Antibiotic-free Treatment of Bacterial Diseases

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Three novel treatments for bacterial diseases show high bactericidal effects, giving hope to a future without antibiotics.

As antibiotic resistance continues to threaten global health, and with the progress of antibiotic discovery having slowed down dramatically in recent years, the demand for alternative methods of treating the ever-growing list of bacterial diseases has become increasingly urgent. In a paper published in Biorxiv in the last quarter of 2015, *Kim & Gaitas* [1] explore three novel methods of eliminating Staphylococcus aureus from blood in vitro. These involve capturing pathogens using an antibody-coated tube, killing pathogens using near infrared light, and killing pathogens using ultraviolet light. The authors investigate the viability of these antibiotic-free techniques as options for the treatment of S. aureus infections and address limitations and future research to improve and expand the application of these methodologies to other bacterial diseases. Fig 1 illustrates the experimental setup.

Staphylococcus aureus is a bacterium responsible for a wide range of diseases including food poisoning and skin and bone infections. Penicillin, which was used to treat S. aureus, was quickly rendered useless as the bacterium evolved to produce penicillinase, an enzyme that breaks the penicillin molecule apart. As a result, penicillinase-resistant antibiotics such as methicillin and oxacillin were developed. Widespread use of these antibiotics led to the emergence of new strains of the bacterium, methicillin-resistant S. aureus (MRSA) and oxacillin-resistant S. aureus (ORSA).

The first technique, called tube capturing, involves capturing – not killing – S. aureus cells using a modified medical tube coated with polyclonal antibodies (pAb). Both ends of the tube are immersed into a solution of whole human blood with S. aureus pathogens added. The blood-bacteria solution circulates through the tube for 5 hours, allowing the pathogens to adhere to the antibodies on the inner surface of the tube, thus removing them from the blood. This technique captured 83.4% of the bacteria. In a paper published in Nature in 2017, *Vinerean et al.* [2] employ the same technique in vivo using rats induced with the S. aureus infection, resulting in a 99.1% capture. They further confirm its feasibility by capturing the food pathogen, *Salmonella typhimurium* , in ground chicken and beef. A second method, photodynamic therapy technique (PDT), requires a conjugate of the polyclonal antibody and a photosensitizer, Chlorin E6 (Ce6). A photosensitizer is a light-sensitive molecule that elicits chemical changes in another molecule. The Ce6-pAb conjugate attaches to the pathogens in the blood-bacteria sample circulating through a modified tube, and is activated by a near infrared light, killing the pathogens. Results of this experiment show a 71.4% reduction in the number of pathogens.

Ultraviolet irradiation is commonly used to kill bacteria and viruses in surgical wounds, drinking water and wastewater treatment. In this experiment, the blood-bacteria sample circulates through a tube passing through an illumination chamber with mirrored walls. UV light illuminates chamber, destroying 61.6% of the

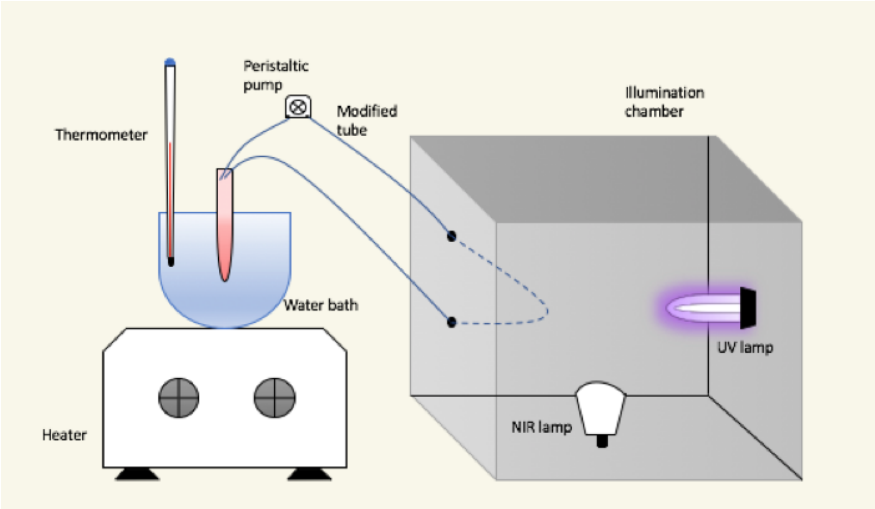

Figure 1: **Figure 1 | Experimental setup.** Test tubes containing the blood sample sit in a temperature-controlled water bath heated to 37°C. A peristaltic pump circulates the blood at constant velocity (0.5mL/min) via a 120cm long and 1.02mm wide medical tube with both ends placed inside the blood sample to complete the circuit. Part of the tube passes inside a temperature-controlled illuminated chamber with mirrored walls to reflect the light. The NIR and UV lamps are located inside the chamber.

pathogens in this case. A plausible explanation for this relatively low result is the absorption of ultraviolet light by haemoglobin in the blood. Haemoglobin absorbs most of the UV light [3], meaning only the pathogens at the surface of the tube get exposed and die. Tube capturing is evidently the most effective out of the three in reducing bacteria count. However, as it does not actually kill bacteria, it would be more beneficial to use it with another technique to prevent repopulation of the uncaptured bacteria. Combining PDT with tube capturing eliminated 87.1% of pathogens, while a UV-tube capturing configuration was more effective, eliminating 89% of bacteria. Using the three methods together was the most successful, reducing the bacteria count by 92%.

Although this research is highly promising, it raises a crucial question. How safe would these techniques be for humans? According to the paper [1], having excess photosensitizer-antibody conjugates in the blood stream pose a potential risk. In the experiment, the sample goes through a wait time where the blood circulates without NIR light for the first 2 hours, giving sufficient time for all the conjugates to bind to pathogens. A similar technique would be followed in humans and animals, allowing enough binding between conjugates and target cells, and the unbound conjugates would be cleared out by the body's natural filtering organs. Further tests are required to determine the appropriate wait time that would maximise efficiency of the process while keeping collateral damage to a minimum. Techniques such as complete blood counts can be carried out to quantify the death of targeted and non-targeted cells.

Despite the widespread application of ultraviolet irradiation for germicidal purposes, health risks such as skin cancer have caused limitations to its use in humans. However, UV light is non-specific, which is advantageous as it clears out most microorganisms. Its effects on other blood components are unknown and would need to be investigated before this technique can be used to kill pathogens in human blood. There is still considerable room for improvement. For instance, additional types of antibodies or binding molecules can be used to create more binding sites and reduce competition. Bioengineering solutions are being developed, which will cater to larger throughputs of blood from humans and other animals. Also, further tests will use binding molecules that target a wide variety of pathogens, thus eliminating the need to identify the pathogen beforehand. Future experiments can extend the use of these techniques to target parasites, fungi, viruses and other microorganisms using suitable binding molecules.

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The significance of this study is immense. Kim & Gaitas' work provides possible solutions to the uncertainty of a future without antibiotics. As resistance spreads more rapidly, it is imperative that we direct our focus to other forms of treatment. These novel techniques would be especially suitable for individuals with antibiotic allergies or immunocompromised patients with diseases such as cancer or HIV. Although many patients may not appreciate the idea of treating bacterial infections by getting wired to a machine while their blood gets pumped through tubes, circumstances may require these seemingly cumbersome treatments in the near future. However, with constant advancements in science and biotechnology, these techniques – and others to come – will eventually be optimized for the suitability of patients.

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