TREATMENT OF BIOFILMS USING SURFACE ACOUSTIC WAVES

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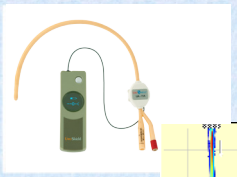
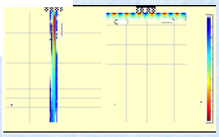
Introduction

Biofilms are bacterial communities embedded in extrapolysaccharide matrix which protects them from hostile environmental conditions. Biofilms are extremely resistant to antibiotics treatments and are a major cause of medical device-associated infections. Therefore immense efforts are being made in a search for alternative ways to eradicate bacterial biofilms. Ultrasound waves have been demonstrated to affect bacterial susceptibility to antibiotics. In the present study we used a new ultrasonic device, UroShield (Nanovibronix corporation), which is attached to a urinary catheter and generates Low-Energy Surface Acoustic Waves (SAW) along the catheter. Our results showed that SAW can reduce the ability of *Pseudomonas aeruginosa* to form biofilms on catheters. Furthermore, a reduction of 90% to 99% in biofilm biomass was observed when biofilms of *P. aeruginosa, Escherichia coli* and *Staphylococcus epidermidis* were treated with gentamicin combined with SAW. Additionally, transcriptome analysis revealed that SAW treatment affects gene expression profile in *P. aeruginosa* biofilm. A number of efflux pump-encoding genes, regulatory genes as well as virulence genes (including type III secretion system) presented with altered expression pattern. Our findings highlight the potential of using the UroShield to reduce as well as treat urinary catheter infections and for the first time demonstrate a clear physiological response to the bioacustic effect.

UroShield® a Low-Energy Surface Acoustic Waves (SAW) generator



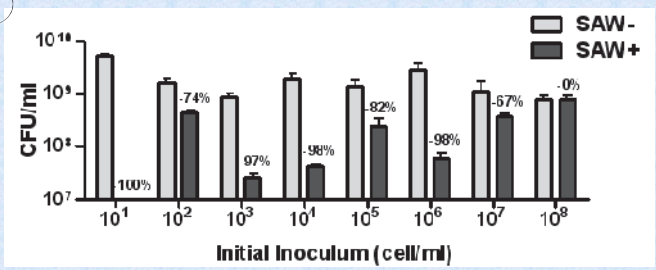
Nanovibronix corporation SAW generator device: (A) An **electronic driver** delivers periodical rectangular electrical pulses to an (B) **actuator** harboring a thin piezo ceramic plate. Low-energy SAW spread from an actuator to (C) **catheter**



The propagation of a pressure wave through medium compares between (A) longitudinal wave that characterize conventional Ultrasound wave which penetrate the medium, and (B) Surface Acoustic Waves (SAW) that travel along the medium interface. (C) blue stands for low pressure and red for high.

1

SAW reduce biofilm formation on catheters

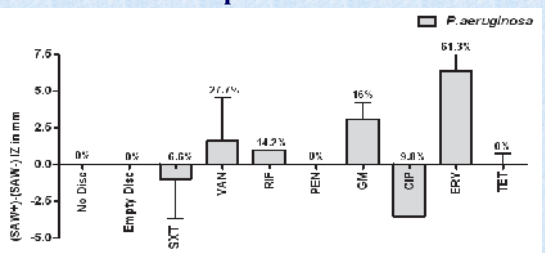


Catheters with and without continuous SAW treatment were challenged with different concentrations of *P. aeruginosa* and the number of biofilm cells that attached to the catheter were determined 72 h post inoculation.

Our results show that SAW can reduce (by 98%) the ability of *P. aeruginosa* to form biofilms on catheters. This inhibition is maintained when the catheters are exposed to bacterial concentrations as high as 10^6 cell/ml. Under an initial inoculum of 10 cell/ml, the SAW treatment kept the catheter sterile.

2

SAW affect the antibiotic susceptibility of *P. aeruginosa* planktonic cultures

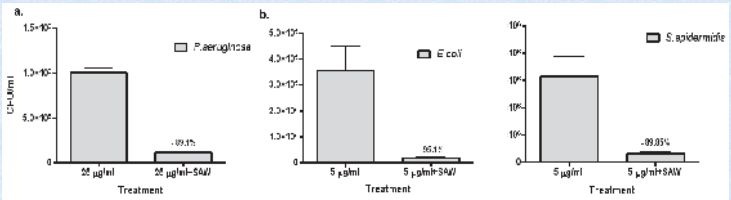


Antibiotic	Code
Sulfamethoxazole+ Trimethoprim	SXT
Vancomycin	VAN
Rifampin	RIF
Penicillin	PEN
Gentamicin	GM
Ciprofloxacin	CIP
Erythromycin	ERY
Tetracycline	TET

Antibiotic efficacy with and without SAW against *P. aeruginosa* was determined with various antibiotics using the disc diffusion assay. A positive value represents an increase in killing in the combined treatment over antibiotic treatment alone.

3

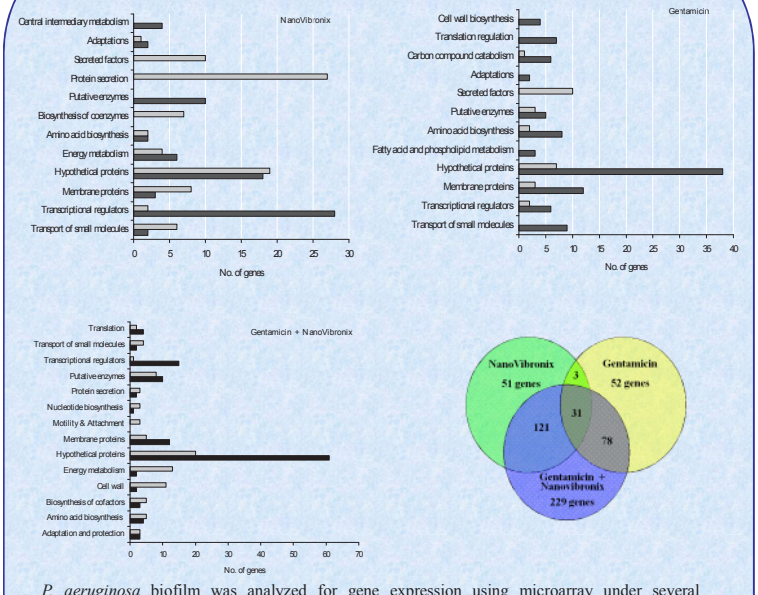
SAW increase the antimicrobial activity of gentamicin against biofilm cells



P. aeruginosa, E. coli and *S. epidermis* were allowed to form biofilm under continuous flow for three days. The biofilm were then treated with gentamicin or gentamicin in the presence of SAW. The presence of SAW decreased the biofilm by nearly 90% in all three species.

4

SAW specifically affect *P. aeruginosa* global transcription pattern



P. aeruginosa biofilm was analyzed for gene expression using microarray under several conditions: SAW alone (top left panel), gentamicin alone (top right panel), and gentamicin in the presence of SAW (bottom left panel). The biofilm were then treated with gentamicin or gentamicin in the presence of SAW. SAW specifically affected transcription pattern of 172 genes. The diagram represents number of genes affected under various conditions.

5

Summary & Conclusions

Our results demonstrate that Surface Acoustic Waves (SAW) are capable of affecting *P. aeruginosa* biofilm formation as well as have a synergistic affect when treating biofilm with antibiotics. Combined treatment of *P. aeruginosa, E.* coli and *S. epidermidis* biofilms with SAW and antibiotics significantly reduced bacterial survival. Moreover, transcriptome analysis revealed that SAW specifically affect transcription pattern of a number of genes. Taken together, these findings highlight the potential of using the UroShield to reduce as well as treat urinary catheter infections.