria (sensitivity), identify bacteria with DNA certainty (specificity), and most importantly do so rapidly. Polymerase chain reaction–based tests can be accomplished in as little as 2 to 3 hours, with other molecular methods taking up to only 6 to 24 hours.[15,16] Regardless of the molecular method used, microorganisms are identified comprehensively, accurately, and in real time.

The idea and methods of polymerase chain reaction originated in 1985, with clinically useful applications emerging by 1990. Texts describing that technology along with clinical methods and applications were available by 1994.[17] Now, two decades later, polymerase chain reaction has matured into the standard means for identification of microorganisms.[18] Most importantly, bacteria growing within a biofilm infection are best identified using molecular methods. Physicians should not dwell on a single molecular technology because the field is so fluid at the present time. Because of the human genome project, very powerful new platforms will emerge over the coming years.

Biofilm phenotype growth states pose insurmountable difficulties for routine clinical cultures. Environmental microbiologists realized long ago that culture methods could not grow what scientists could see with their microscopes.[19] Through molecular methods, they have defined an environmental microbial reality where 99 percent of bacteria belongs to biofilm communities. Biofilm scientists have also discovered that biofilm phenotype bacteria often do not grow with clinical culture methods even though they are alive (viable but not culturable).[20] Finally, biofilm infections are overwhelmingly polymicrobial and therefore can never be adequately evaluated by a clinical culture that is structured to identify only one organism.

Biofilm formation by bacteria, yeast, and fungus has an extensive basic science literature.[21–24] Biofilms have existed since the dawn of time, and have been found as stromatolites in the early fossil record from 3.25 billion years ago. Interestingly, these biofilms still exist off the coast of Australia, seemingly unchanged over that vast time. Evidently, biofilm developed a successful strategy from the very beginning, discovering that it was much better to cooperate than to compete.

Biofilm, regardless of the species that comprises the whole, have basic features in common. First, there is usually attachment of the bacteria to a surface. Surfaces, whether the wound bed, suture, or an implanted medical device, favor bacterial attachments and therefore biofilm formation. Attachment to a surface is the first committed step and the most potent signal for biofilm formation.

Second, the bacteria secrete substances to protect the biofilm from environmental dangers such as bacteriophage, ultraviolet light, and desiccation in the natural world. In a host environment, this extracellular polymeric substance secreted by each bacterium provides protection against white blood cells, antibodies, and even therapeutic antibiotics in the host environment.[25] The molecules secreted by each biofilm bacteria are usually polysaccharides, which prompted the name "glycocalyx" for the extracellular polymeric substance. However, in a host setting, the matrix may be composed of polysaccharides, host DNA (mainly from neutrophils), bacterial DNA, bacteria proteins (e.g., biofilm accumulation protein), or host components usually associated with plasma (e.g., fibrin, albumin). Also, the biofilm has the ability to mix each of these components to suit its needs, or even change the matrix composition to confront different treatments or threats.

A third property of biofilm is that each bacterium in the biofilm has a different physiology or growth state, which is under direction of the quorum sensing signaling system. Quorum sensing molecules produced by an individual bacterium can act on that bacterium, other bacterium of the same species, other bacteria of different species, or even the host. Quorum sensing is unique to biofilm and therefore is a defining property demonstrating biofilm. The main role of quorum sensing is to direct gene expression of the different members throughout the biofilm. This is best illustrated by the varying rates of growth of individual bacteria throughout the community. The base region attached to the host surface has essentially no metabolic activity, whereas the environmental edge has significant metabolic activity, and there are various growth states in between. The molecular mechanisms under quorum sensing control that allow the cooperation of diverse microorganisms are well established.[26,27]

Finally, biofilm has the ability to reconstitute itself after a catastrophic event that destroys almost all of its members of bacteria. The remaining fragments (which are protected by the glycocalyx) will reattach, become metabolically active, and then signal through quorum sensing pathways to rise up and reconstitute the biofilm in the exact same host niche. Fortunately, while the biofilm reconstitutes itself, it is more vul-

nerable to host immunity and to treatments, thus creating a therapeutic window.

Today, the clinical importance of the relationship between planktonic phenotype and biofilm phenotype bacteria is beginning to be appreciated by physicians. Although chronic infections are usually polymicrobial, it is easier to understand these infections if one starts by looking at a single-species biofilm.

P. aeruginosa possesses a large contingent of genes that can allow an individual bacterium to organize into a community protected by a self-secreted matrix with heterogeneous phenotypes and growth states of its members. This genetic capacity allows for the metamorphosis of an individual bacterium into a sessile community to pursue a cooperative existence with the same or different species with mutual benefits and synergies.

There are several known molecular pathways under quorum sensing control for biofilm to inflame the host substrate, thus providing nourishment through exudate instead of host cell death.[28] There are several general quorum-sensing systems (e.g., lasIR, rhl) that regulate *Pseudomonas* biofilm behaviors (e.g., attachment, extracellular polymeric substance production, differentiation) and regulation of virulence factors.[29] Also, a myriad of two-component systems (e.g., PhoP-Q, GacA-S, RetS, LadS, and AlgR),[30] mainly under the control of general quorum-sensing systems, exist that integrate environmental information to provide fine control of virulence factors and antibiotic resistance. However, maintenance of the substantial genetic material for two divergent infection strategies, planktonic (predatory) versus biofilm (parasitic), is costly and sometimes conflicting.

It has been demonstrated in *Streptococcus pneumoniae*,[31] *Haemophilus influenzae*,[32] and *P. aeruginosa*[33] that individual bacterium share only a portion of the noncore set of genes present in the entire group. The total genes present in the group are termed the supragenome. The distributive-genome hypothesis suggests that by carrying only a portion of all the genetic resources available, there is less energy cost to an individual bacterium and there is creation of genetic diversity, both of which provide for fitness and survivability.[34] Taking this one step further is the finding in the biofilm disease cystic fibrosis that there is a clear genetic adaptation over time to jettison planktonic (predatory) mechanisms.[35] *P. aeruginosa* in a long-standing cystic fibrosis infection "almost universally" lacks the genes for motility, T3SS, exotoxin, proteases, and other virulence factors that are necessary for a single-cell lifestyle.[36] For *P. aeruginosa*, this mutation is mainly accomplished over time by deselecting the quorum-sensing regulator lasIR.[35] This demonstrates the distinct difference between the genes responsible for acute (planktonic) infection versus those responsible for chronic infection (biofilm).

Chronic wounds may be a specific example of a chronic infection.[37] Chronic wounds have significant biofilm on their surface, whereas acute wounds have very little biofilm on their surface.[38] The literature suggests that an infection is being produced by biofilm phenotype bacteria when bacteria adhere to a surface and are highly resistant to appropriate antibiotics and biocides.[39,40] Also, the presence of multiple different species, all existing in the same limited host habitat, strongly suggests biofilm. Finally, the inflammatory response produced by biofilm phenotype bacteria tends to wax and wane, persisting over time.[3,28] All of these properties of biofilm-host interaction are characteristic of chronic wounds.

Studies have demonstrated that the chronic wound bed possesses host cells that are senescent[41] and have increased proinflammatory cytokines,[42] elevated matrix metalloproteases,[43] and excessive neutrophils.[44] Chronic wounds are stuck in a persistent inflammatory state. All of these consistent and persistent molecular and cellular findings are easily explainable as downstream events produced by biofilm infection. However, all biofilm infections are not the same, as the microbial constituents of biofilm show marked variability from wound to wound, with thousands of different species already identified.[45–48] This suggests that an individual wound will possess its own unique wound biofilm that must be diagnosed before therapy.

Using clinical cultures to diagnose the wound bioburden has been shown to be of no clinical value because multiple studies have shown that the results from agar cultures do not correlate with any improvement in wound healing outcomes.[49–51] However, there are data available that demonstrate that a specific and correct diagnosis of the wound bioburden using molecular methods can lead to improved wound healing outcomes.[52]

There is no question that host impairments must be managed aggressively—a strategy that will never change. Host factors such as poor perfusion, venous insufficiency, pressure issues, nutrition, and systemic diseases all impact not only the formation but also the resolution of a cutaneous wound; therefore, each one of these host impairments is a "right target" for aggressive management.

There is agreement that all chronic wounds possess a bioburden in which microbes play an important role. Gaining acceptance in the wound care community is the realization that the bacteria that contribute to the bioburden are organized into a biofilm.[38,53] If biofilm is present on all chronic wounds, biofilm becomes a treatment target that is relevant for each wound. Regardless of the amount of negative influence the resident biofilm is exerting on a particular wound, by specifically targeting the biofilm, even "minor" inhibitions to healing are quashed, and healing outcomes are improved.[52] This demonstrates that biofilms are indeed a "right target."

Biofilm-based wound care was developed to specifically target biofilm defenses and the individual microorganisms that constitute the biofilm.[54,55] Biofilm-based wound care, conceptually, is the use of multiple concurrent strategies in a dynamic fashion that attempts to combine different strategies to suppress wound biofilm. These strategies include débridement (i.e., sharp, energy transfer, ultrasound, and biological), antibiofilm agents (i.e., addressing attachment such as by applying topical lactoferrin,[40] degrading the matrix such as by using topical xylitol,[40] and disrupting quorum sensing by applying quorum sensing inhibitor such as hamamelitannin[56]), and cidal agents (antibiotics and biocides). By disrupting biofilm in a host setting, any residual microorganisms must change their growth state to reestablish the biofilm. While these microorganisms are reconstituting the biofilm, they are more vulnerable to conventional therapies such as antibiotics and biocides and less conventional treatments such as antibiofilm agents.[57]

A diabetic foot ulcer is an excellent example of a chronic wound that responds well to management using biofilm principles.[46] By opening up tunneling, undermining, and so forth, surfaces necessary for biofilm growth are reduced. Then, the exposed surface must be débrided aggressively to physically disrupt the protective matrix. Molecular diagnostics can then be used to define the microorganisms present and can help direct topical and systemic therapies. With the matrix breached, coordinated treatments can be successful.

Because biofilms differ based on the microorganisms present, it becomes important to identify not only what species are present but also their relative amounts in the biofilm. Molecular methods are now available that can rapidly and reliably accomplish this. With the knowledge of the microorganisms present, systemic and/or topical antibiotics can be chosen. With most diabetic foot ulcers, especially those that may result in a major limb amputation, more aggressive treatment with systemic antibiotics at high doses along with topical agents is warranted.

We routinely use gels formulated at a compounding pharmacy to target the main microbial populations within the wound biofilm with antibiotic concentrations 500 to 1000 times the minimal inhibitory concentration. To these gels, other active agents that work synergistically with antibiotics can be added to suppress the wound biofilm. These agents are typically termed antibiofilm agents in that they do not directly target the bacteria, but target biofilm behaviors, which improves the potency of the antibiotics.[58] These antibiofilm agents, along with antibiotics, have been found to be very compatible with most selective biocides such as silver, cadexomer iodine, and other commercially available topical biocides.

CASE HISTORY

A 43-year-old diabetic construction worker suffered a concrete burn to the dorsum of his right foot approximately 2 weeks before our initial meeting. Contrary to stereotypes, the patient had never smoked or used alcohol products. He has been a type 1 diabetic since his mid teens that had produced a significant generalized peripheral neuropathy but very few other comorbidities. He had excellent circulation and no other major medical problems but was insulin dependent (Fig. 1, *above left*).

The patient had some concrete get into his boot during a pour. The chemical burn quickly resulted in an infection of his right foot that required hospitalization for incision and drainage along with intravenous vancomycin and Zosyn (Wyeth, Madison, N.J.). At the time of surgery, cultures were taken and the patient was found to have bone involvement of the fourth metatarsal. Over the next 3 days, the treating physicians escalated from a recommendation for a below-knee amputation to a declaration, so that the patient finally left the hospital against medical advice.

The patient was seen the day he left the hospital in our clinic for wound care. Our wound care required only surface débridement, at which time a sample was taken for molecular identification of the bacteria present in the wound. At the same time, we called the hospital for the culture results, which were not back that day but eventually did show no growth. However, our molecular results returned that day, showing large amounts of bacterial DNA, of which 89 percent was methicillin-resistant *Staphylococcus aureus*, with 11 percent being minor populations.

Even though the patient had a very high risk for amputation, we had no way to start intravenous antibiotics that day. The patient was started on doxycycline 100 mg, one tablet orally two times per day along with topical treatments. The topical treatments consisted of a nano lipid-based gel (Sanguitec; South Eastern Medical Technologies, Savannah, Ga.) in which linezolid 1%, amikacin 1%, and clindamycin 1% were added. The gel was applied to the wound on a Monday, Wednesday, and Friday basis and then covered with Acticoat (Smith & Nephew, London, United Kingdom) to provide a selective biocide.

At 4 weeks, the patient's wound showed tremendous healing as much through wound contraction as through angio-

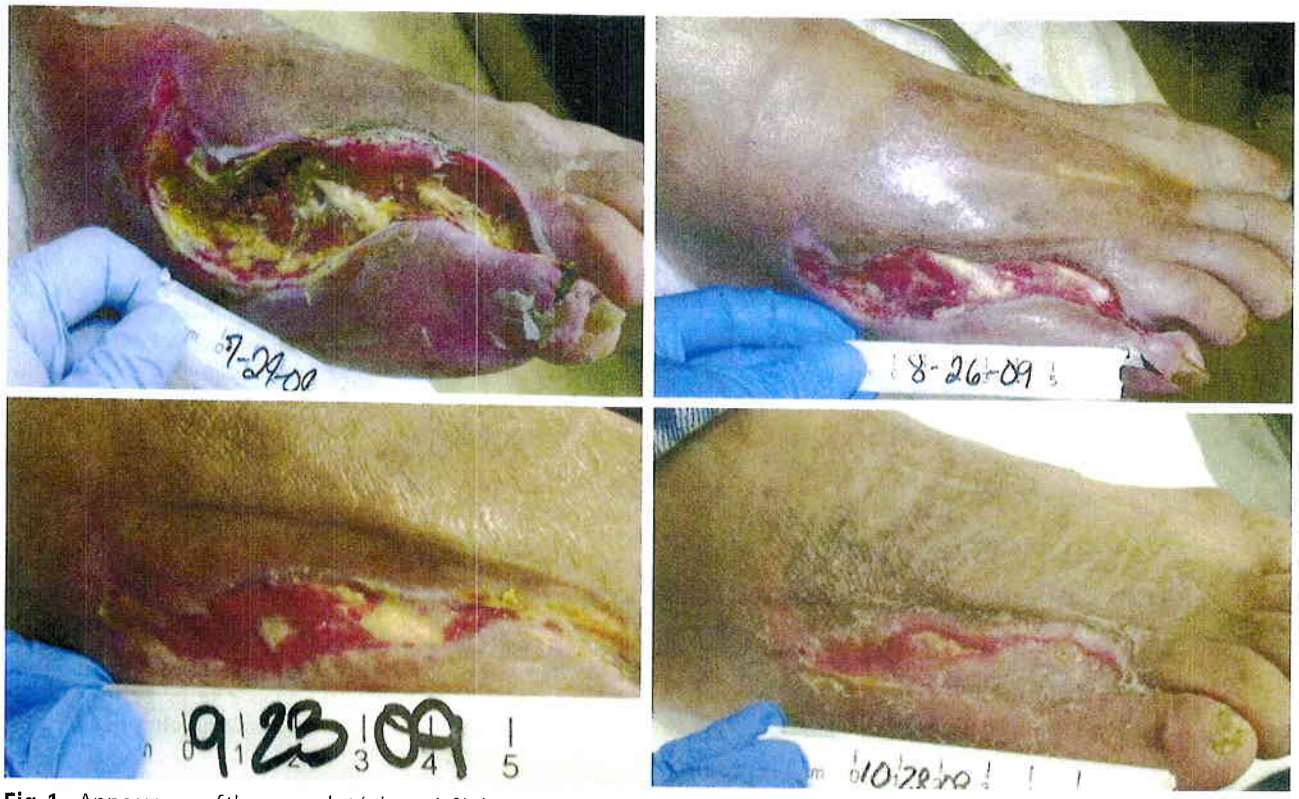

Fig. 1. Appearance of the wound at (*above, left*) the initial visit and progress at (*above, right*) 4 weeks, (*below, left*) 8 weeks, and (*below, right*) 12 weeks.

genesis (Fig. 1, *above, right*). At approximately 8 weeks, the patient was back at work (Fig. 1, *below, left*). By 12 weeks, we released him from care (Fig. 1, *below, right*). No follow-up molecular evaluation or reformulation of the gel was necessary, probably because of the lack of bacterial diversity in the original wound biofilm.

CONCLUSIONS

Molecular diagnostics has led to a much more efficient use of these antibiofilm strategies and has provided important direction for changing treating agents. This allows the system to be dynamic. The strategies for targeting biofilm phenotype bacteria are much more robust than planktonic treatments, and biofilm-based treatments will easily cover any planktonic phenotype microorganisms present. This makes biofilm-based wound care a fail-safe system, because by treating for the "worst" phenotype, all phenotypes are addressed adequately.

Unlike acute infections, biofilm can reconstitute itself from minor constituents. This ability of biofilm to reinvent itself requires the physician to be persistent. Suppression of wound biofilm, along with management of host impairments, is an appropriate target for the physician for more effective wound healing outcomes. By targeting the wound biofilm with powerful diagnostic tools and personalized wound care, wound healing outcomes are significantly improved.[52] This improvement in wound healing outcomes by specifically addressing biofilm demonstrates that biofilm is indeed a right target.

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Discussion: The Role of Biofilms: Are We Hitting the Right Target?

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It is our pleasure to discuss the masterful review of Drs. Wolcott and Dowd for the special wound healing supplement of *Plastic and Reconstructive Surgery*. Our premise will be that bacteria lack the "mental aptitude" necessary to distinguish between human body sites and that the basic bacterial strategy that these authors counteract so successfully in their wound healing practice operates in many chronic infections. The corollary will be that the clinical approach they have pioneered in wound healing can pay equally large dividends in the management of orthopedic infections.

Wolcott and Dowd posit that the bacteria that inhabit chronic wounds set up complex polymicrobial biofilm communities that can only be detected by culture techniques when they happen to detach a sufficient bolus of planktonic cells that can be grown on conventional culture media. The problem in orthopedic infections is even more invidious, as the fact that biofilm bacteria cannot be recovered by culture techniques means that prosthetic joints and fracture repairs may (and often do) yield negative culture results even when multiple clinical signs point to infection. This conundrum has both legal and ethical consequences, when the surgeon knows that an infection is present and must proceed with surgical intervention, despite negative culture results and a lack of information on antibiotic resistance.

The only methods approved by the U.S. Food and Drug Administration for the detection and identification of the bacteria that cause human infections are cultures that depend on the ability of cells of hundreds of different species to grow and produce visible colonies when they are placed on the surface of moist agar media. As Wolcott and Dowd point out very clearly, this 150-year-old technology detects one or two of the dozens of bacterial species that may be present in a wound, and we now know that it may fail completely in the detection of bacteria that are present in very large numbers in orthopedic infections. Cases in point include an infection of a broken elbow repaired by six surgical procedures over a span of 4.5 years that never yielded positive intraoperative culture results[1] but in which direct observation by modern microscopic techniques showed the presence of huge numbers of *Staphylococcus aureus* growing in well-developed biofilms. These biofilm communities lie at the root of the problem, because the bacterial cells that grow in them change their patterns of gene expression (phenotype) to suit their collegial "lifestyle," and become incapable of growth on agar plates.[2] The scope of the problem caused by the lack of sensitivity of cultures in the detection of biofilm bacteria comes into sharp focus when we realize that virtually all device-related infections and 65 percent of all chronic infections treated by physicians in the developed world are caused by bacteria growing in biofilms.[3]

Culture techniques have enabled the virtual conquest of acute epidemic bacterial diseases in the developed world and have facilitated the development of the vaccines and antibiotics that defend this position. Wolcott and Dowd's title suggests (correctly) that the target has shifted, and that our infection control strategies must accommodate this shift if our patients are to continue to be protected. Cultures detect the swarms of individual planktonic bacterial cells that cause acute infections (e.g., cellulitis), and antibiotics kill the majority of these sensitive cells before they can overwhelm the host, if both are invoked in good time. However, acute bacterial infections are increasingly rare, and they have been largely replaced by device-related and other chronic infections in which the bacteria grow in slime-enclosed biofilms.[3] Biofilm bacteria are profoundly resis-

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tant to antibiotics, so surgeons have reacted by removing all infected hardware and affected tissues and by using very high doses of antibiotics to kill any planktonic "stragglers."[4] However, the problem of accurate detection and identification of bacteria in chronic biofilm infections remains.

Wolcott and Dowd have pioneered the use of polymerase chain reaction amplification to detect bacterial DNA and the use of 16S ribosomal RNA sequencing to identify the species of bacteria that are present in wounds, to direct their therapeutic strategies against the correct targets. The technology of bacterial detection and identification is currently in a state of flux, with almost monthly changes in approach and equipment, and we have chosen to use polymerase chain reaction amplification combined with rapid identification by matching base ratios to a database.[5] However, the salient point is that these modern molecular methods detect bacterial pathogens by their DNA—which differs from human DNA—and identify them by methods similar to those used to great effect in modern forensic medicine. We can confirm Wolcott and Dowd's conclusions that DNA-based molecular methods can detect pathogenic bacteria with much better sensitivity than cultures (100 percent versus 30 percent), with commensurate improvements in accuracy. These methods can also detect and identify bacterial antibiotic-resistance genes more rapidly than can be done by cultures. Thus, the stage is set for a trial period in which cultures will be used in parallel with DNA-based molecular techniques, as has already been initiated at the Mayo Clinic, and for the eventual replacement of cultures by these modern technologies for the detection and identification of pathogenic biofilm bacteria. In this profound shift from the venerable culture methods and the acute infection paradigm, to modern DNA-based molecular technologies and the biofilm infection paradigm, Wolcott and Dowd will have played a primary and perceptive role.

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