

THE BIOFILM REVOLUTION

Abstract #1

“The Efficacy of Compound A in Killing Planktonic and Biofilm Cells Using Modern Methods Suitable for FDA Submissions”

By

J. William Costerton, PhD, Consultant

REPORT

Title : THE EFFICACY OF COMPOUND A IN KILLING PLANKTONIC AND BIOFILM CELLS, USING MODERN METHODS SUITABLE FOR FDA SUBMISSIONS.

Prepared for Dr. Larry Funt, Coalition of Health-Care Professionals

Respectfully Submitted :

A handwritten signature in black ink, appearing to read "J.W. Costerton". The signature is written in a cursive style with a horizontal line extending from the end.

J. William Costerton, PhD., Consultant

Experimental work conducted by Bacterin Inc., Guy Cook, President

Dated : 05.28.01

Abstract of Report #1

Prologue

“The pivotal difficulty in the traditional methods (*used for determining the efficacy of antimicrobial agents in killing bacteria growing in biofilms*) is their reliance on the “colony-forming-units” (CFU) that produce colonies on agar, after the sessile (biofilm) population has been removed from the surface and separated (as much as possible) into individual cells. This removal is incomplete, and the separation steps are inadequate, so that efficacy data based on “scrape-and-plate” culture methods are misleading and tragic consequences have already affected hundreds of recipients of medical devices incorrectly characterized as being colonization resistant.”

“Fortunately, at a time when biofilms are being recognized as being responsible for all device-related infections and fully 65% of all bacterial infections (Costerton, et al., 1999), the scientists and engineers of the Center for Biofilm Engineering (CBE) have developed new technologies. These new methods, which are made possible by the superior resolving power of the Scanning Confocal Laser Microscope (CSLM), are unequivocal because they rely on direct observations and avoid the extrapolations traditionally used in this field. The FDA has recently invited several proponents of these new direct technologies to Washington, D.C. to help the agency draft new guidelines for the assessment of the efficacy of antibacterial agents in killing biofilm bacteria.”

Aim of The Present Study

“The Coalition of Health-Care Professionals (COHP) already has data proving that its Compound A kills planktonic cells of many bacterial species. This study was designed to use the new methods of direct observation, to determine whether this agent kills biofilm cells with similar efficiency.”

Results

“When we exposed *de facto* biofilms of 8 bacterial species, and 1 fungal species, to Compound A we obtained complete killing of the sessile cells in all cases.” (these species included: *E. coli*, *C. albicans*, *E. cloacae*, *E. faecalis*, *K. oxytoca*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, and *S. aureus*) “All of these preparations of *de facto* biofilms from static liquid cultures contained some planktonic cells, that had also settled onto surfaces, and these direct observations confirm the traditional microbiological data that show that Compound A kills planktonic cells of all these species.”

Conclusions

“Certain conclusions can be based strictly on the data presented in this report. These include confirmation that Compound A is effective in killing planktonic cells of all of the 8 bacterial and one fungal species included in these studies. These data also show that Compound A kills sessile cells of all these species when they are growing in aggregates in liquid media, which constitute *de facto* biofilms in that they are present in slime-enclosed masses... The agent killed sessile cells, even when they were growing in the middle of very substantial aggregates.... These data indicate that Compound A is effective in killing bacteria in aggregates derived from biofilms, such as the aggregates that are released by mechanical cleaning of surfaces (teeth or pipes) that are heavily colonized by bacterial biofilms. We test a very large number of putative anti-biofilm biocides, and the only agents that have been successful against these entrenched organisms have been oxidizing compounds whose use in the medical context is contraindicated.”

“The principals of COHP have shown me data from other sources, whose origins I trust and whose methods I recognize, and I can reach another broader set of conclusions when I combine these data with those in this report. I believe that Compound A has excellent efficacy in killing planktonic bacteria and yeast, of virtually all species. I believe that this agent also has a unique capability of killing sessile bacteria and yeast in aggregates that constitute *de facto* biofilms, and that provide complete protection from killing by most of the anti-biofilm agents we have tested. I think that the agent is bacteriacidal, in itself, but that it also exerts a physical effect on biofilms, because it often changes the state of the biofilm within which the target organisms are living, and rolls it up or fragments it.”