Effective Prevention of Microbial Biofilm Formation on Medical Devices by Low-Energy Surface Acoustic Waves

Zadik Hazan, Jona Zumeris, Harold Jacob, Hanan Raskin, Gera Kratysh, Moshe Vishnia, Naama Dror, Tilda Barliya, Mathilda Mandel, and Gad Lavie

Nanovibronix Corporation, Nesher, and Institute of Hematology and Blood Center, Sheba Medical Center, Tel-Hashomer, Israel

Received 4 April 2006/Returned for modification 9 May 2006/Accepted 18 August 2006

Low-energy surface acoustic waves generated from electrically activated piezo elements are shown to effectively prevent microbial biofilm formation on indwelling medical devices. The development of biofilms by four different bacteria and Candida species is prevented when such elastic waves with amplitudes in the nanometer range are applied. Acoustic-wave-activated Foley catheters have all their surfaces vibrating with longitudinal and transversal dispersion vectors homogeneously surrounding the catheter surfaces. The acoustic waves at the surface are repulsive to bacteria and interfere with the docking and attachment of planktonic microorganisms to solid surfaces that constitute the initial phases of microbial biofilm development. FimH-mediated adhesion of uropathogenic Escherichia coli to guinea pig erythrocytes was prevented at power densities below thresholds that activate bacterial force sensor mechanisms. Elevated power densities dramatically enhanced red blood cell aggregation. We inserted Foley urinary catheters attached with elastic-wave-generating actuators into the urinary tracts of male rabbits. The treatment with the elastic acoustic waves maintained urine sterility for up to 9 days compared to 2 days in control catheterized animals. Scanning electron microscopy and bioburden analyses revealed diminished biofilm development on these catheters. The ability to prevent biofilm formation on indwelling devices and catheters can benefit the implanted medical device industry.

Indwelling device-related infections constitute a major cause of morbidity and mortality in hospitalized patients, adding considerably to medical costs. Microbial biofilms readily develop on all types of devices, urinary, endotracheal, intravenous, and other types of catheters and implants inserted into more than 25% of patients during hospitalization. The incidence of bacterial infections in patients with urinary catheters is approximately 5 to 10% per day, with virtually all patients who undergo long-term catheterization (>28 days) becoming infected (13, 14, 17).

The first stage in biofilm formation from planktonic microorganisms is attachment to solid surfaces (6). Attachment stimulates microbial aggregation and proliferation to form microcolonies. The colonies excrete an encasing exopolysaccharide “slime,” which consolidates the attachment to surfaces, and the microaggregates differentiate into characteristic biofilms (20). Quorum-sensing molecules that generate concentration gradient-dependent signals that control and alter expression of a large number of genes also aid biofilm differentiation (15, 25). Encasing the extracellular polysaccharide matrix of biofilms regulates exchange of ions and nutrients with the surrounding environment. This regulation contributes to increases of up to 1,000-fold in biofilm resistance to antibiotics compared to planktonic bacteria (9, 11) and protects the biofilms from biocides, surfactants, and predators. Microbial biofilms also present serious challenges to the immune system because expression of bacterial antigens within the encasing polysaccharide matrix is suppressed and the colonies are highly resistant to phagocytosis by polymorphonuclear cells (12). Altogether these properties render biofilms exceedingly difficult to eradicate and explain the severity, persistence, and high levels of morbidity associated with the infections that they produce.

The harsh and potentially fatal consequences of microbial biofilm infections generated efforts to prevent their formation, particularly on indwelling medical devices using chemical and mechanical approaches. Catheters coated with hydrogel, silver salts, and antimicrobials have been evaluated; however, they provide minimal reduction in infection incidence (21). Mechanical approaches to preventing biofilm formation have utilized ultrasonic energy, yet the focus has thus far been on increasing biofilm sensitivity to antibiotics (18). The combination of ultrasound with antibiotics was found effective only in reducing the burden of Escherichia coli biofilms in animal models, falling short of providing a comprehensive solution to the biofilm problem (3).

We devised an innovative approach in which we generate low-energy elastic acoustic waves of practically nonthermal range from electrically activated piezo ceramic elements. The vibration energy is transmitted directly to indwelling medical devices in an integrated unit. Our aim was to achieve dispersion of the acoustic energy on entire surfaces of indwelling medical devices with different consistencies and structures. We analyzed the physical and power requirements for harnessing these waves to prevent microbial attachment and biofilm formation. The findings were consolidated into piezo actuators generating low-power acoustic waves at frequencies ranging from 100 to 300 kHz. The results of studies evaluating the efficacy of these actuators in preventing biofilm formation on indwelling medical devices from several microorganisms, in vitro and in animal models, are presented.
Low-energy SAW spread from an actuator to catheters, covering all surfaces with waves at amplitudes between 0.2 and 2 nm. These noncavitational power intensities of 103 mW/cm2 produced acoustic pressure amplitudes ranging from 0.16 kPa at the edge of the catheter to 0.21 kPa at the center. Fresh media containing 105 CFU/ml of several types of bacteria (from ATCC) were pumped continuously from chemostats at 0.5 ml/min and a temperature of ±30°C for 3 days. The segments were fixed in 4% buffered formaldehyde, rinsed four times with PBS, and dehydrated incrementally with 25% to 100% aqueous ethanol gradients. Following drying in a Bio-Rad C.P.D 750 critical point dryer, the samples were mounted on metal stubs and coated with a gold layer, and three different areas on each catheter were examined by SEM. Surfaces of SAW-treated catheters (left panels) are compared to nontreated controls (right panels).

The acoustic pressure amplitudes of the waves vary on different parts of urinary catheters (body, balloon, and tip) as shown in a simulation of their transversal vector directed perpendicularly to the catheter surface is detected around the balloon with maximal acoustic intensity of 200 mW/cm2 and amplitudes of 300 to 800 nm. Consequently, all catheters are covered with a virtual vibrating coat (24). Consequently, all catheters are covered with a virtual vibrating coat (24).

<table>
<thead>
<tr>
<th>Microbial species</th>
<th>Bioburden (CFU/cm²) of microbial biofilm developing on 16Fr Foley catheter</th>
<th>Log reduction</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>4.07E+04</td>
<td>0.79</td>
<td>0.000</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1.52E+04</td>
<td>1.22</td>
<td>0.050</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>6.48E+04</td>
<td>1.15</td>
<td>0.001</td>
</tr>
<tr>
<td>Entrococcus faecalis</td>
<td>4.42E+04</td>
<td>0.75</td>
<td>0.013</td>
</tr>
<tr>
<td><strong>SAW-treated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>4.55E+04</td>
<td>0.79</td>
<td>0.000</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>9.02E+02</td>
<td>1.22</td>
<td>0.050</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>4.78E+03</td>
<td>1.15</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Log reduction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Three-centimeter sections were prepared from each catheter, sonicated at 20 KHz and 3 to 4 W in two 30-second pulses to shed the catheter-associated biofilms and disperse them in solution for titration. Microbial counts correspond to overall load on 3-cm-long catheter sections.

** Standard deviations refer to differences in bacterial loads between the control and the SAW-treated in the three repetitions of each analysis.

** Log reduction values and P values compare the bioburden for control and SAW-treated catheters.

** are 3 orders of magnitude lower than the thresholds beyond which cavitation is produced (frequency f = 100 kHz at acoustic intensities of 0.5 × 102 to 2 × 103 mW/cm2) (5, 8).

Evaluation of biofilm prevention on urinary catheters by SAW in vitro. Sections of 16Fr Foley catheters 6 cm long (siliconized latex, Unomedical, Denmark) were attached to piezo actuators, sterilized with isopropyl alcohol, and placed in 25-ml tissue culture flasks (Corning) through an opening created at the top of the flask. Several commercial microbial strains (E. coli, ATCC 25922, Entrococcus faecalis ATCC 19433, Candida albicans ATCC 10231, and Proteus mirabilis ATCC 49300 supplied by Hyblabs, Rehovot, Israel) were cultured over-
night in Bacto tryptic soy broth (TSB) (Difco). The log-phase cultures were brought to a concentration of 10⁵ CFU/ml determined by optical density at 640 nm and confirmed by plate counts. The selected bacteria were brought to a concentration of 10⁷ CFU/ml in a mixture of (a) 50% of a solution containing 8 g of TSB and 8 ml fetal calf serum (Gibco) in 1 ml of phosphate-buffered saline (PBS) (Gibco) and (a) 50% heat-sterilized human urine obtained from a healthy donor and placed in a chamber in which the flask was connected and sealed with plastic covers. The media were passed over the catheters in the flask continuously for the 3-day duration of each experiment. Flow was achieved via a peristaltic pump at a rate of 0.5 ml/min under a temperature of ~30°C with the input medium replaced daily (batch system). Signals for surface acoustic nanowaves were monitored twice daily in the active chambers using a highly sensitive hydrophone. After 3 days, the catheter segments were rinsed and cut into two halves. One half was subjected to sonication at 20 kHz and 3 to 4 W output (model 550 sonicator; Fisher Scientific) to shed the biofilm off the catheter. The overall biofilm on catheter surfaces was assayed by plate counts on blood agar plates of removed biofilm mass from 5 cm sections of the catheters. Other sections were left intact for biofilm assessment by scanning electron microscopy (SEM).

Preparation of catheter samples for SEM. Catheter samples were fixed in 4% buffered formaldehyde (Futarom, Israel) and rinsed four times with phosphate-buffered saline (GIBCO). Critical drying was performed with ethanol at concentrations increasing from 25% to 100% in doubble distilled water. The samples were dried in a critical point dryer (Bio-Rad C.P.D. 750), mounted on metal stubs, and coated with a gold layer. Three different points were examined in each catheter by SEM at three magnifications: 1,000, 3,500, and 7,500, from four different fragments of the catheter and processed for SEM as indicated above. The outer and inner surfaces were evaluated separately at three different magnifications: 500, 1,000, and 3,500. Catheters removed from rabbit urinary bladder were sectioned into 1-cm-long fragments of the body, balloon, and tip of each catheter and processed for SEM as indicated above. The outer and inner surfaces were evaluated separately at three different magnifications: 500, 1,000, and 3,500 (from four different animals in each experimental group).

RESULTS

Prevention of microbial biofilm formation by surface acoustic waves. We examined the effects of low-energy SAW on biofilm formation by four common clinically relevant types of microorganisms on several types of surfaces, including 16Fr plastic catheters (data not shown), indicating that these element-generated elastic waves can be adjusted to prevent microbial adhesion and biofilm formation on surfaces with different consistencies and shapes.

Surface acoustic waves interfere with adhesion of planktonic microorganisms to cellular surfaces. Our analysis of mechanisms by which SAW interfere with bacterial biofilm formation focused on the hypothesis that SAW target the adhesion of planktonic bacteria to surfaces, the first step in the biofilm formation process. To evaluate the effects of SAW on bacterial adhesion, we used the mannose receptor-specific adhesion of uropathogenic E. coli bacteria to guinea pig erythrocytes as a model; the adhesion occurs via type 1 pili, FimH lectin and culminates in RBC aggregation (22). In this system, bacterial adhesion was selected from clinical isolates at the microbiology laboratory of the Sheba Medical Center. The bacteria were analyzed for the ability to form biofilms and the ability to induce guinea pig red blood cell (RBC) aggregation. Bacteria (10⁷) were applied to a 4% guinea pig erythrocyte suspension in saline (0.9% NaCl) in 50-mm Miniplast petri dishes to which a single SAW actuator has been attached. Surface acoustic waves at a power intensity of 0.5 mMw/cm² are shown to enhance mannose receptor-specific bacterial adhesion to RBC. The samples in panels A and B were photographed 3 h after administration of bacteria and initiation of treatment with SAW. Exceedingly large RBC aggregates formed, as shown in Fig. 3B (middle panel), which were susceptible to dissociation with D-mannose (Fig. 3B, right panel).
RBC. Power intensities of 0.1 and 0.2 mW/cm², generating vibra-
resumption of the SAW treatment (not shown), although it was
other cells is thus not damaged by SAW. Once aggregation has
fimbriae. The bacterial mechanism for adhesion to RBC and
chanical, readily reversible following SAW deactivation, and
indicate that inhibition of RBC aggregation by SAW is me-
titor the plates with time-lapse photography. Guinea pig eryth-
substrates.

We deactivated the SAW treatment and continued to mon-
itor the plates with time-lapse photography. Guinea pig eryth-
rocyte aggregation resumed 10 min after SAW ter-
mination, a rate similar to RBC aggregation in control plates
(12 min ± 3 min; difference not significant). These findings
indicate that inhibition of RBC aggregation by SAW is me-
chanical, readily reversible following SAW deactivation, and
does not diminish the functionality of the FimH lectin on
fimbriae. The bacterial mechanism for adhesion to RBC and
other cells is thus not damaged by SAW. Once aggregation has
taken place, RBC aggregates could no longer be dissociated by
resumption of the SAW treatment (not shown), although it was
reversed by α-mannose.

We next examined the correlation between levels of SAW
energy that were applied and E. coli-induced RBC aggregation.
SAW activated with 0.05 to 0.20 mW/cm² effectively prevented
RBC aggregation (Fig. 3A); however, increasing the output to
beyond a 0.35-mW/cm² threshold converted the inhibition into a
significant enhancement of bacterial attachment. Exceedingly
large RBC aggregates formed as shown in Fig. 3B, which were
susceptible to dissociation with α-mannose (Fig. 3B) and gradu-
ally dissolved upon cessation of the SAW treatment (not shown).
Hence, SAW applied at power intensities beyond approximately
0.35 mW/cm² can activate FimH force sensor activity in a manner
similar to force sensor activation seen when shear force is applied
to uropathogenic E. coli bacteria (22).

Prevention of microbial biofilm formation on urinary cath-
eters with acoustic nanowave actuators in an animal model in
vivo. The ultimate preclinical determination of whether SAW-
generating piezo actuators can interfere with microbial biofilm
formation on urinary catheters in clinical settings is in animal
studies. We inserted 10Fr Foley catheters attached with a piezo
actuator at the extracorporeal portion of the catheter into the
urinary bladders of male rabbits in a sterile manner. The de-
vices were activated for up to 9 days in four of eight tested
rabbits (in three separate experiments). Urine samples were
collected daily, the bacterial load was titrated, and time to bacteriuria was determined. Urine samples from rabbits with
SAW-treated catheters remained sterile for 5, 7, and 9 days (26
cumulative days of sterile urine) despite the extensive contam-
nation of the perineal area with feces. Furthermore, the bac-
teria that did develop in some rabbits was mostly of low
titer, whereas three of four control rabbits developed bacte-
riuria of ≥10⁷ CFU/mL within 2 or 3 days and the fourth had a
titer of ≥10⁶ CFU/mL on day 7. The average number of days
to development of urinary tract infection, defined as bacteriuria of ≥10⁶ CFU/mL, was 7.3 ± 1.3 days for the SAW-treated
rabbits versus 1.5 ± 0.6 days in the untreated controls (P <
0.0009 by two-tailed Student’s t test; n = 4) (Table 2).

At the end of the experiments, the animals were sacrifi-
ced, the bladder and urethra were cut open, and the cath-
eters were removed carefully, avoiding disruption of the
biofilms. Biofilm content was examined by SEM. Analyses of the
internal surfaces of recovered catheters revealed strong
inhibition of bacterial biofilm formation on the surfaces of
catheters treated with SAW (Fig. 4A). In contrast, control
group catheters were covered with various densities of mi-
crobiota despite the shorter durations of catheter-
inization (in two of the animals, the catheters were in place for
only 3 or 4 days) (Fig. 4B).

Evaluation of the integrity of mucous membranes by histo-
logical and ultrastructural analyses in all control and SAW-
treated animals revealed that the treatment with SAW did not
produce any histopathological changes. Furthermore, uroepi-
theial integrity was found to be less affected by trauma and

- **TABLE 2. Time to bacteriuria in rabbits with 10Fr Foley catheters and SAW-generating piezo actuators**

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Bacterial titer (CFU/ml) on:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>SAW-treated rabbits</td>
<td></td>
</tr>
<tr>
<td>163</td>
<td>0</td>
</tr>
<tr>
<td>229</td>
<td>0</td>
</tr>
<tr>
<td>265</td>
<td>0</td>
</tr>
<tr>
<td>143</td>
<td>0</td>
</tr>
<tr>
<td>Control rabbits</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>31</td>
<td>4</td>
</tr>
<tr>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>144</td>
<td>70</td>
</tr>
</tbody>
</table>

* Rabbits had 10Fr Foley catheters inserted. The catheters were attached to SAW-generating piezo actuators at the extracorporeal body of the catheters. Animals
that developed bacteriuria were removed and their participation in the experiments was terminated due to limitations imposed by the Animal Welfare and Care Commit-
better conserved in the SAW-treated animals than in the controls (data not shown).

**DISCUSSION**

The remarkable flexibility by which microorganisms adapt to changing environments or become insulated from environmental hazards has been the core of shortcomings in the ability of chemical approaches to prevent microbial biofilm formation on implanted medical devices. Efforts to target or eradicate biofilms therefore include mechanical approaches, which thus far have mainly been aimed at increasing the penetration of antibiotics into microbial colonies (3, 18).

We have contemplated utilization of mechanical vibration energy to interfere with early events in the biofilm development process—the adhesion of planktonic microorganisms to surfaces. By preventing adhesion, we sought to abort their subsequent firm attachment to substrates (1), gene expression reprogramming, and synthesis of the corresponding protein products that transform the lifestyle of microorganisms from the planktonic to sessile form (2, 4, 19). We also speculate that chaotic microstreaming produced in fluids by the ongoing vibrations hampers the development of coherent concentration-dependent gradients of quorum-sensing molecules. Disruption of such gradients is likely to interfere with cell-cell communications among microorganisms, virulence factor production, and other postattachment biofilm developmental processes. The outcome is prevention of colony differentiation and biofilm formation (7, 10, 16).

We show that low-energy elastic acoustic waves transmitted directly to extracorporal portions of implanted medical devices can interfere effectively with attachment of planktonic microorganisms to surfaces and prevent biofilm formation for extended time intervals. The mechanical nature of this treatment implies that the elastic waves must be powered continuously throughout the duration of device implantation to prevent attachment of planktonic bacteria. Disruption of the vibration energy is found to promote renewed adhesion of bacteria to these surfaces, indicating that the effects of SAW are readily reversible and do not diminish the functionality of bacterial adhesion mechanisms. For example, the fimbrial adhesion of E. coli to guinea pig RBC following disruption of SAW.

**tions between microorganisms, virulence factor production, and other postattachment biofilm developmental processes. The outcome is prevention of colony differentiation and biofilm formation (7, 10, 16).**

**tions between microorganisms, virulence factor production, and other postattachment biofilm developmental processes. The outcome is prevention of colony differentiation and biofilm formation (7, 10, 16).**

We propose the following hypothesis to explain the low-energy SAW-mediated biofilm prevention phenomenon. Attachment or repulsion of bacteria in the 10-nm range near surfaces is an outcome of van der Waals and hydrophobic attraction forces being counteracted by electrostatic repulsion (6). This phenomenon known as the Z potential of the surface is critical to bacterial adhesion mechanisms. For example, the fimbrial adhesion of E. coli to guinea pig RBC following disruption of SAW.

**tions between microorganisms, virulence factor production, and other postattachment biofilm developmental processes. The outcome is prevention of colony differentiation and biofilm formation (7, 10, 16).**

We propose the following hypothesis to explain the low-energy SAW-mediated biofilm prevention phenomenon. Attachment or repulsion of bacteria in the 10-nm range near surfaces is an outcome of van der Waals and hydrophobic attraction forces being counteracted by electrostatic repulsion (6). This phenomenon known as the Z potential of the surface is critical to bacterial adhesion mechanisms. For example, the fimbrial adhesion of E. coli to guinea pig RBC following disruption of SAW.

**tions between microorganisms, virulence factor production, and other postattachment biofilm developmental processes. The outcome is prevention of colony differentiation and biofilm formation (7, 10, 16).**

We propose the following hypothesis to explain the low-energy SAW-mediated biofilm prevention phenomenon. Attachment or repulsion of bacteria in the 10-nm range near surfaces is an outcome of van der Waals and hydrophobic attraction forces being counteracted by electrostatic repulsion (6). This phenomenon known as the Z potential of the surface is critical to bacterial adhesion mechanisms. For example, the fimbrial adhesion of E. coli to guinea pig RBC following disruption of SAW.

**tions between microorganisms, virulence factor production, and other postattachment biofilm developmental processes. The outcome is prevention of colony differentiation and biofilm formation (7, 10, 16).**

We propose the following hypothesis to explain the low-energy SAW-mediated biofilm prevention phenomenon. Attachment or repulsion of bacteria in the 10-nm range near surfaces is an outcome of van der Waals and hydrophobic attraction forces being counteracted by electrostatic repulsion (6). This phenomenon known as the Z potential of the surface is critical to bacterial adhesion mechanisms. For example, the fimbrial adhesion of E. coli to guinea pig RBC following disruption of SAW.

**tions between microorganisms, virulence factor production, and other postattachment biofilm developmental processes. The outcome is prevention of colony differentiation and biofilm formation (7, 10, 16).**

We propose the following hypothesis to explain the low-energy SAW-mediated biofilm prevention phenomenon. Attachment or repulsion of bacteria in the 10-nm range near surfaces is an outcome of van der Waals and hydrophobic attraction forces being counteracted by electrostatic repulsion (6). This phenomenon known as the Z potential of the surface is critical to bacterial adhesion mechanisms. For example, the fimbrial adhesion of E. coli to guinea pig RBC following disruption of SAW.