Antimicrobial Photodynamic Therapy
– A Promising Alternative to Treatment of Prosthetic Joint Infections?

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Introduction

Periprosthetic Joint Infection (PJI) is one of the greatest challenges to orthopaedics. It is associated with high patient morbidity and a considerable financial burden to the healthcare system.

Incidence is increasing as arthroplasty is performed on greater numbers of high-risk patients:

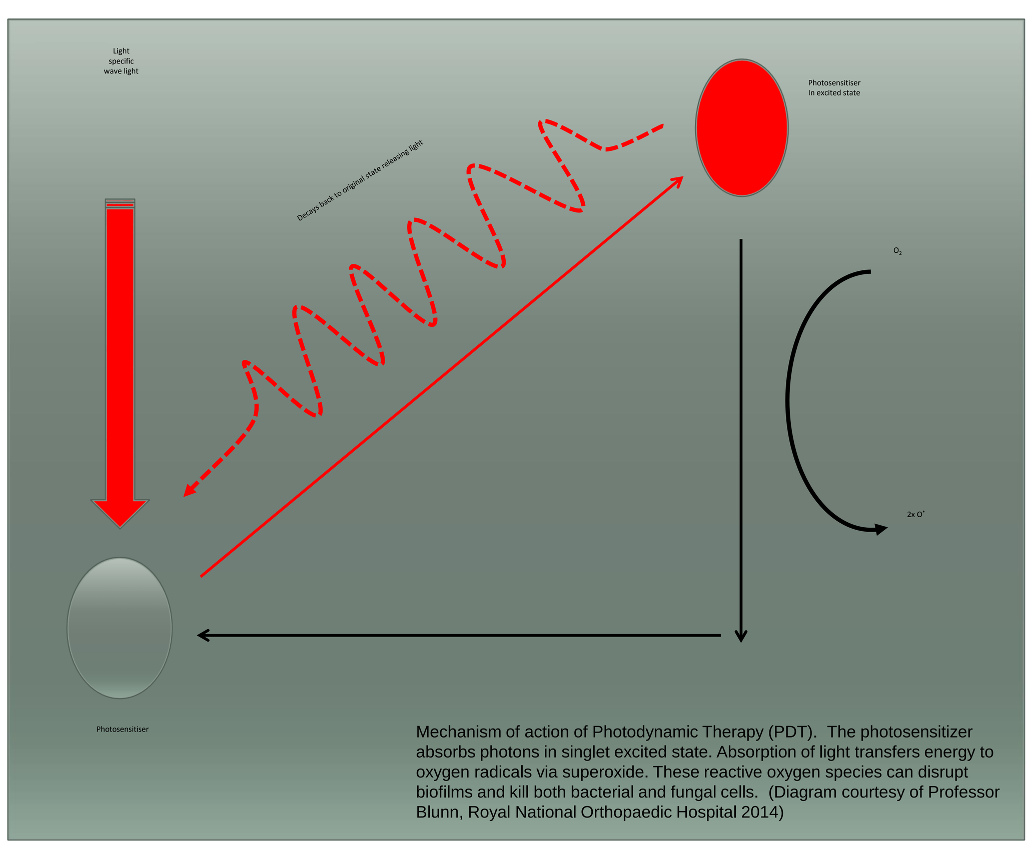
- 0.28% to 4% primary hips infected
- 0.39% to 3.9% primary knees infected
- NJR 2013: 10,040 revision hips – 12% (1222) for infection
  6009 revision knees – 22% (1322) for infection

Thus far preventative measures have not been successful. Current treatments using antibiotics and surgery are long, invasive, and often ineffective. They are also very expensive, exceeding the cost of primary surgery tenfold.

Increasing antibiotic resistance of microorganisms and the inefficacy of antibiotic treatment against biofilms on prosthetic implants means alternative treatments are needed.

Photodynamic Therapy (PDT)

Involves the application of a photosensitiser to a localised area and subsequent exposure to light which activates it and kills targeted cells. It has known antimicrobial qualities providing rapid results with few systemic side-effects and no bacterial resistance.



Aim

Investigate the use of PDT as a means of eradicating strains of bacteria that commonly cause prosthetic joint infections (*methicillin- sensitive staphylococcus aureus (MSSA), methicillin- resistant staphylococcus aureus (MRSA), staphylococcus epidermidis,* and *pseudomonas aeruginosa*) both as planktonic culture and in a biofilm on polished titanium and hydroxyapatite (HA)-coated titanium.

Hypothesis

Photodynamic therapy is an effective means of eradicating common strains of bacteria that cause PJI within planktonic culture, and in a biofilm culture on both polished titanium surfaces and HA-coated surfaces.

Methods

- Cultures of ***S.aureus, MRSA, S.epidermidis*** and ***Pseudomonas aeruginosa*** grown from a single colony
- Photosensitizer = Methylene Blue (MB)
- Light = 655nM laser with lens for dispersion

Pilot Studies

Initial pilot studies were done to determine what concentration of the photosensitizer Methylene Blue (MB), and what laser power was the most effective PDT. In both studies the agar plates underwent qualitative visual analysis to detect the amount of bacteria eradicated.

a)Lawns of all bacteria on agar plates were exposed to PDT with increasing concentrations of MB (0.1mM - 0.5mM) and 15 jcm-2 laser.

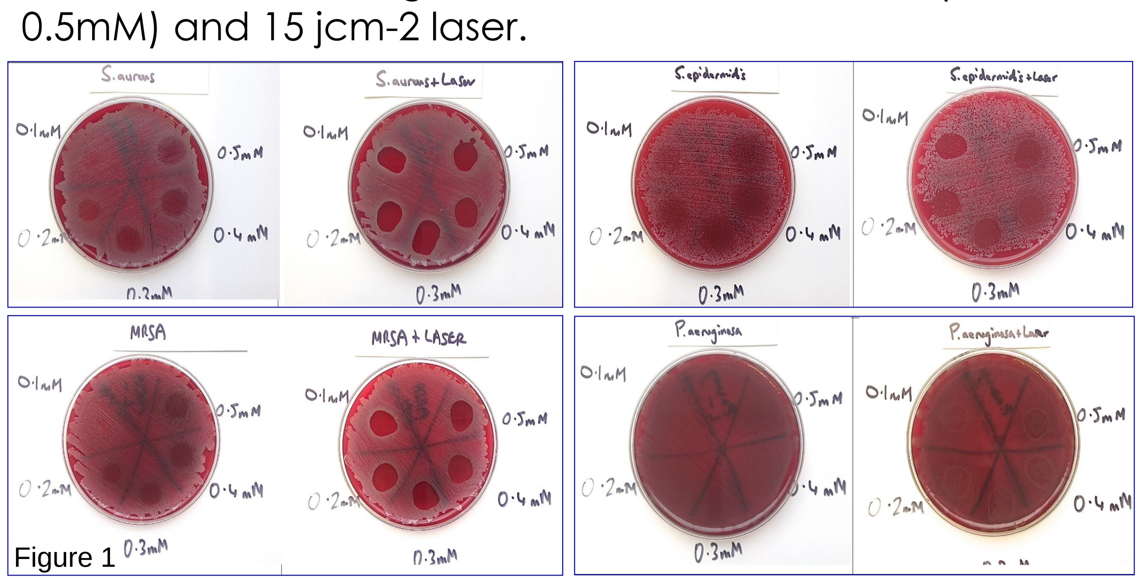


Figure 1

Results demonstrated that PDT is more effective than MB alone for 5 bacteria. All *staphylococci* colony forming units (cfu) were eradicated even at the lowest strength of 0.1mM MB (fig 1). Whilst with *p.aeruginosa* and *a.baumannii,* increasing the MB concentration had an improved bactericidal effect.

b) Lawns of s.*epidermidis* were treated with PDT with 0.3mM MB and increasing laser power (15-45 jcm-2). The higher the power of the laser used in PDT the more bacteria were killed (Fig 2). Laser power ≥35 jcm^{-2} eradicated *s.epidermidis*.

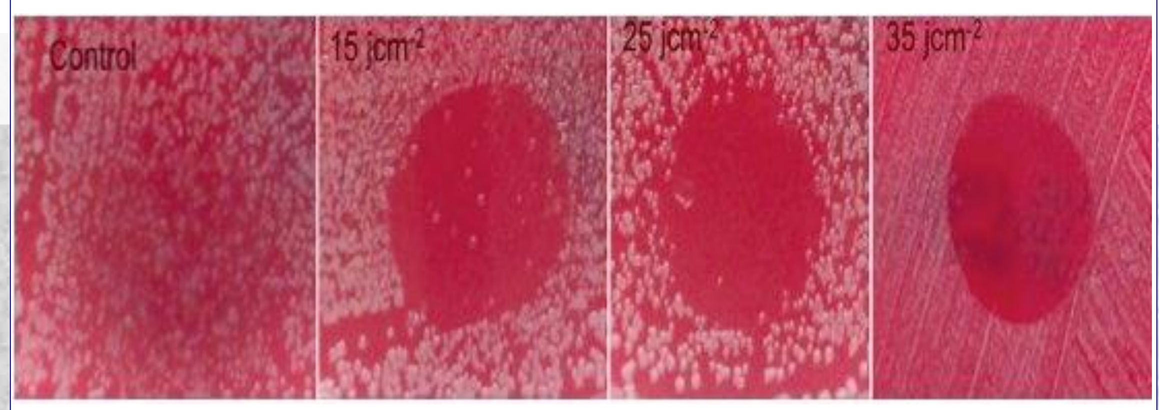


Figure 2: Results show that the higher the power of the laser used in PDT the more bacteria were killed. Laser power ≥35 jcm^{-2} eradicated *s.epidermidis*.

Planktonic Culture Study

The first experiment treated planktonic cultures of 4 bacteria grown in well-plates with 4 treatment regimes (figs 3 and 4) - PDT (MB+L+), photosensitizer alone (MB+L-), laser alone (MB-L+), and control (MB-L) using 0.3mM MB and 35jcm^{-2} laser. Following treatment the wells were analysed for the number of bacteria present using a standard serial dilution technique.

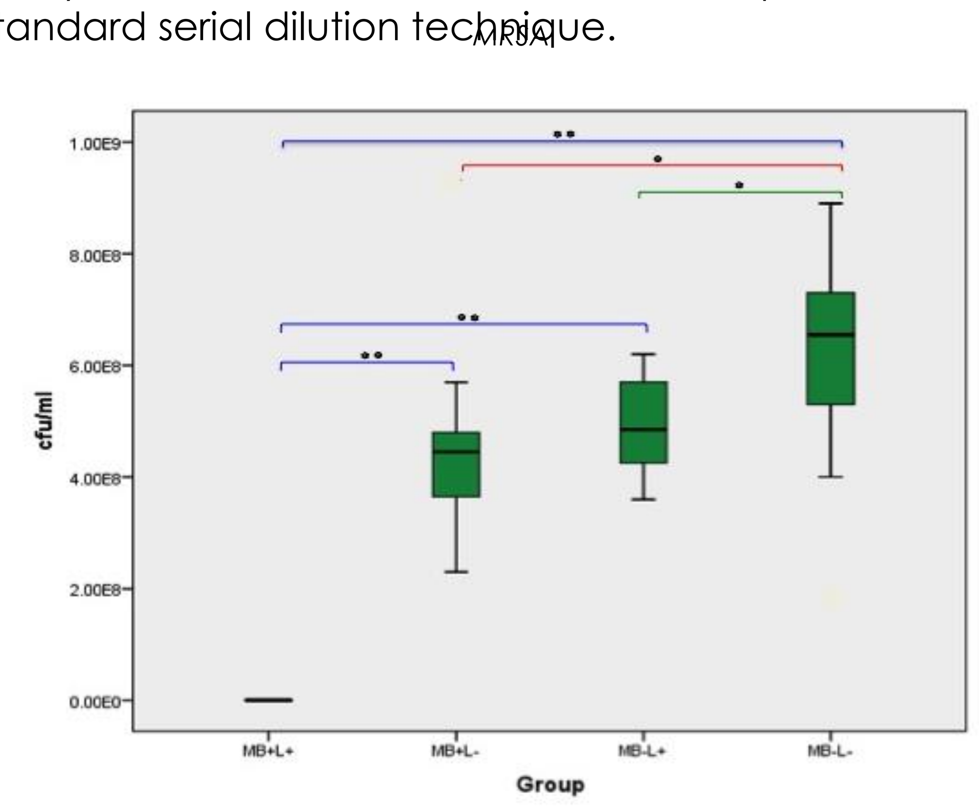


Figure 3: planktonic MRSA bacteria (cfu/ml) remaining within the culture following the different treatments

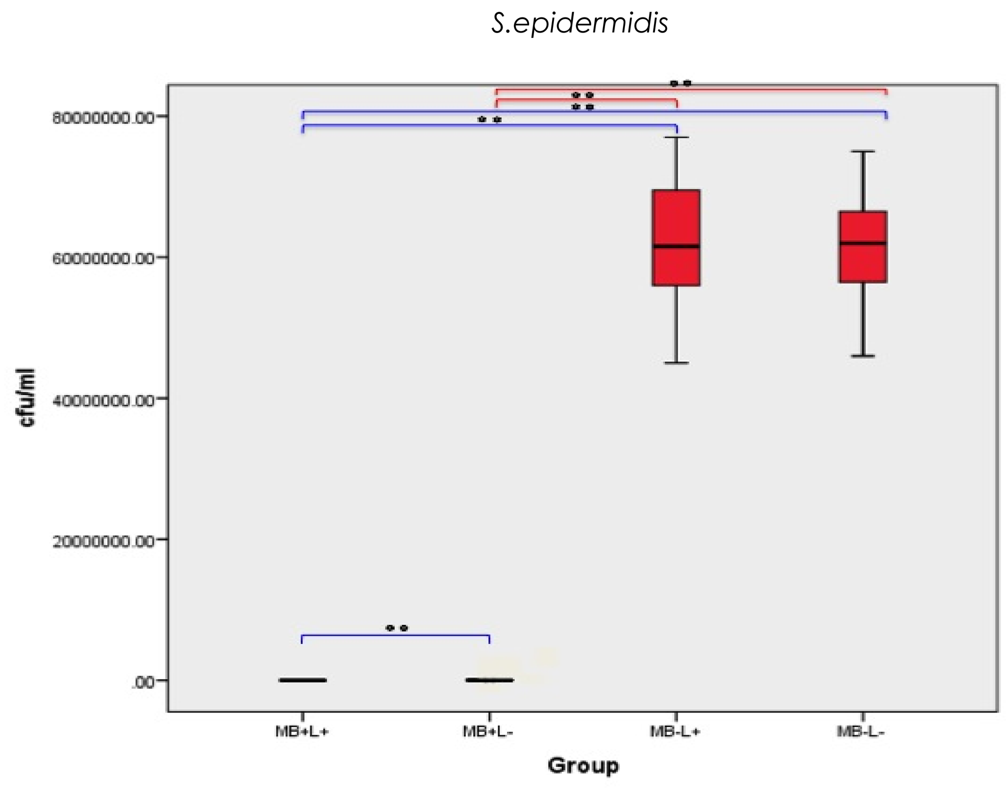


Figure 4: planktonic S.epidermidis bacteria (cfu/ml) remaining within the culture following the different treatments

PDT had a significant bactericidal effect against planktonic *MRSA* and *s.epidermidis* compared to MB alone, laser alone, or the control. MB alone killed more bacteria than the control for both *MRSA* and *s.epidermidis* and more than laser alone for *s.epidermidis*. Laser alone was more effective than the control against *MRSA* but not *s.epidermidis*.

Polished Titanium Biofilm Study

The second experiment investigated the effectiveness of 4 treatment regimes on bacterial biofilms on polished titanium.

- Biofilms were formed for each of the 4 bacteria by placing polished titanium discs in planktonic culture and subjecting them to "oxidative stress"
- Discs removed from "reactor" and washed vigorously with sterile saline leaving the biofilm adherent to the disc surface
- Discs treated with 4 treatment regimes: PDT, MB+L-, MB-L+, MB-L-
- The bacterial biofilms were treated (as per planktonic culture). Following treatment the discs were washed and ultrasonicated to displace biofilm bacteria and standard serial dilution was performed to quantify remaining bacteria (fig 5).

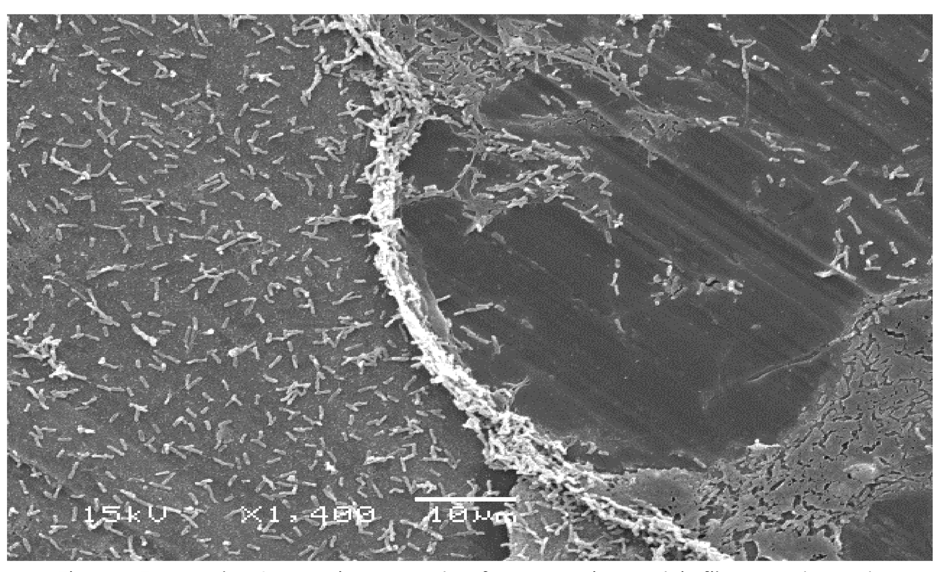


Figure 5: An electron micrograph of p.aeruginosa biofilm produced

PDT was shown to have significantly higher bactericidal effects than MB alone, laser alone, and the control for all four strains of bacteria within biofilms on titanium discs (figs 6 – 8). MB alone killed more bacteria than laser alone and the control for all four strains. Laser alone killed more bacteria than the control for all three of the staphylococci bacteria but not for *p.aeruginosa*.

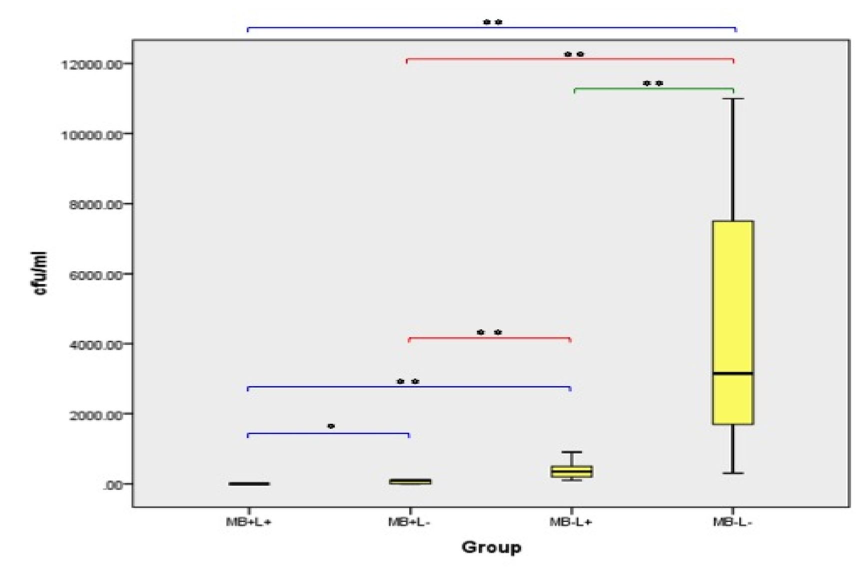


Figure 6: MSSA bacteria (cfu/ml) remaining within the culture following the different treatments

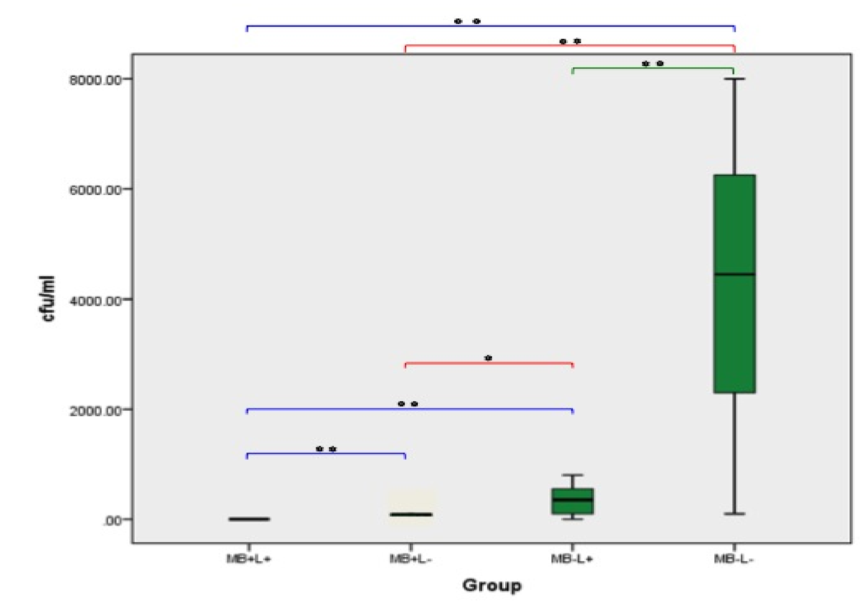


Figure 6: MRSA bacteria (cfu/ml) remaining within the culture following the different treatments

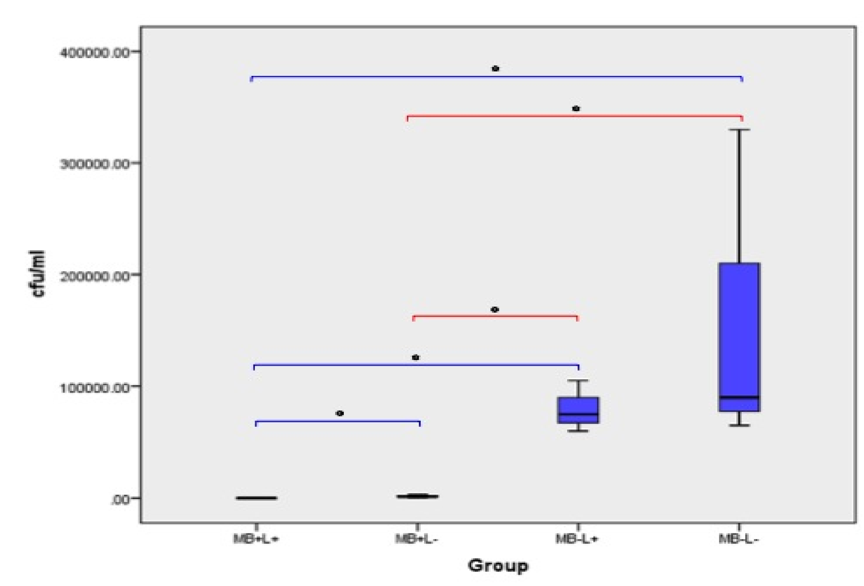


Figure 7: P.aeruginosa bacteria (cfu/ml) remaining within the culture following the different treatments

Hydroyapatite-coated Titanium Biofilm Study

The experiment was then repeated for *p.aeruginosa* biofilms on HA-coated discs.

PDT was shown to have significantly higher bactericidal effects than photosensitiser alone, laser alone and the control against *p.aeruginosa* on HA-coated titanium discs. Photosensitiser alone and laser alone did not significantly eradicate more *p.aeruginosa* bacteria than the control. PDT is less effective against biofilms on HA-coated surfaces (fig 8). The hypothesis can only be partially accepted because bacteria were not eradicated in planktonic *MRSA* or HA-coated *p.aeruginosa* biofilms.

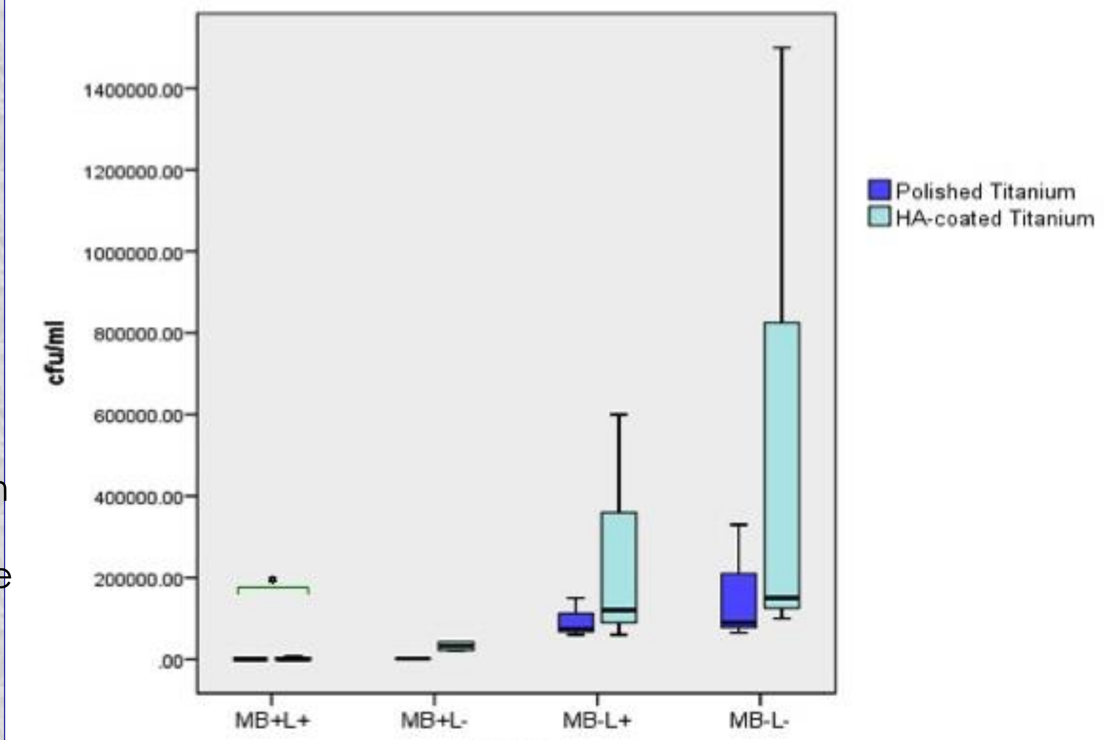


Figure 8: Comparison of the effect of PDT against *p.aeruginosa* biofilms on Polished v HA-coated titanium

Conclusions

- PDT is effective at killing common organisms involved in periprosthetic infection on agar plates, in planktonic culture and in simple biofilms grown *in-vitro*
- PDT may be a significant therapeutic advance in the war on infection in the age of increasing antibiotic resistance
- PDT has the potential to be developed into a surgical treatment that may improve the success rates of single stage revisions and DAIR procedures for infection
- **Future Work** is needed to test the efficacy of PDT across the whole range of causative organisms and in polymicrobial culture.

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