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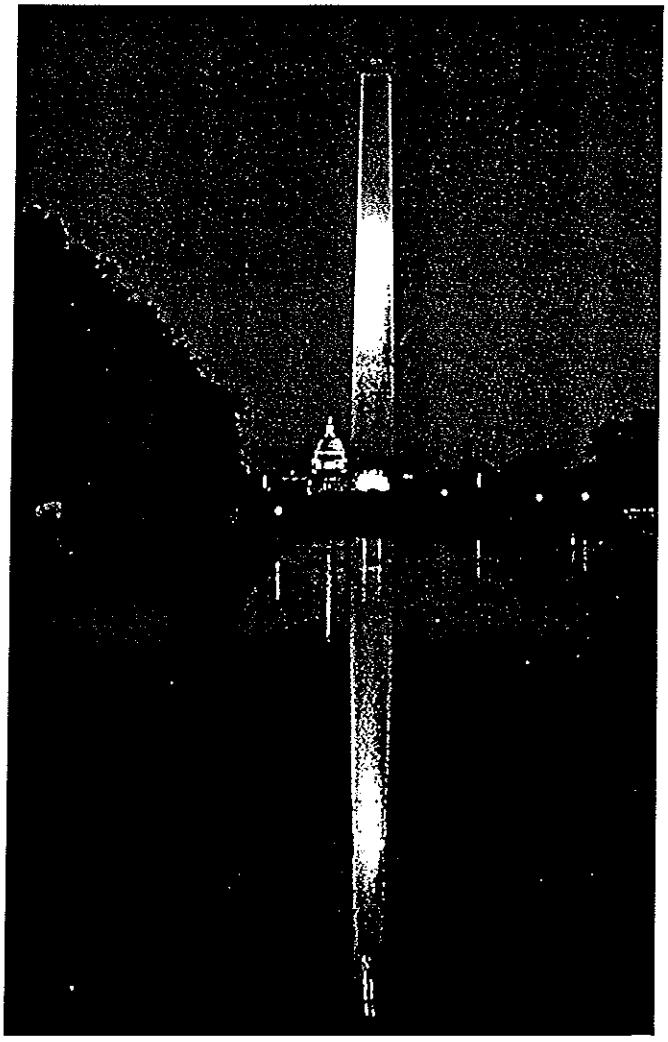
ASSOCIATION FOR THE ADVANCEMENT OF MEDICAL INSTRUMENTATION

AAMI 97: 32nd Annual Meeting & Exposition

June 7–11
Washington, DC



- *Cryoprobe for Minimally Invasive Procedures*
- *Impact of Health Care Trends*
- *Multiple-modality Therapy for Tension Headaches*

REFERENCES

1. *This paper was one of the top-rated presentations at AAMI's second conference on Designing, Testing, and Labeling Reusable Medical Devices for Reprocessing in Health Care Facilities, held 13–15 November, 1996, in Los Angeles, CA.*
2. *This poster and the other four described here will appear in this issue or the July/August issue of* BI&T *and were selected as the most outstanding at the recent AAMI reprocessing conference. Anne Booth's work was the top-rated poster.*
3. *Association for the Advancement of Medical Instrumentation. Designing, Testing, and Labeling Reusable Medical Devices for Reprocessing in Health Care Facilities: A Guide for Manufacturers. AAMI Technical Information Report 12, Arlington, VA: AAMI, 1994.*

Cleaning Techniques for Medical Devices: Biofilms

J. WILLIAM COSTERTON, PHD

We at the Center for Biofilm Engineering examine bacteria on surfaces. So far, the National Science Foundation has spent or committed $18 million of research money on our study of bacteria on surfaces in the hope that what we do might be useful to industry.

The Center combines microbiologists and engineers—a very potent combination. We use a new form of microscopy—the confocal scanning laser microscope (CSLM)—to look at living biofilms. Rather than removing bacteria from the surfaces and counting them on plates, we image them directly using the CSLM and a number of stains. We can then look at a surface very directly when we try to sterilize it, and actually see which organisms are alive and which are dead.

CLEANING

We are particularly keen on cleaning surfaces because if bacteria get a chance to establish themselves and to hide behind their own or other polymers, they can become remarkably resistant to sterilization processes.

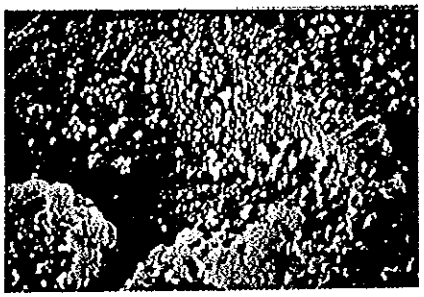


Figure 1. Scanning electron micrograph of the surface of the lead wire of a cardiac pacemaker removed from a patient with a severe Staphylococcus aureus *bacteremia. A thick biofilm composed of cells of* S. aureus *embedded in an exopolysaccharide matrix material had formed on the surface of this device. In the process of the removal and sampling of the device, the biofilm was sectioned so that the spherical bacterial cells and their dehydration-reduced polysaccharide matrix are clearly seen. Each spherical cell approximately 1.0 μm in diameter.*

We once encountered problems with a patient suffering from olecranon bursitis who had a pacemaker and who developed bacteremia. Knowing that he had an old pacemaker, we began to think about what might be happening inside him. He had an alcohol problem, the pacemaker was disused, and the batteries were dead. But we thought there probably was bacterial accretion on the plastic and metal surfaces in his heart—in other words, the cardial pacemaker. We treated him for 3 weeks with 16 grams a day of cloxacillin. This beat back the bac-

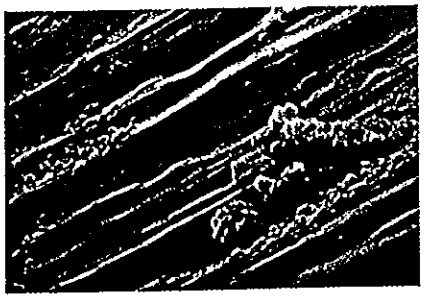


Figure 2. Scanning electron micrograph of the surface of the battery pack of the pacemaker from the patient described in figure 1. Living bacteria could be cultured from these relatively small microcolonies, after six weeks of very intensive antibiotic therapy, even though they are composed of less than 100 cells of S. aureus *embedded in a slimy exopolysaccharide matrix that has been radically condensed by the dehydration necessitated by this type of microscopy.*

teremia, but it kept returning every time we stopped the antibiotics. Finally, we took out the pacemaker and saw the face of the enemy for the first time. Figure 1 shows the scraped area that yielded 10^9 living cells of *Staphylococcus aureus*. You can see all over the surface of the pacemaker the biofilm that we were trying to kill. Any antibiotic that would kill all of the cells in this biofilm would have to penetrate to the bottom of the very thick sessile population of bacteria.

Now think about what you're trying to kill on endoscopes and other medical devices. If you've got something buried in its own slime that's had a chance to grow, and it's also hidden behind other slime, it becomes almost impossible to kill. In this case, we had an elegant example of the resistance of bacterial biofilms. Figure 2 shows the spherical cells of *S. aureus* and the dried slime they usually hide behind. Even this little pocket of bacteria had resisted the effects of 16 grams a day of cloxacillin.

RESISTANCE EXPERIMENTS

How resistant are these bacteria? We conducted an experiment using professional and graduate student urine—a magic mixture. We were trying to be as realistic as we possibly could by not only using real urine, but ultimately real bacteria. If you experiment with an American Type Culture Collection strain that's been transferred 1,500 times in the lab, you will find it is not going to be very adherent or resistant to anything; these are pussycat strains that are easy to kill. You won't achieve realistic results unless you actually use "wild" strains of bacteria on their first transfer after being isolated from a natural source.

We took some catheter material and started passing wild strains of bacteria past it. Eight hours later, the surface of the material was completely buried—8 cells and 15 mi-

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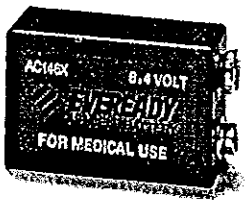

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crons deep. So if the medical devices you've been reusing are in a moist environment after use, bacteria will grow overnight, and they can easily reach this level of accretions on the surface.

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How resistant are these bacteria to antibacterial agents? We attempted to kill *Pseudomonas aeruginosa* with tobramycin. Planktonic cells exposed for a contact time of 8 hours to 50 micrograms per ml of the agent would be deader than doornails. But biofilm cells, the sessile cells, were exposed to 1,000 micrograms per ml for 8 to 12 hours with no appreciable kill.

We isolated bacteria from medical devices in patients so that the bacteria would grow planktonically—the usual way of growing them in a fluid medium. A certain dose of antibiotic will kill the bacteria. Then we put the bacteria in biofilms and went after them with the same antibiotic. The rule of thumb is that 1,500 times more of an agent is required to kill the entrenched biofilm bacteria on a surface than the floating planktonic form.

ELECTRIC CURRENT

Science wasn't doing us much good at this point; we knew that bacteria were remarkably resistant to sterilizing agents if they were entrenched, but we didn't know what to do about it. We wallowed in a collective depression for 6 or 8 years wondering about this. We were waiting for diffusion to take a chemical agent from the solution down through all the slimy mess and kill these bacteria that were either in their own biofilms or hiding behind organic soils. But the problem was that diffusion takes a long time.

The idea we began to formulate was hatched by a microbiologist (me) and a urologist, Tony Khoury. Neither of us had a physics background at all—I failed high school physics; Tony took only one course in physics at university. But we thought that maybe we could accelerate the process of chemical sterilization on surfaces by invoking physics, particularly direct current (DC) fields.

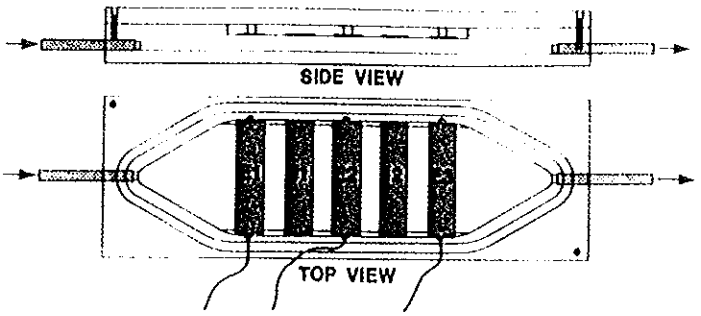


Figure 3. Diagram of an in vitro *flow cell containing five stainless steel foils in a wrap-around electrode design. These foils are mounted in a plastic flow cell and two of them (E1 and E3) are connected to the power source so that they compose one electrode, while E2 comprises a single electrode of the opposite polarity. Polarity of the electrodes is reversed at 64-second intervals. The other two stainless steel foils (I1 and I2) are not connected to the power source, and they simply constitute "inserts" into the DC field.*

In the first and simplest experiment, we used a stainless steel electrode backed off with a platinum electrode and built a biofilm in one of our biofilm sampling devices. The biofilm was approximately 15 cells thick. We then imposed the following electrical regimen on that biofilm: The driving voltage was 3 volts, and the field strength was 15 milliamps per cm^2.

We decided to accelerate the diffusion process with the DC field, and we were able to do this with the biofilm *P. aeruginosa.* We were working at two and a half times the minimal inhibitory concentration (MIC) of the floating planktonic bacteria. Of course, if we put in this amount of tobramycin, we would have no effect on the biofilm in the absence of the DC field. But when we imposed the antibiotic on the bacterium in the presence of a DC field, we got a complete kill.

We wondered if this kill was unique to *Pseudomonas,* so we tested *Escherichia coli,* and the kill was accomplished even more quickly. Then we went to a gram positive organisms, *S. epidermidis,* with, in this case, five times the planktonic MIC, and we found ourselves with a complete kill of the bacteria. This has since been extended to show that we can kill the human immunodeficiency virus in .05% glutaraldehyde. We can kill the spores of various protozoa. It seems that anything hampered by penetration difficulties can very much benefit from this enhanced penetration by the DC field.

It was mysterious to us at first because we found that the DC field by itself had no effect on the bacteria at all. If we had no antibacterial agents, no biocide, and no antibiotic present,and we colonized the surface in the presence of the electrical field, we got slightly better colonization than we did in the absence of the DC field. (Remember, this is a very small DC field.) However, when we added the antibacterial agent, we got a spectacular kill in the presence of the field and no significant kill without the field.

So it seemed as if we were enhancing the penetration of the agent. Working with 3M Company, we examined some other electrode configurations. Previously, we'd either put the bacterial biofilm on the cathode or the anode, or switched back and forth. But we found that if we put a plastic surface between the anode and the cathode and simply had the DC field go past this nonconductive surface, we got an equally efficient kill. So the biofilm doesn't have to be right on the cathode or anode; it can be anywhere in the DC field.

At this point, the patent for this method was filed, and it has since been issued. We then exposed our work to electrical engineers, and they looked in absolute horror at how we had designed our experiments; they were utterly contemptuous. They're still frowning when they hear about our first experiment designed by Frank Johnson. The first electrical engineer in a long series, Johnson designed what he called a wrap-around electrode, shown in Figure 3.

WRAP-AROUND ELECTRODE

One electrode, E2, is wired to the generator as the anode or cathode. The opposite charge is put on E1 and E3, and the I1 and I2 are passive inserts in the system. In this wrap-around electrode, a current is generated between E2 and E1 and E3. I1 and I2 are not connected to the electrical field at all; they are just sitting inside it. And on the outsides of E1 and E3 are very small current densities; we calculate them to be less than 150 microamps per cm^2.

These surfaces were fouled with bacterial biofilms. When the current was switched on in the presence of an antibacterial agent, a complete kill resulted. That is, we could not detect any living organisms on any of the surfaces, including the outsides of E1 and E3. These are the calculated and measured current densities, and they go down to 100 microamps per cm^2. The field doesn't have to be very strong to enhance the penetration of these agents and kill the bacteria.

For companies interested in this process, we produced an *in vivo* flow cell that we could put inside a rabbit. We put very small amounts of current density on these elements. Then we brought an antibiotic in and took it out in the drainage bag.

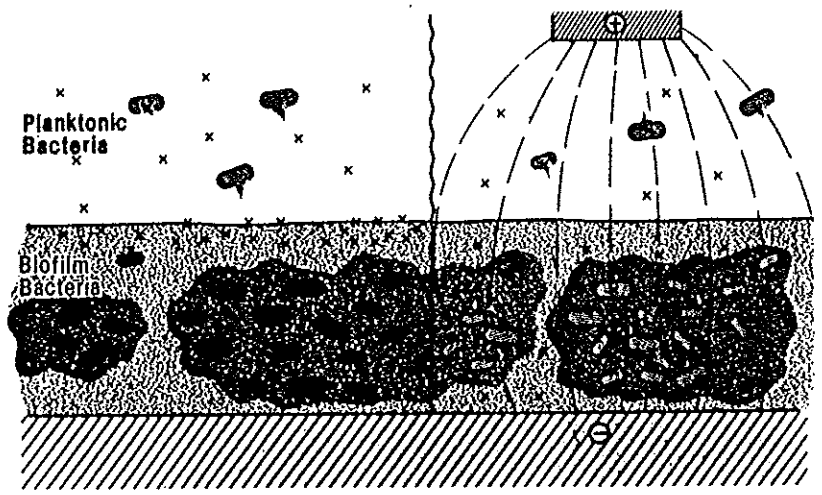


Figure 4. Conceptual diagram to explain our working hypothesis concerning the bioelectric effect. In the absence of the DC field (left) the antibiotic molecules (x) readily contact and kill planktonic bacterial cells, but they cannot penetrate the exopolysaccharide matrix of the biofilm and kill the bacterial cells within the especially dense slime of the microcolonies. In the presence of the DC field, the antibiotic molecules (x) are driven into the microcolonies of the biofilm, by a process similar to electrophoresis, and the antibacterial agent kills all of the bacteria within these sessile communities.

There are three electrodes and passive elements; some bacteria survived on one of the passive elements. So we actually killed biofilm bacteria on electrodes in the peritoneum of a rabbit using the voltages that we finally chose.

The mechanism of this effect, the bioelectric effect, appears to be as follows: With floating planktonic bacteria and an antibacterial agent, it is very easy to access the bacteria and kill them at very low concentrations. Sterilants, disinfectants, and antibiotics are designed to do exactly this. But when bacteria get on surfaces, they can hide behind their own or other polymers, causing a serious penetration problem. This greatly retards sterilization. If we then use a DC current, most of these molecules become charged (we can actually see them if we tag them with fluorescein and go deeper and deeper into the biofilms, killing right down to the surfaces.

STERILIZER PROTOTYPES

It seems that electrophoresis or assisted electrophoresis (Figure 4) is the major mechanism of this bioelectric effect. So far, we have built prototypes of various kinds of sterilizers. In a prototype of a sterilizer for contact lenses, we take contact lenses with biofilms on them, cement them on the end of planchettes, and put wired electrodes on either side of the vessel. We can get a complete kill of viruses, bacteria, spores, fungal spores, and protozoan cysts in this type of an arrangement.

Just recently, we examined the characteristics of a biofilm using an anionic indicator, which tells us whether a number of cations are trapped in the biofilm itself. A phase microscope of a biofilm shows that if you put in the anionic indicator it is usually very heavily attracted to the whole matrix, proving that a number of cations are in the biofilm normally. If we hit a biofilm with the electrical current, we drive off huge amounts of calcium and magnesium. We don't find the cation reaction in the biofilm after the electrical treatment. So besides driving the agents in, the electrical treatment seems to drive out almost all of the divalent cations. Those of you who have conducted sensitivities in different concentrations of calcium and magnesium can understand what a profound effect this would have on bacterial sensitivities to sterilants.

To sum up, we are exploring sterilization on surfaces, specifically electrically assisted sterilization. To chemically sterilize bacteria in biofilms or accretions of any kind, we wait for the diffusion of the chemicals we've chosen—we wait for the detergents, the enzymes, and the sterilants to get in. We don't have to wait for the sterilants to get in if we drive them in with a DC field. (Continued on page 247.)

J. William Costerton assumed directorship of the Center for Biofilm Engineering in January 1993. He came to Montana State University—Bozeman following 23 years at the University of Calgary, and he was appointed an associate professor of biology there in 1970. He became a full professor in 1975. In 1980, he was appointed to the Alberta Oil Sands Technology and Research Authority research chair in microbiology, and in 1990 to the Natural Sciences and Engineering Research Council industrial research chair in microbiology. He received his PhD in bacteriology in 1960 from the University of Western Ontario. His interest in microbial ecology led him to study bacteria attached to surfaces and biofilms. His research since has dealt with biofilms in many environments—from mountain streams, to industrial systems, to medical devices implanted in humans.

Validation of Hospital-based Sterilization Processes

by Anne F. Booth

Continued from page 226.

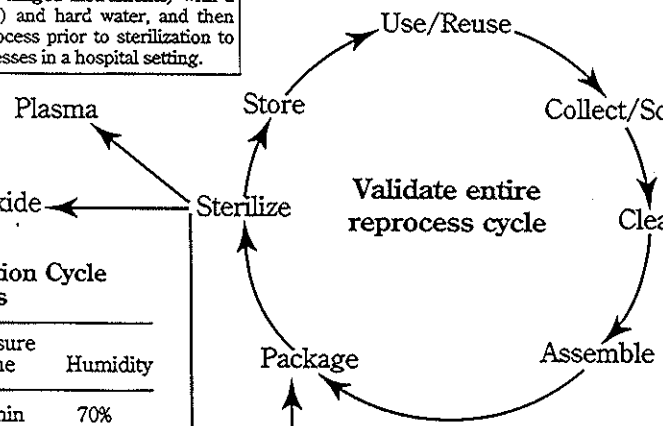
Abstract

Manufacturers of reusable devices are responsible for demonstrating that their devices are suitable for sterilization by hospital-based sterilization processes. To do this, hospital methods must be determined and understood, then validated using an in-use hospital scenario. This requires a life cycle approach based on the evaluation of the entire reprocessing cycle, including initial device use, recovery, decontamination and cleaning, packaging, sterilization, storage and reuse. With the standard industrial overkill methodology of inactivation of six logs of organisms in one-half cycle in mind, manufacturers then add hospital-specific preparation activities to the validation protocol. Inoculate difficult-to-sterilize devices (lumen and hinged instruments) with a simulated challenge that includes serum (blood) and hard water, and then subject these devices to the routine cleaning process prior to sterilization to accurately assess the efficacy of sterilization processes in a hospital setting.

Protocol Elements

1. Ensure proper equipment function.
2. Select biological challenge.
3. Select hospital chemical indicator/self contained biological indicator.
4. Choose difficult-to-sterilize devices.
5. Ensure efficacy using blood/hard water spore suspension.
6. Select at least two cleaning and sterilization methods.
7. Use full load configuration.
8. Validate cycles using 1/2 approach with three acceptable runs.

Reprocessing Cycle



Typical Ethylene Oxide Sterilization Cycle Parameters in Hospitals

Type	EO	Temperature	Exposure Time	Humidity
100%	883 mg/L	131° F (55° C)	60 min	70%
EO	883 mg/L	99° F (37° C)	120 min	70%
EO	450 mg/L	130° F (55° C)	3 hours	30–80%
CO_2		100° F (38° C)	7 hours	30–80%
EO	600 mg/L	130° F (55° C)	2 hours	30–80%
HCFC		100° F (38° C)	5 hours	30–80%

Cleaning Methods Used in Hospitals

Ultrasonic	60–140° F	pH 8–11	Rinse & dry
Washer-disinfector	Washing active Detergent/H_2O	pH 7–11	Rinse 180–200° F
Washer-sterilizer	Detergent/H_2O >150° F		Flash > 285° F

Biological Indicator Challenge Systems

Ensure efficacy by inoculation of hinged devices with blood/hard water spore suspension; then expose to cleaning.

Place BIs within device lumens or within a syringe barrel or within a length of tubing.

Select customary hospital challenge BI + CI
Use self-contained early readout systems
(tortuous path for EtO)

Select customary full hospital load with wrapped/cased items

Standard Steam Sterilization Cycles in Hospitals

Load	Gravity 121°C/250°F	Gravity 132°C/270°F	Vacuum 132°C/270°F
Unwrapped hard goods	≥ 15 minutes	≥ 3 minutes	≥ 3 minutes
Unwrapped hard goods	≥ 30 minutes	≥ 10 minutes	≥ 4 minutes
Packs with fabric	≥ 30 minutes	NA	≥ 4 minutes
Wrapped hard goods	≥ 20 minutes	≥ 10 minutes	≥ 4 minutes
Container systems	≥ 40 minutes	≥ 3 minutes	≥ 4 minutes

Anne F. Booth, MS, is a quality good manufacturing practices (GMP) and sterilization specialist with more than thirteen years of experience in the field of medical device manufacturing. She established a consulting business, Booth & Associates, in 1993, which provides GMP and sterilization programs for medical device manufacturers.