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Three inventions to clean ship hulls, decontaminate hospital floors, and treat wounds, using air, sound and water

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This paper describes three ultrasonic inventions linked by the means to remove biofilms, and so prevent problems of societal importance. The first is cleans flat surfaces, or surfaces with long radii of curvatures (e.g. ship hulls). Macroscopic marine biofouling takes hold following the development of a bacterial biofilm on the hull. The device inhibits the maturation of the biofilm, so reducing the establishment of macroscopic marine biofoulant. The device presents a water-filled cavity to the surface and within it creates a modal sound field that ensures that an acoustic pressure antinode is presented over the surface to be cleaned (the 'target'). Examples are presented for the removal of marine biofoulant grown on surfaces representative of vessel hulls (for which the device is designed to minimize the leakage of ultrasound into the surrounding marine environment). The second device uses a similar principle to remove antibiotic-resistant bacteria (here, MRSA - methicillin-resistant Staphylococcus aureus) from surfaces representative of hospital floors and walls. A third device eliminates the need for the surface to be flat, allowing the removal of biofilm from difficult-to-heal wounds, opening up the possibility of healing previously incurable wounds using just water, sound and air.

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1. INTRODUCTION

This paper describes three inventions linked by the means to remove biofilms, and so prevent problems of societal importance: marine biofouling (e.g. of ship hulls); flat surface cleaning (e.g. solar panels, floors of hospital and power stations) and the cleaning and healing of currently incurable wounds.

All are linked through the mode of action, which is to activate pulsation and surface waves on microscopic air bubbles submerged in water and transmitted to the surface to be cleaned (the 'target') with no, or minimal, contact between the device and the surface.[1] The peak-to-peak time history of the pressure variation on the target can be controlled to generate either inertial, or non-inertial cavitation (depending on whether the cleaning requires, and the surface can tolerate, the effect of inertial cavitation; or whether the surface would be damaged by inertial cavitation but the contaminant could be removed by the bubble wall surface waves that can be stimulated during non-inertial cavitation).[2] In each device, the ability to predict and control the conditions for generating surface waves,[3],[4] and use Bjerknes forces to ensure the bubbles hosting those surface wave migrate into crevices to clean them,[5] is vital.

All are also linked through their ability to remove bacterial biofilms. For many years, the prevailing perception of bacteria prevalence was as individual cells (the 'planktonic' form), but in recent years the importance of the formation of biofilm, where communities of bacteria colonize a surface, has become more widely appreciated. Cells within biofilms form a structure that offers mutual protection and antibiotic resistance, and indeed multiple species of bacteria, and even inter-kingdom biofilms of bacteria, fungi and inert particles, can co-exist to make these contaminants more difficult to remove from a surface. Moreover, if the surface has a complex structure or is porous, traditional wipes or brushes can find it very difficult to penetrate the pores/crevices to remove biofilm from there.[6] The cavitation described in the preceding paragraph can, under the influence of Bjerknes forces, penetrate the pores and crevices to remove the biofilm.[7] In the examples shown here, only non-inertial cavitation is excited on the target, so that unlike mechanical brushing or scrubbing, or inertial cavitation in an ultrasonic cleaning bath, the cleaning action does not produce pits or scratches on the target surface (Figure 1). This is important because pits and scratches make it easier for future biofilms to become established on the surface: if that happens during brushing or use of an ultrasonic cleaning bath, repeated cleaning becomes progressively more difficult (Figure 2).

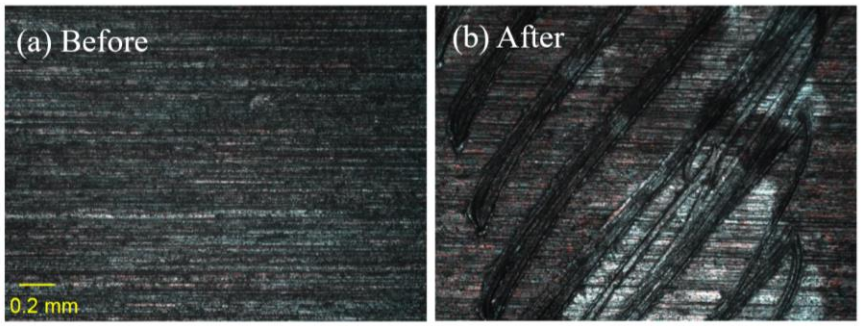

Figure 1. Aluminium sheet treated by traditional power scrubbing, (a) before and (b) after treatment (where scratches are visible).

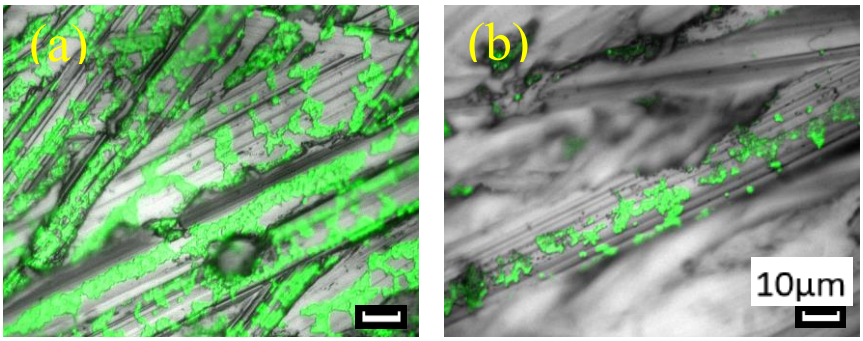

Figure 2. SYTO-9 stained Methicillin-resistant Staphylococcus aureus (MRSA) contamination on the same region stainless steel surfaces, (a) before cleaning, and (a) after cleaning by model ethanol wipes (which fail to remove the bacteria from the deepest crevice).

Figures 1 and 2 relate to metal hulls, but the rubberized anechoic tiles of submarines are particularly important. Biofouling can reduce speed, a particularly important issue on hunter submarines like the one shown in Figure 3(a). Figure 3(b) shows damage to rubber anechoic tiles caused by a mechanical rotating brush used to remove marine biofoulant.

Figure 3. (a) A fast attack submarine, showing significant biofouling that will reduce its speed and efficiency. At the Naval Base Point Loma, Calif. (Jan 10, 2006), the crew of the Navy's only commissioned floating dry-dock, ARCO (ARDM-5), uses mooring lines to pull in Los Angeles-class, fast-attack nuclear submarine, USS Helena (SSN-725). After mooring, the floating dry-dock will blow water out of its ballasts allowing for complete access to the submarine. The crews of ARCO and Helena along with civilian contractors will perform scheduled maintenance procedures on the boat. U.S. Navy photo by Journalist Seaman Joseph Caballero). (b) Damage to a rubber tile caused by removing biofouling from it with a circular rotating brush. The image is not meant to imply that such a cleaner or damage occurs in the situation shown in (a).

Despite the consequences of potential damage, mechanical cleaning methods are of interest because biocides are harmful to protected species, and marine biofoulant must be reduced on most vessels because fouling reduces sonar performance if it occurs over a sonar source/receiver, and across the hull it increases drag[8] and reduces fuel efficiency by up to 50% with associated fuel penalties and negative environmental impacts.[9] The United States Navy is estimated to spend between $180M and $260M per year combatting the effect of biofouling on ships and submarines.[9] To prevent the spread of invasive species, examples of increased biosecurity controls include regulations requiring all vessels entering New Zealand waters to present records of marine biofouling management (generally cleaning 30 days prior to arrival) on arrival in a New Zealand port. In March 2017 *DL Marigold* (a 33,000 ton bulk carrier) was required to leave New Zealand waters (and then barred from Fijian waters) due to marine biofouling, preventing the vessel from unloading its cargo.

Biofilms also give pathogens increased resistance to decontamination measures, especially on surfaces that becomes increasingly scratched through repeated cleaning. In the US, hospital-acquired infections cost $9.8 billion annually,[10] with at least 1.7 million hospital-acquired infections (of which 16% were reported as resistant to the antibiotics commonly used to treat them[11]).[12]

For these reasons, a mechanical method of removing biofilms from macroscopically and locally flat surfaces (such as ship hulls and hospital floors) would be useful. Whilst some biofilms in the correct time and location can be highly beneficial to humanity, the remainder of the paper will outline the three inventions linked by the need to remove biofilms for societal benefit. The first device is an ultrasonic device for cleaning flat surfaces, or surfaces with long radii of curvatures (such as ship hulls). The second device uses a similar principle to remove antibiotic-resistant bacteria (here, Methicillin-resistant *Staphylococcus aureus*, MRSA) from surfaces representative of hospital floors and walls. The third device eliminates the need for the surface to be flat, allowing the removal of biofilm from difficult-to-heal wounds, opening up the possibility of healing previously incurable wounds using just water, sound and air.

2. MATERIALS AND METHODS

A. HULL CLEANING

Figure 4(a) shows a schematic of the ship hull cleaner. Macroscopic marine biofouling takes hold following the initial development of a bacterial biofilm on the hull. The device inhibits the maturation of the biofilm, so reducing the subsequent establishment of macroscopic marine biofouling. In the device used here, 70 kHz was chosen, although the architecture provides for a range, balancing the need to establish an appropriate modal field in the cavity, and reduce the likelihood for adverse effects on marine life (fish,[13] cetaceans[14] and benthic species[15]). The device presents a water-filled cavity to the surface and within it creates a modal sound field that ensures that an acoustic pressure antinode is presented over the surface to be cleaned (the 'target'). Examples are presented for the removal of marine biofoulant grown on surfaces representative of vessel hulls (for which the device is designed to minimize the leakage of ultrasound into the surrounding marine environment).

Traditionally, hull cleaning has focused on intermittent removal of the mature biofoulant. However, by recognizing the importance of the biofilm in producing the foundation to which macroscopic biofouling becomes established, a procedure of deploying an autonomous device to cover the hull whilst it is in harbor, would prevent the establishment of a mature biofilm and so remove the foundational pre-requisite for macroscopic biofouling (Figure 4(b)). This tactical proposal is supported by the fact that most marine weeds are grown in harbor rather than when cruising at high speed.[16] The chamber walls reduce the leakage of sound into the environment, an increasing concern with conventional hull treatment technologies.[17,18]

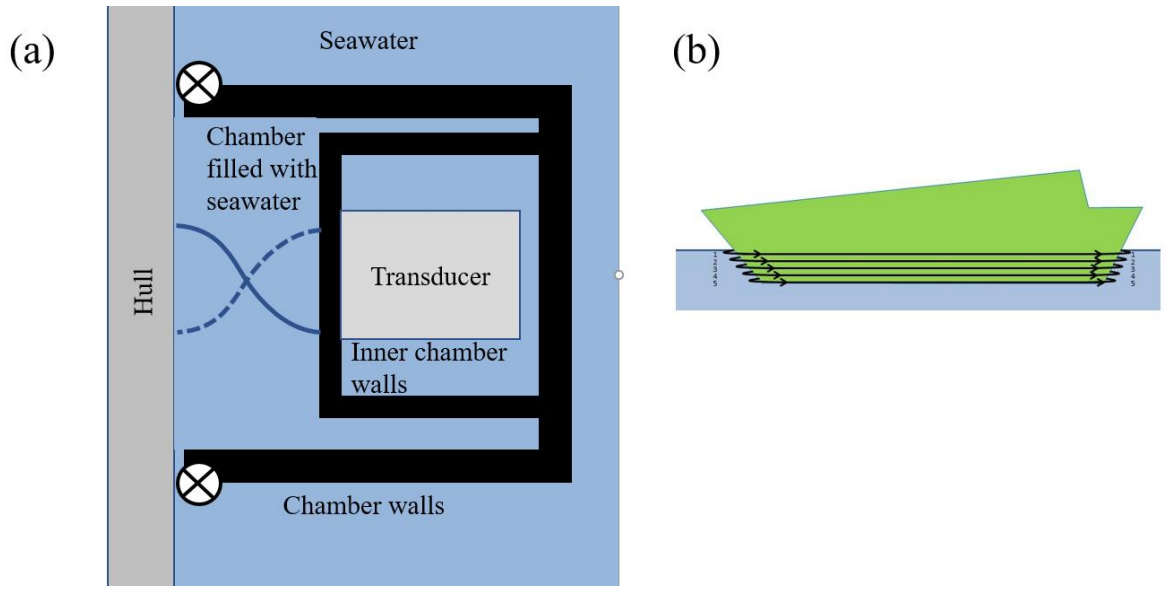

Figure 4. (a) Schematic of the hull cleaning device, and (b) method for covering a ship hull. The typical footprint of a single cell is around 0.1 m by 0.1 m although they can be made larger with multiple transducers. Cells like this can also tesselate to cover a larger overall footprint.

Sample plates of area (100 cm^2) of aluminium, rubber and steel were either submerged, left untreated, cleaned once a week, or cleaned twice a week. The cleaning time was set to 1 minute which would allow for the entire sample area to be under the cleaning chamber for sufficient time for the surface cleaning action to take place. The footprint area of the cleaning chamber is 36 cm^2. The whole surface of the sample could be cleaned by repositioning the device 4 times. This ensures every part of the sample receives 15 seconds of cleaning action within this 1-minute period.

The rate of fouling was dependent on the material, and the decision was made to end the test when the control became heavily fouled, which was 41 days for aluminium, 50 days for rubber, and 36 days for steel.

The thickness of foulant on each sample was measured using an Episcopic Differential Interference Contrast (EDIC) microscope, which has the light source above the sample, and a very narrow depth of field, so that by measuring the travel required to focus on the top of the foulant compared to focusing on a small, intensively cleaned area of hull, and doing this on 10 sites over the surface, the average thickness can be calculated.

B. FLOOR CLEANING

The second invention has similar features to that of the ship hull cleaner shown in Figure 4, although is adapted for use normally on near-horizontal targets in air (e.g. on floors, solar panels etc.), requiring a membrane within the chamber to reduce the flow of water out of the device. Since the intention is to clean floors where minimal contact is preferred (e.g. to reduce the spread of infection from one floor to another in a hospital) the wheels of the trolley holding the cell can be made of antimicrobial material (e.g. copper). A frequency of 68 kHz was chosen in this case, although again the design suits a range of transducers, dependent on the balance of establishing an appropriate modal field within the cavity, and avoiding adverse responses (particularly in humans as a result of the leakage of ultrasound into the air[19,20]).

Overnight cultures of Methicillin-resistant *Staphylococcus aureus* (MRSA) (200 µL per tile) were inoculated on the bathroom tiles and were incubated for 1 hour at 37 Celsius. Once the MRSA was attached to the bathroom tiles they were either cleaned with the device (30 s) or running water (30 s), the residual MRSA on the tiles were dyed for 1 hour at 37 Celsius. The MRSA was visualised via SYTO 9 (Invitrogen, UK) nucleic acid staining for 5 min at room temperature, excess, unbound STYO 9 dye was removed via subsequent PBS (phosphate buffered saline) and deionised water. Visualisation of the residual MRSA contamination was carried out using sensitive epi-fluorescent microscopy and image analysis.

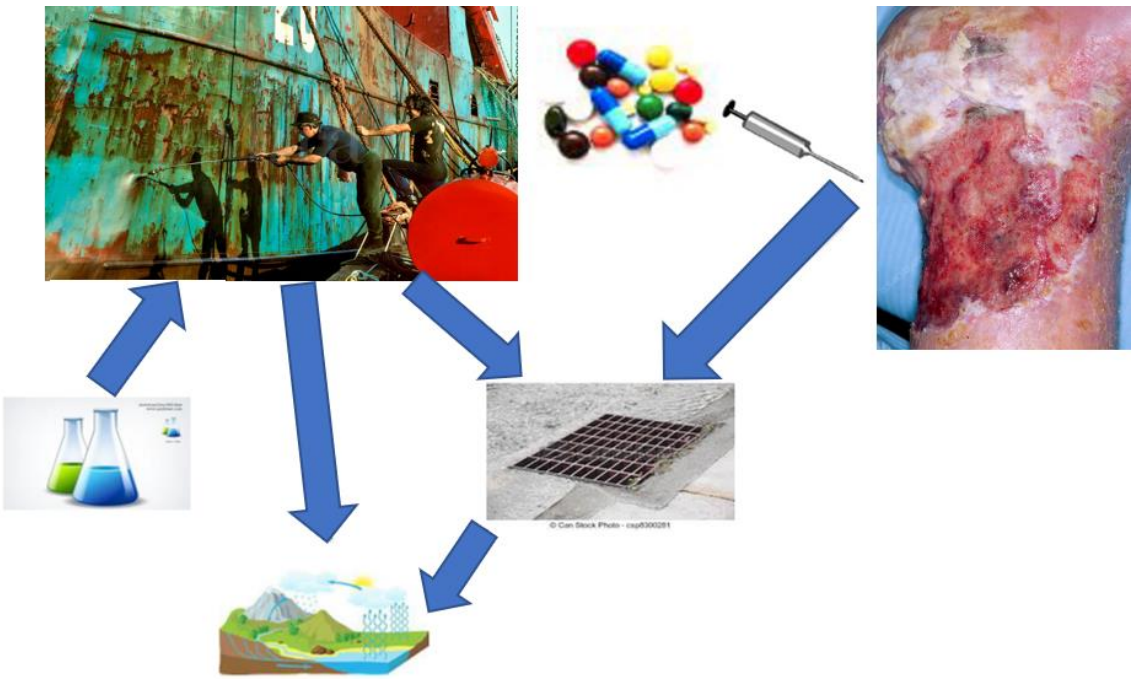

Figure 5. Schematic showing how the use of chemical agents to combat microbes leads to greater likelihood of subsequent colonizations of wounds, hull and hospitals, by species resistant to that chemical.

C. WOUND CLEANING

All three devices transmit sound and microbubbles through a water channel to the target. However, whereas the water channel for the first two inventions takes the form of a water cushion giving a few millimeters stand-off of the solid cleaner from the target. the third device increases the stand-off top several centimeters by using a water stream. This LAWS (Liquid Acoustic Wound Stream) device uses a stream, flowing to the surface at around 2 litres/minute with a stand-off distance of around 1 cm. This is so that the device can treat the structure topography of a wound bed, whilst avoiding touching the wound.

Pre-wounded reconstituted human epithelial tissues (EpiDerm Full Thickness, MatTek Corp., Ashland, Massachusetts) were examined microscopically following Haematoxylin and Eosin (H&E) staining. The tissues are derived from human neonatal foreskin tissue to form a multi-layered highly differentiated model of human skin and contains both keratinocytes and fibroblasts. These wound models were either washed with plain water or 0.9% saline through the LAWS device either with the sound turned on or off at a rate of 2 L/min as described in the results. Control wound models were untreated.

Details of the experiment are given in an earlier paper.[21] Previously, its 132-135 kHz ultrasound efficacy to remove contaminants from hard inert surfaces was shown for a range of applications, including cleaning baby equipment,[1] railway components[22-24], surgical instruments[25,26] and tools,[22] bone prior to transplant[26] and pipework/packaging associated with food and beverages.[1,6,22,27] Food itself has been cleaned without damage (including salad[28] and hay[29]), as have other soft targets including hands.[22]

Both hard (e.g. particulate[22-24]) and softer contaminants have been removed. Softer ones include glues,[22] greases[23] and lubricants[7], amyloid prion in brain tissue,[25,26] and biofilms (including those associated with dental[26,30], marine[31] and gastronomic[28,29] surfaces). The effective tackling of biofilms using only sound, air and water meant that, unlike the use of conventional antimicrobial treatments (antibiotics, antivirals, antifungals etc.), the use of such technology should not so readily promote the rise of AntiMicrobial Resistance (AMR).[27,32,33] Figure 5 schematically lays out possible route covering multiple mechanisms (not detailed in the figure) by which run-off containing chemical agents (biocides, antimicrobial agents etc.) contributes to resistance to those agents becoming (over microbial generations) more prevalent in the wider environment from which colonizations and infections are seeded.

One such route is natural selection. Consider a wider world where, initially, there has been an absence of such chemicals. Here, the resistant strains compete with other microbes (that are susceptible to those chemical agents) for resources (food, space etc.). However, if the runoff contains chemicals that suppress the populations of susceptible strains, over generation natural selection can generate an environment in which the resistant strains are more prevalent than they would otherwise be, because the populations of the susceptible strains have been suppressed. This makes subsequent colonizations more difficult to remove by the same treatment.

Another route comes from the fact that the chemical agents in the run-off might be diluted such that they have sub-minimal inhibitory concentrations when they come in contact with the microbes. This can cause genetic changes that imbue the microbe with greater resistance, again leading to an increased chance of a future infection or colonization being resistance. Worst yet, through quorum sensing, microbes with resistance can, through genetic transfer, enable previously susceptible microbes to gain resistance.

These are just some of the mechanisms by which allowing chemical agents that are meant to combat microbes into run-off, can lead to greater chance of future infections/colonizations by resistant microbes.

3. RESULTS

Figure 6(a) shows representative samples of aluminium, rubber and steel hulls, that were untreated, or cleaned each week either once or twice. The visual differences in fouling in Figure 6(a) are quantified in Figure 6(b) through measurement of the thickness of the foulant layer.

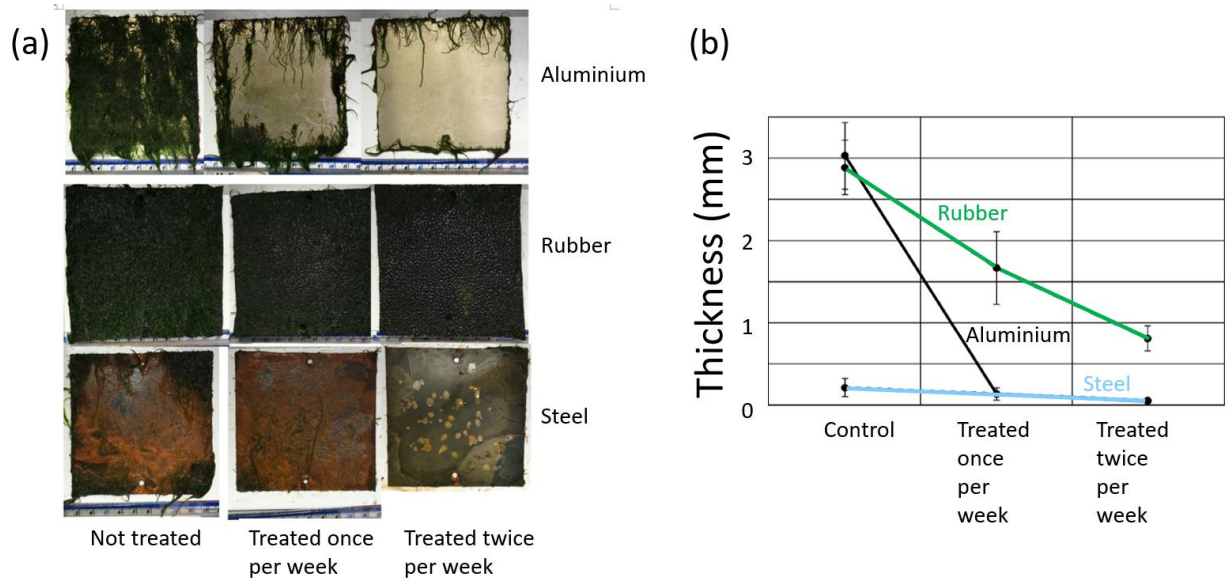

Figure 6. (a) A representative plate of aluminium (top row), rubber (middle row) and steel (bottom row), after being submerged and left as an untreated control (left column), treated once a week for 1 minute (middle column) or treated twice a week for 1 minute each treatment (middle column). (b).

Figure 7 uses microscopic photos to indicate the success of the floor cleaning invention in removing MRSA [panel (c)] compared to cleaning with running water [panel (b)] when both are compared to the control [panel (a)].

Figure 8 shows the results *P. aerugi*nosa inoculated into the wound bed and the model cultured for 24 hours at 37_C and 5% CO_2 to produce an early-stage biofilm. The micrographs in Figure 8 demonstrate the effect of washing the Epiderm FT model with either saline or a LAWS treatment. Control sections were not washed. The wash with saline alone appears to have spread the bacteria more evenly across the wound model but without removing a significant quantity of bacteria. The reduction achieved after 2 minutes of washing with a LAWS is statistically significant with $P \leq 0.001$.

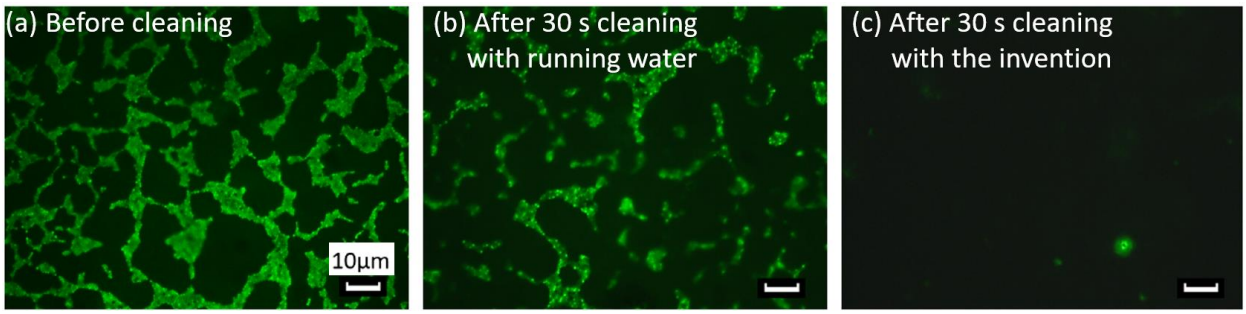

Figure 7. Microscopic photos of Methicillin-resistant *Staphylococcus aureus* (MRSA) coated bathroom tiles, (a) before cleaning, (b) after cleaning with running water only, or (c) after cleaning with the device.

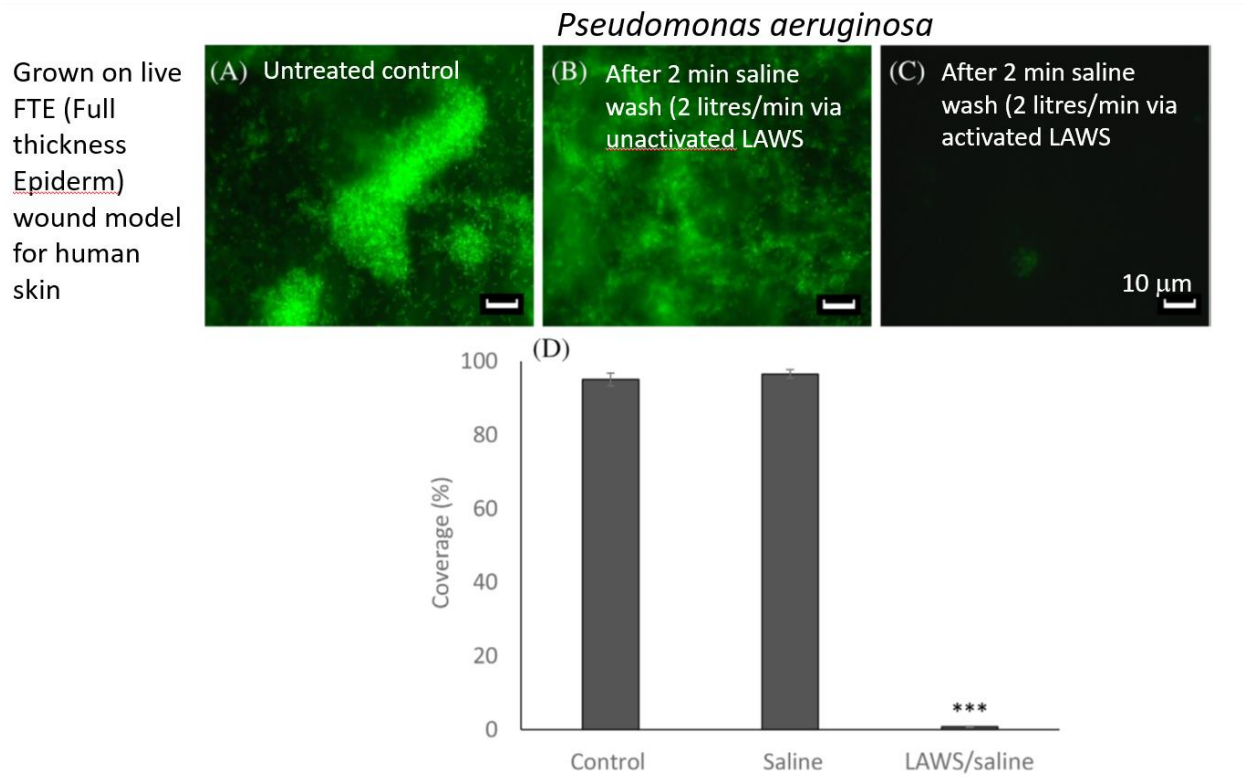

Figure 8. Epiderm full thickness (EFT) wound models. Representative episcopic differential interference contrast (EDIC)/EF micrographs of green fluorescent protein (GFP)-tagged Pseudomonas aeruginosa pMF230 biofilm within the EFT wound models: (A) with no treatment, (B) after a 2 minutes saline wash at a flow rate of 2 L/min, and (C) after a 2 minutes liquid acoustic wound stream (LAWS)/saline treatment at a flow rate of 2 L/min. Scale bars represent 10 µm. Image analysis (D) of the EDIC/EF micrographs demonstrating the percentage coverage of GFP tagged P. aeruginosa pMF230 biofilm within the EFT wound models after 24-hour incubation at 37_C. Error bars represent the SEM (n = 3), One-Way analysis of variance/Tukey post-hoc test demonstrated ***P ≤ 0.001 when compared with the nontreated controls. Reproduced from Reference 21.

Figure 9 shows the results of examination of the H&E-stained sections of uninfected wound models allows measurement of the length of the tongue of reepithelialisation. The EpiDerm FT wound model is known to heal with the addition of human growth serum, and measurement of the length of the tongue is a method of quantifying healing in this model. There was no significant difference in tongue length between the control (no wash) and saline wash samples but the difference between the LAWS saline treated models and the controls is significant ($P \leq .05$). No acoustically-derived damage to the EFT was seen in histological examinations of the sections.

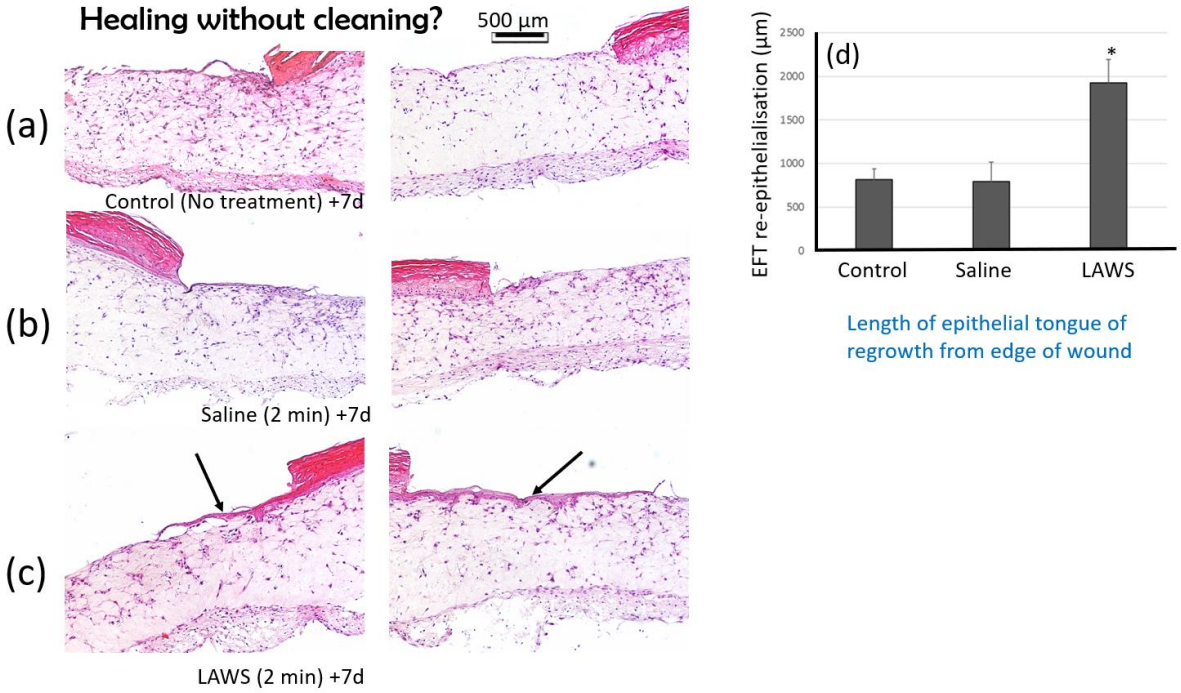

Figure 9. Epiderm full thickness (EFT) wound models that have been wounded (note there is only partial coverage of each sample by the upper (outer) layer), and presented here as Haematoxylin and Eosin (H&E) stained sections from the EFT wound models. The sections are 4µm thick. The wounds were kept clean after wounding, and imaged here after 7 days. The upper row (a) shows two control samples having no treatment. The middle row (b) shows two samples that were treated for 2 minutes after wounding by a saline stream run at 2 L/minute through the device, but without the sound activated. The lower row (c) is treated exactly the same as for row (b), but this time with the sound activated. The black arrows in the micrographs in row (c) highlight the re-epithelialisation tongue observed in these sections. Panel (d) shows data from image analysis measurements (E) of the extent of reepithelialisation 7 days post treatment are shown. Error bars represent the SEM (n = 3), One-Way analysis of variance/Tukey post hoc test demonstrated *$P \leq .05$ when compared with the non-treated controls. Reproduced from Reference 21.

4. CONCLUSIONS

The data presented in this paper show three inventions that transmit acoustic waves down a body of water surrounded by air to cause cleaning, as the acoustic waves excite microbubbles in the stream. The body of water in the first two inventions is a shift film or cushion of water, by which demonstrations were given of cleaning ship hulls and hospital floors. The third invention has a greater stand-off-distance, of around 1 cm, to accommodate the topography and infection control needs associated with a wound. In addition to clear evidence of cleaning, it demonstrated tentative evidence of healing irrespective of biofilm cleaning which is potentially caused by the either changes in growth factors caused by the acoustical signal penetrating the skin, and/or the mechanobiological stimulation (caused by the acoustically excited microbubbles) of the keratinocytes. Further research into the mechanisms of improved healing is currently in progress.

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