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EDITORIAL

From Koch's postulates to biofilm theory. The lesson of Bill Costerton

Garth D. Ehrlich[1], Carla Renata Arciola[2]

[1] Executive Director of the Center for Genomic Sciences, Allegheny-Singer Research Institute, Professor of Microbiology and Immunology, Drexel University College of Medicine, Allegheny Campus, Pittsburgh, PA - USA

[2] Head of the Research Unit on Implant Infections, Rizzoli Orthopaedic Institute, Bologna, Italy and Professor of General Pathology, Department of Experimental Pathology, Faculty of Medicine, University of Bologna, Bologna - Italy

ABSTRACT

The clinical diagnoses of implant infections pose insurmountable difficulties for cultural methods because of their frequent failure when bacteria are growing in biofilms. In 1978 Bill Costerton warned that chronic infections in patients with indwelling medical devices were caused by bacteria growing in well-developed glycocalyx-enclosed biofilms and that bacteria within biofilms resist antibiotic therapies and immune host defenses. Costerton's "biofilm theory" opened two lines of scientific endeavor: the study of the biochemistry and genetics of biofilm formation and function; and, on the other side, the search for new methods for medical diagnosis and treatment of biofilm-centered implant infections.

This Editorial and the entire 2012 issue "Focus on Implant Infections" are dedicated to the memory of Bill Costerton, recognized worldwide as the Father of Biofilms for his innovation and body of work on infections caused by sessile bacteria. Bill Costerton was a great scientist, heedful both to the biological aspects of biofilms and to the medical challenges of new diagnostic methods and modern therapeutic approaches to implant infections. But, most of all, he was a charming Maestro *for the large number of colleagues and students whose enthusiasm for the science he was able to nourish. Bill passed away on May 12th, 2012 and the entire science community mourns the death of a friend and a leader.*

KEY WORDS: Implant infections, Biofilm, IBIS, MALDI-TOF, Anti-infective materials, Neutrophil, Staphylococcus internalization

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In 1978 Bill Costerton established a profoundly new microbiological paradigm, the biofilm theory, with the publication of an article in Scientific American, in which he stated that bacteria stick on available surfaces in glycocalyx-enclosed biofilms and that these sessile bacterial populations become predominant in natural, industrial and, particularly, in medical ecosystems (1).

As he himself often narrates, his attention to bacterial biofilms originates from an embarrassing tumble on the icy waters of an "alpine" creek near the Bugaboo Spire glacier in British Columbia. While it is commonly reported that alpine rivulets contain less than ten bacteria per milliliter, he noted that the granite cobblestones were covered by a slimy slippery mud, which, after careful microscopic observations, was demonstrated to be constituted by a sessile biofilm, in which bacteria greatly exceeded in number the floating planktonic bacteria in the same ecosystem. Bill's attention turned soon to medical devices, where he noticed that chronic infections in patients bearing implantable devices were caused by bacteria growing in well-developed glycocalyx-enclosed biofilms. Moreover, he observed that bacteria within biofilms are inherently resistant to modern, even aggressive,

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antibiotic chemotherapies. These findings shifted the research community's attention from cell-wall structures, constituting the interface of planktonic bacteria with the environment, to the biofilm, which is the interface of sessile bacteria with their environment (2). As Costerton himself stated in his Current Contents 1989 commentary, when most medical and industrial problems caused by planktonic bacteria have been solved by conventional microbiology, the residual problems in these areas involve biofilms, so that their solution will require a new understanding of the ecology, physiology and physiopathology of these tenaciously adherent bacterial populations.

The launch of the "biofilm theory" opened two lines of scientific endeavor: the study of the biochemistry and genetics of biofilms and their formation and, on the other side, the challenge to medical diagnostics and the treatment of biofilm-centered infections.

The first line of research is summarized in two of Costerton's articles, dealing on production and regulation of biofilm (3) and on the molecular mechanisms of biofilm formation (4). Both articles consider the biofilm formation in the context of implant infections, the practical arena in which bacteria find their protective niche and their supremacy, the battlefield on which they must be opposed and won.

Progresses in the scientific knowledge of the structural molecules (exopolysaccharides, proteins, teichoic acids, and extracellular DNA) that compose the biofilm matrix, of the genetics and regulation of biofilm production, and on the complex networks of molecular signals that control polymicrobial bacterial populations in biofilms are necessary to find efficacious strategies to overcome biofilm-centered infections.

In particular, the knowledge of the quorum sensing communication systems employed among bacteria to sense and regulate population density and to determine biofilm architecture by specific secreted pheromones or autoinducers may open the way to interfere with bacterial growth by "jamming" the bacterial communication signals, as in a sort of war of nerves (5).

The second line of scientific interest opened up by Costerton's insights deals with the medical diagnosis and treatment of implant infections. Bacterial growth in biofilms creates insuperable difficulties not only in the treatment of the infection, owing to the high antibiotic resistance of bacteria embedded in biofilm, but even in ascertaining the state and the nature of the infection to reach a proper diagnosis of the bacterial (and fungal) species causing the infection when using traditional culture methods. The only laboratory techniques approved by the U.S. Food and Drug Administration to detect and identify bacteria responsible for human infections are cultures, which are entirely dependent on the ability of bacteria to grow and produce visible colonies when seeded on the surfaces of moist agar media. But this 150-year-old technology detects at best, under ideal circumstances, only one or two of the dozens of bacterial species that may be present in a wound and it may fail completely in the detection of bacteria present in very large numbers in orthopedic infections (6, 7).

In the vast majority of implant infections biofilm bacteria cannot be recovered by culture techniques. This obstacle necessitates a shift from the *acute infection paradigm*, based on culture methods, to the *chronic biofilm infection paradigm*, (8) based on DNA molecular technologies. In implant infections, in particular in orthopedics, the rapid, sensitive, and specific identification of the etiological agents is necessary for instituting efficacious therapeutic measures. Rapidity is important because determining if there is an infection is vitally important as both sterile and non-sterile conditions can have similar presentations. Highly accurate microbial species and strain-level identifications of the infecting pathogens are then needed to determine the virulence potential, the antibiotic resistance profiles, and to predict the biofilm-forming capacity of the etiological agent in order to best develop productive therapeutic approaches. These can include local and systemic antibiotic therapy, surgical debridement, and lastly the removal and replacement of the implant.

While the unequivocal characterization of an infection as a biofilm infection is based on microscopic demonstration of matrix-embedded microbial communities *in* or *on* the affected tissues or prostheses, diagnostic modalities are not practical for routine clinical use as they are both costly and time intensive, and moreover they require invasive diagnostic procedures (9). Thus, DNA-based molecular methods have been developed to provide rapid identification of all microbial pathogens that due not relay on cultural methods. Costerton and his colleagues have reviewed the plethora of molecular techniques that could replace cultures in the diagnosis of bacterial diseases and have concluded that the IBIS PLEX-ID technology may be a valid tool for routine diagnosis in orthopedic surgery (10). Besides the PLEX-ID, another mass spectroscopy-based technology, MALDI-TOF, has garnered some diagnostic interest, but this technology, while rapid and useful for speciation (11), still requires a

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colony on a plate for analysis and, thus, suffers from all of the disadvantages of microbial culture (12).

As mentioned above, the recognition of the role of biofilms in the irreducibility of prosthesis infections establishes the rationale for the surgical treatment of implant-associated infections. Owing to the strong resistance of biofilms to host defenses and antibiotic therapy, the resolution of these infections often relies only on the complete removal of the infected prosthesis (13). However, thanks to increasing knowledge with regard to the molecular components of the biofilm matrix (14-16) and to the mechanisms of biofilm production and regulation, together with improved understandings of the pathogenesis of implant-infections, new horizons are opening up in the field of preventive and therapeutic strategies. These include anti-infective or infection-resistant materials that are also able to promote osseointegration (17-20), disaggregating biofilm agents (21), the development of biofilm-specific vaccines (22), the new photodynamic antibiofilm therapies (23) and the combined antibiotic-antibiofilm therapies (24), which collectively could revolutionize the management of periprosthesis infections (4, 10, 25). Ongoing investigations of the host innate defense mechanisms operating in chronic implant infections are helping to clarify the mechanisms by which neutrophils can attack and disaggregate staphylococcal biofilms (26, 27). This new line of research has opened up because of new knowledge regarding the composition and the structure of biofilms. These recent understandings with regard to the pathogenesis of osteolysis and osteomyelitis in biofilm-centered infections arise from the investigations on biofilm susceptibility to the neutrophil attack with the consequent "frustrated phagocytosis" (28, 29) and from the studies on the adhesin-mediated interactions of staphylococci with bone cells (30-33).

In 1884 Robert Koch and Friedrich Loeffler formulated the four postulates that gave the theoretical and practical bases on which to establish a causal relationship between a microbe and a disease (34). From a theoretical point of view Koch's postulates descend from the deterministic concept of material and efficient cause that, in medicine, in the early 1800s, gained ground first in Bichat morbid anatomy (35) and then on Virchow's cellular pathology (36). The extension of this traditional paradigm in microbiology led to Koch's criteria, according to which the cause is an agent that is always identifiable in the lesions, it is absent in healthy subjects, can be isolated in a pure culture, and causes the disease by inoculation into animals. Limitations of Koch's postulates have been largely discussed and have been analyzed in depth by Alfred S. Evans, who tried to extend the Koch's postulates to the fields in which the infectious agent does not appear immediately responsible for a disease but its implication is sustained by epidemiologic criteria (37). In a subsequent paper, Evans underlined how technology influenced the evolution of the concept of causation in bacteriology, virology and immunology (38). But neither Evans nor the numerous brilliant scientists that he calls as witnesses in his papers raise the problems associated with the biofilm niche in which there are almost always multiple agents. Not only are there often multiple agents, but the same type of infection in different patients can have very different microbial compositions. These problems are compounded by the fact that most or all of these agents will remain hidden if evaluated only using cultural methods. The inapplicability of the second Koch's criterion that the pure culture for the infective agent to be detected poses insurmountable difficulties for routine clinical diagnosis of biofilm-centered infections. But the Costerton's "biofilm theory" has given an explanation for the failure of culture methods in the diagnosis of implant biofilm-centered infections and has promoted the search for new methods in the diagnosis (39-42) and in the control (43) of implant infections.

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Address for correspondence:
Prof. Carla Renata Arciola
Head of the Research Unit on Implant Infections,
Rizzoli Orthopaedic Institute,
Via di Barbiano 1/10
40136 Bologna, Italy
e-mail: carlarenata.arciola@ior.it

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