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■ INFECTION

Induction heating for eradicating *Staphylococcus epidermidis* from biofilm

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Aims

Induction heating is a noninvasive, nonantibiotic treatment modality that can potentially be used to cause thermal damage to the bacterial biofilm on the metal implant surface. The purpose of this study was to determine the effectiveness of induction heating on killing *Staphylococcus epidermidis* from biofilm and to determine the possible synergistic effect of induction heating and antibiotics.

Methods

S. epidermidis biofilms were grown on titanium alloy (Ti6Al4V) coupons for 24 hours (young biofilm) and seven days (mature biofilm). These coupons with biofilm were heated to temperatures of 50°C, 55°C, 60°C, 65°C, 70°C, 80°C, and 90°C for 3.5 minutes and subsequently exposed to vancomycin and rifampicin at clinically relevant concentrations.

Results

For the young biofilm, total eradication was observed at 65°C or higher for 3.5 minutes followed by 24 hours of vancomycin 10 mg/l and rifampicin 1 mg/l. For the mature biofilm, total eradication was observed at 60°C for 3.5 minutes followed by 24 hours of vancomycin 10 mg/l and rifampicin 1 mg/l. Total eradication was also observed at 60°C for 3.5 minutes followed by 24 hours of vancomycin 1 mg/l and rifampicin 1 mg/l followed by another thermal shock of 60°C for 3.5 minutes (two thermal shocks).

Conclusion

Induction heating of Ti6Al4V coupons is effective in reducing bacterial load in vitro for *S. epidermidis* biofilms. Induction heating and antibiotics have a synergistic effect resulting in total eradication of the biofilm at 60°C or higher for clinically relevant concentrations of vancomycin and rifampicin.

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Keywords: Infection, Prosthetic joint infection, Total joint replacement, Induction heating, Antibiotic

Article focus

- Non-contact induction heating (NCIH) of metal implants is a noninvasive, non-antibiotic treatment modality that can potentially be used to cause thermal damage to the bacterial biofilm on the metal implant surface.
- We used NCIH to expose biofilms of *Staphylococcus epidermidis* to temperatures of 50°C, 55°C, 60°C, 65°C, 70°C, 80°C, and 90°C for 3.5 minutes and subsequently determine remaining viable bacteria.
- We determined the possible synergistic effect of thermal dose by induction heating

and antibiotics for clinically relevant doses of vancomycin and rifampicin.

Key messages

- NCIH and antibiotics have a synergistic effect (for 60°C and 65°C) resulting in total eradication of the biofilm at 60°C or higher for clinically relevant concentrations of vancomycin and rifampicin.
- The combination of NCIH and antibiotics is the key to full eradication, because neither induction heat nor antibiotics alone were able to achieve full eradication.

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- Fragmentation of the thermal dose is feasible: vancomycin and rifampicin prevent regrowth of the biofilm after the first thermal shock, so that the second thermal shock can eradicate the biofilm completely.

Strengths and limitations

- We applied clinically feasible temperature range, heating duration, and antibiotic concentrations.
- Multiple experiments on 24 hour and seven day biofilms of *S. epidermidis*, which is one of the most commonly isolated pathogens from infected implants.
- Our experiments are in vitro work and may not translate entirely to in vivo situations.

Introduction

Prosthetic joint infection (PJI) is a major problem in both elective orthopaedic and acute trauma surgery. Patients with infected implants will undergo long and extensive treatments consisting of often multiple surgical procedures and antibiotic courses for several months.^{1,2} This treatment is maximally invasive and therefore impossible in patients with high comorbidity factors. Furthermore, increasing antibiotic resistance of bacteria raises concern and limits choices of antibiotics.³⁻⁶ A recent study has uncovered international spread of near pan-drug-resistant *Staphylococcus epidermidis*.⁵ This finding is particularly disturbing because *S. epidermidis* is one of the most commonly isolated pathogens from infected implants.⁷ It is therefore vital that new treatments for the prevention and treatment of biofilm infections in implants are being developed.

Non-contact induction heating (NCIH) of metal implants is a new and emerging treatment for infected metal implants.⁸⁻¹¹ NCIH uses pulsed electromagnetic fields (PEMFs) to induce so-called 'eddy currents' within metal objects which causes them to heat up. This heat can be used to cause thermal damage to the bacterial biofilm on the metal implant hence killing the bacteria and weakening the biofilm. NCIH only actively heats the metal implant and has no direct heating effect on the surrounding tissue, so it can potentially be used as a non-invasive treatment for PJI. Several studies have shown the effectiveness of NCIH of a metal implant in reducing bacterial load in vitro.⁸⁻¹¹ However, it is presently unknown whether NCIH can reduce or eradicate *S. epidermidis* from biofilm on a titanium alloy (Ti6Al4V), which is commonly used for joint implants.

The purpose of this study is to determine the effectiveness of NCIH on killing *S. epidermidis* from biofilm and to determine the possible synergistic effect of NCIH and antibiotics.

Methods

Induction heating and temperature control. *S. epidermidis* biofilms were grown on Ti6Al4V coupons of 38 mm × 25 mm of 1 mm thickness and exposed to a PEMF of 97 kHz

at a maximum of 65 W from a custom build induction heater. Since the type of implant material influences bacterial adhesion and total biofilm burden, we used Ti6Al4V coupons, the main material used in joint implants.¹² The induction heater features a pancake-type coil of nine turns of copper litz wire with an inductance of 12 mH. For non-contact temperature measurement and temperature control, we used a microcontroller board based on the ATmega328 (Arduino Uno; Adafruit Industries, New York, New York, USA) and infrared (IR) temperature sensor (MLX90614; Melexis, Ypres, Belgium). Temperature was recorded four times per second (4Hz) real-time and stored in a data file on a laptop. Figure 1 shows an arrangement of the non-contact induction heater and temperature control system.

Temperature measurement with the IR sensor is affected by emissivity of the surface of, in this case, the Ti6Al4V coupon with biofilm. Emissivity of an object is the ratio of the amount of radiation actually emitted from the surface to that emitted by a blackbody at the same temperature.¹³ To validate the IR temperature measurements a K-type thermocouple was used in order to compare the temperature measured with the IR sensor to the temperature measured with the thermocouple. These measurements were taken after the PEMF had been switched off, because the PEMF may affect the thermocouple's measurements.⁸⁻¹¹ To optimize uniform heating of the coupons we used an IR thermal camera (T440 Thermal Imaging Camera; FLIR Systems, Wilsonville, Oregon, USA) to develop a heating protocol. This protocol was based on a duty-cycle approach to allow the heat to conduct to colder areas on the coupon ensuring more uniform heating (see Figure 1).

Ti6Al4V coupons with biofilm were heated to temperatures of 50°C, 55°C, 60°C, 65°C, 70°C, 80°C, and 90°C for 3.5 minutes. The duration of 3.5 minutes was chosen to allow comparison to previous results of induction heating planktonic microorganisms.¹⁰ Experiments were repeated at least five times unless otherwise indicated.

Biofilm growth and quantification. *S. epidermidis* (American Type Culture Collection (ATCC) 14990) biofilms were grown on Ti6Al4V coupons at 37°C for 24 hours (young biofilm) and seven days (mature biofilm) in a polypropylene container equipped with bacteria filter (1 micron PTFE hydrophobic membrane; Medical Filtration Solutions, Preston, UK) to allow for sterile ventilation. A biofilm was produced by immersing the coupons in 100 ml of growth medium (brain heart infusion (BHI)), inoculated with *S. epidermidis*, for four hours at 37°C to allow adherence, and then transferred into another 100 ml of medium (BHI) and incubated for 24 hours or 300 ml for seven days medium at 37°C.

Prior to thermal shock by the induction heater, the coupons were washed with phosphate-buffered saline (PBS; Fresenius Kabi, Graz, Austria) solution in a Petri dish (Greiner Bio-One, Frickenhausen, Germany) to remove

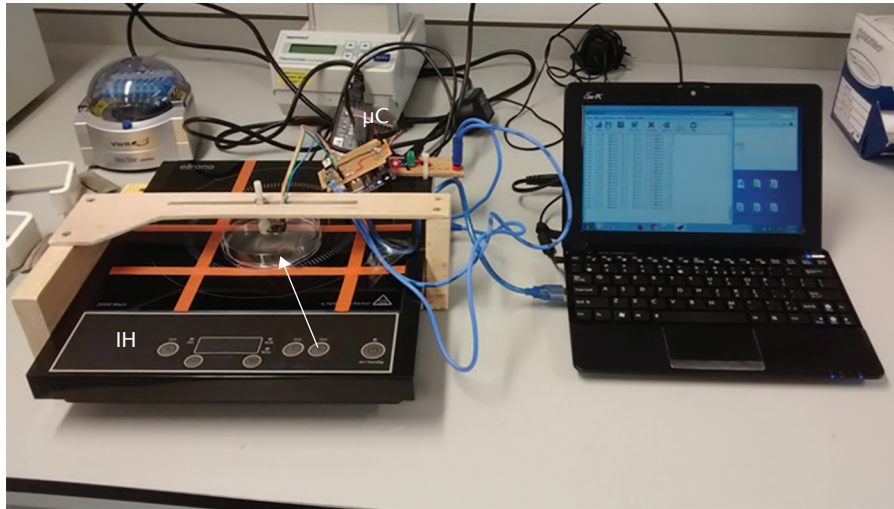


Fig. 1a

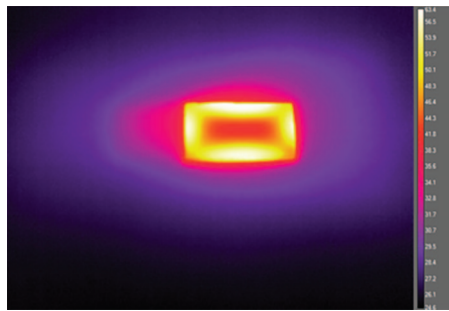


Fig. 1b

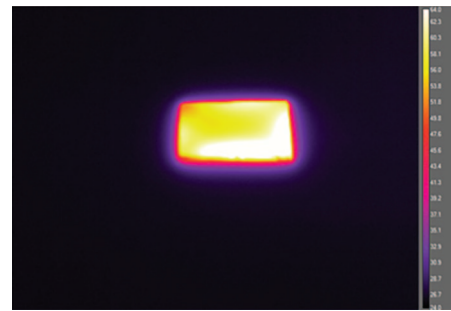


Fig. 1c

a) Photograph of the arrangement of the induction systems and a titanium alloy (Ti6Al4V) coupon in a Petri dish. An arrow shows the Ti6Al4V coupon with biofilm and the infrared temperature sensor directly above it for non-contact temperature measurements. b) Thermal image showing heat distribution before using study heating protocol. c) Thermal image showing heat distribution after using study heating protocol. IH, induction heat for non-contact heating; μ C, micro-controller for temperature control and communication (data logging) with the laptop.

any planktonic bacteria. Subsequently, the coupons were exposed to the thermal shock as described above. After the thermal shock the coupons were washed again with PBS solution in a Petri dish and directly afterwards placed in a 50 ml centrifuge tube with 20 ml PBS solution. This tube, including the coupon, was sonicated (D-78224 Ultrasonic Cleaner; Elma Schmidbauer, Singen, Germany) for five minutes at 35 kHz to dislodge the bacteria from the biofilm into suspension. Afterwards a dilution series of the supernatant was cultured for 48 hours at 37°C in order to determine the colony forming units (CFUs) per cm².

We also included two control conditions without induction heating: these coupons went through all the steps, however the induction heater was not switched on. In a second control condition the coupons also went through all the steps, except for induction heating, and were exposed to 0.5% chlorhexidine with 70% ethanol (C₂H₅OH; both materials produced by Added Pharma, Oss, The Netherlands) for 3.5 minutes.

Heat and antibiotic experiments in 24 hour biofilm model.

To study the possible synergistic effect of antibiotics and induction heating, the Ti6Al4V coupons with biofilm

were also exposed (after induction heating) to high or low concentrations of vancomycin (high = 20 mg/l, low = 10 mg/l) and rifampicin (high = 10mg/l, low = 1 mg/l) for 24 hours at 37°C. The combination of vancomycin and rifampicin was chosen in order to represent clinical practice of adding rifampicin to the antibiotic treatment in case the causative microorganisms are sensitive to rifampicin.¹⁴ After thermal shock by induction heating, instead of proceeding with sonication the coupons were separately placed into another polypropylene container equipped with bacteria filter with 50 ml fresh BHI growth medium containing vancomycin and rifampicin. The coupons were subsequently incubated for 24 hours at 37°C, washed with PBS solution in a Petri dish, placed into a 50 ml centrifuge tube with 20 ml PBS, and sonicated and enumerated as described above. The high antibiotic group received 20 mg/l vancomycin and 10 mg/l rifampicin into the medium, whereas the low antibiotic group received 10 mg/l vancomycin and 1 mg/l rifampicin into the medium. These high and low concentrations were chosen to represent clinically relevant concentrations in plasma.^{15,16} We also included control conditions without

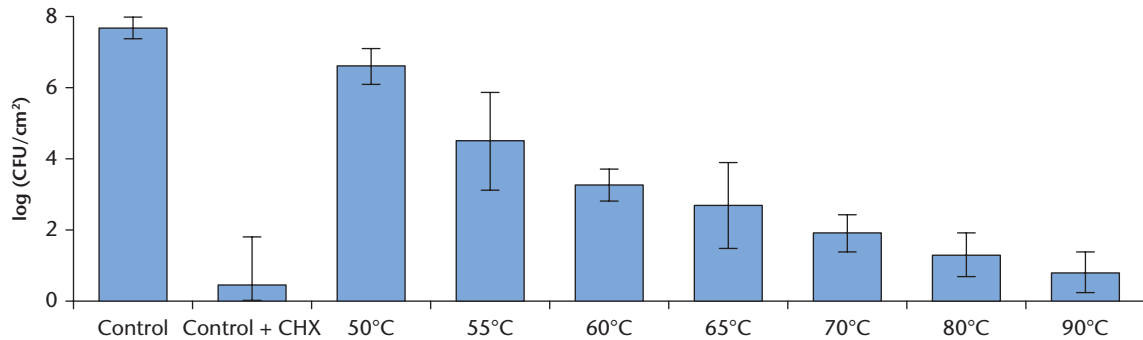


Fig. 2

Graph showing the relationship between temperature exposure and log colony forming units (CFUs) per cm² for 24 hour *Staphylococcus epidermidis* biofilms. The bacteria were exposed to the target temperature for 3.5 minutes. Data are presented as means and corresponding 95% confidence intervals of at least five experiments per group. CHX, chlorhexidine.

induction heating but with the earlier mentioned high or low concentrations of antibiotics.

Heat and antibiotic experiments in seven day biofilm model.

To study the possible synergistic effects of antibiotics and induction heating in a mature biofilm model, we exposed seven day biofilms to thermal shock by induction heating and afterwards to concentrations of vancomycin and rifampicin, which can be expected in the bone.^{17,18} For the seven day model a biofilm was produced by immersing the coupons in 100 ml of growth medium (BHI), inoculated with *S. epidermidis* for four hours at 37°C to allow adherence, and then transferred into 300 ml of medium (BHI) and incubated for seven days at 37°C. During these seven days the medium was not replenished. Apart from using a seven day biofilm model and lower concentrations of vancomycin and rifampicin the experiments were identical to the antibiotic experiments in a 24 hour biofilm model, described above. Experiments were repeated at least four times.

Statistical analysis. Intraclass correlation (ICC) was used to compare the temperature measured with the IR sensor with the temperature measured with the K-type thermocouple (B+B Thermo-Technik, Donaueschingen, Germany). Statistical analyses, when appropriate, were performed using analysis of variance (ANOVA; SPSS version 23, IBM, Armonk, New York). In line with recent recommendations, means and corresponding confidence intervals (CIs) are reported, while p-values are not reported.¹⁹

Synergy between thermal shock and antibiotics was defined as > 2 log decrease in CFUs/cm² between the combination and its most active constituent.^{20,21}

Results

The ICC of the temperature measured with the IR sensor and the temperature measured with the K-type thermocouple is 0.99 (95% CI 0.99 to 1.00), indicating near perfect agreement.

The 24 hour biofilm model. In the control group without induction heating, 4.7×10^7 CFUs/cm² were observed (total of 26 experiments). For induction heating alone reductions of 1.0 log, 3.2 log, 4.4 log, 5.0 log, 5.8 log, 6.4 log, and 6.9 log in CFUs/cm² were observed for 50°C, 55°C, 60°C, 65°C, 70°C, 80°C, and 90°C, respectively (ANOVA analysis). In the chlorhexidine group a reduction of 7.2 log in CFUs/cm² was observed (Figure 2).

Without induction heating there were 467 CFUs/cm² in the high antibiotic group and 147 CFUs/cm² in the low antibiotic group, which signify 5 log and 5.5 log reductions for the high and low antibiotic groups, respectively, compared to the control without antibiotics. For the high antibiotic group there was a 6.8 log reduction for 50°C and a 7.5 log reduction for 60°C. Total eradication was observed at 65°C or higher (Figure 3).

For the low antibiotic group there was a 5.6 log reduction for 50°C and a 7.2 log reduction for 60°C. Total eradication was observed at 65°C or higher (Figure 4).

Regarding antibiotic concentration and heat, only for 50°C was there a difference in reduction between the high and low antibiotic groups: 6.8 log reduction for the high group and 5.6 log reduction for the low group since their CIs did not overlap (Figure 4).

There was a synergistic effect between the antibiotics and thermal shock for 60°C and 65°C, since the combination had more than 2 log reduction compared to the most active constituent. For 50°C the effect was additive and for 90°C synergy could not be evaluated because fewer than 2 log CFUs/cm² were left after 90°C heating for 3.5 minutes.

The seven day biofilm model. In the control group without induction heating, 1.3×10^8 CFUs/cm² were observed (total of 14 experiments). After exposure to antibiotics for 24 hours (no heat) there was a reduction of 7.6 log CFUs/cm² for vancomycin 10 mg/l and rifampicin 1 mg/l (ANOVA analysis; Figure 5). There was no reduction for vancomycin 1 mg/l and rifampicin 1 mg/l or vancomycin

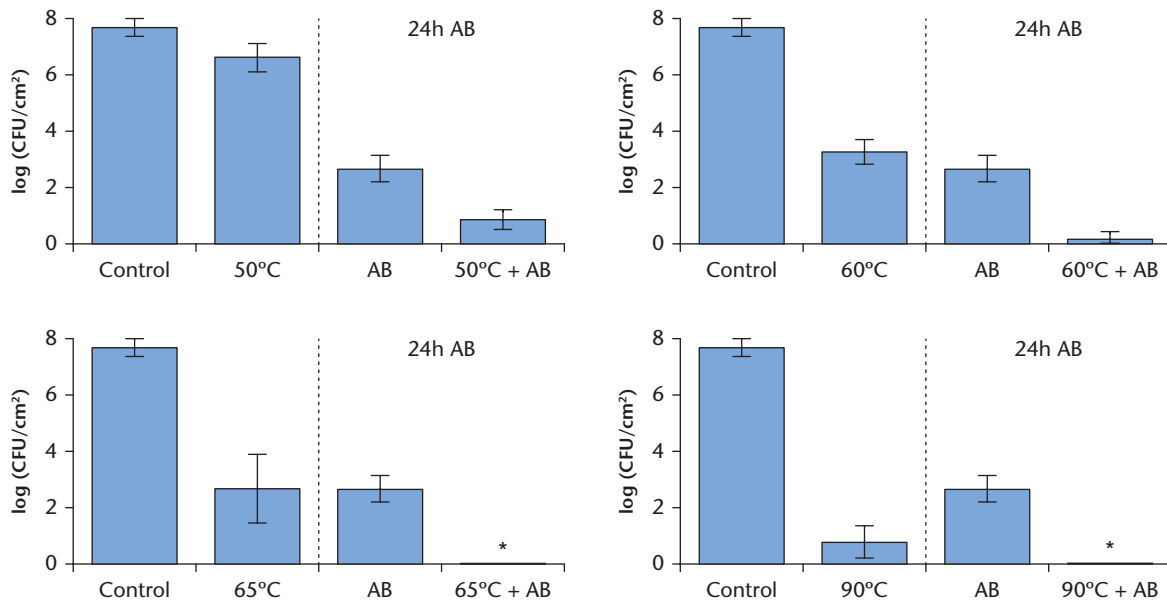


Fig. 3

Graphs showing log colony forming units (CFUs) per cm² for 24 hour *Staphylococcus epidermidis* biofilms with (24h AB) and without antibiotics after thermal exposure. The bacteria were exposed to the target temperature for 3.5 minutes. Data are presented as means and corresponding 95% confidence intervals of at least five experiments per group. AB, vancomycin 20 mg/l and rifampicin 10 mg/l for 24 hours after thermal shock from induction heater. *Full eradication.

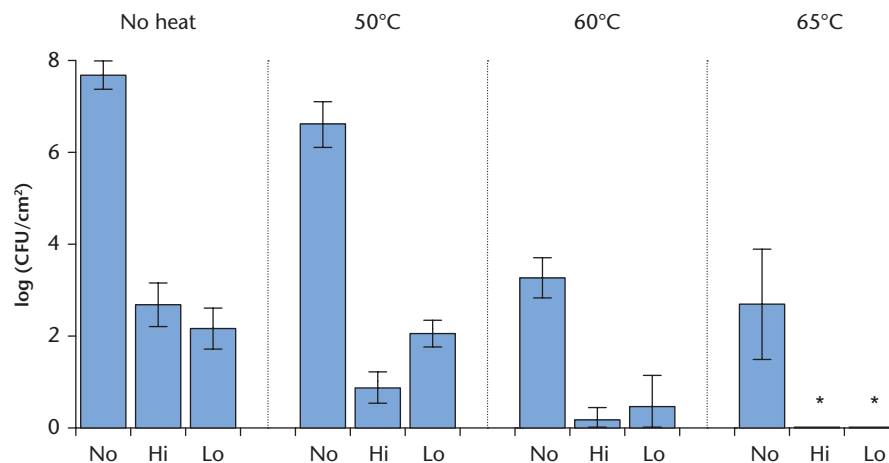


Fig. 4

Graph showing the log colony forming units (CFUs) per cm² for 24 hour *Staphylococcus epidermidis* biofilms with no antibiotics, and high and low concentrations of antibiotics after thermal exposure. The bacteria were exposed to the target temperature for 3.5 minutes. Data are presented as means and corresponding 95% confidence intervals of at least five experiments per group. Plating was performed immediately after the thermal shock. No, no antibiotics, only thermal shock; Hi, thermal shock followed by high concentration antibiotics: vancomycin 20 mg/l and rifampicin 10 mg/l for 24 hours; Lo, thermal shock followed by low concentration antibiotics: vancomycin 10 mg/l and rifampicin 1 mg/l for 24 hours. *Full eradication.

1 mg/l and rifampicin 0.1 mg/l compared to the control group (ANOVA analysis; Figure 5).

With induction heating (no antibiotics) at 60°C during 3.5 minutes and one minute, reductions of 6.7 log and 5.2 log in CFUs/cm² were observed, respectively (ANOVA analysis).

With induction heating at 60°C during 3.5 minutes followed by vancomycin 1 mg/l and rifampicin 1 mg/l for 24 hours, a 6.8 log reduction in CFUs/cm² was observed. For 60°C during 3.5 minutes followed by vancomycin

1 mg/l and rifampicin 0.1 mg/l for 24 hours, growth of 2.9 log was observed.

No viable bacteria were detected after 60°C for 3.5 minutes followed by 24 hours of vancomycin 10 mg/l and rifampicin 1 mg/l (Figure 6). No viable bacteria were detected after 60°C for 3.5 minutes followed by 24 hours of vancomycin 1 mg/l and rifampicin 1 mg/l followed by another thermal shock of 60°C for 3.5 minutes (total of two thermal shocks; Figure 6).

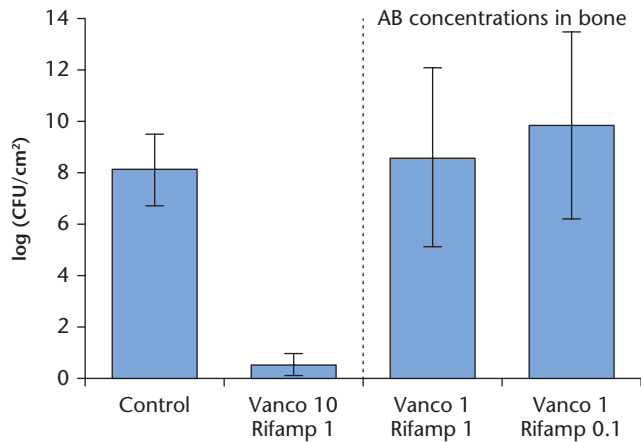


Fig. 5

Graph showing the log colony forming units (CFUs) per cm² for seven day *Staphylococcus epidermidis* biofilms without thermal exposure. Data are presented as means and corresponding 95% confidence intervals of at least four experiments per group. Vanco 10, vancomycin 10 mg/l; Vanco 1, vancomycin 1 mg/l; Rifamp 1, rifampicin 1 mg/l; Rifamp 0.1, rifampicin 0.1 mg/l.

For 60°C synergy could not be evaluated because fewer than 2 log CFUs/cm² were left after 60°C heating for 3.5 minutes.

Discussion

The results of our study show that induction heating of titanium coupons is effective in reducing bacterial load in vitro for *S. epidermidis* biofilms. Induction heating and antibiotics have a synergistic effect (for 60°C and 65°C) resulting in eradication of the biofilm at 65°C or higher for both high and low concentrations of vancomycin and rifampicin for 24 hour biofilms. In the seven day biofilm

model eradication was observed for 60°C followed by vancomycin 10 mg/l and rifampicin 1 mg/l. Eradication was also observed for 60°C followed by vancomycin 1 mg/l and rifampicin 1 mg/l (to represent concentrations that can be expected in the bone),^{17,18} followed by a second 60°C thermal shock. A possible explanation for the prevention of regrowth of the biofilm after the first thermal shock could be the presence of vancomycin and rifampicin in the medium. A second thermal shock was able to eradicate the biofilm completely.

The observed synergistic effect between heat and antibiotics is in accordance with Hajdu et al,²² who have shown enhancement of the antibacterial activity of antimicrobial agents against staphylococcal biofilms by increasing the ambient temperature. Ricker and Nuxoll²³ have also demonstrated this synergistic effect of antibiotics and heat in a *Pseudomonas* film for erythromycin, tobramycin, and ciprofloxacin. Both Hajdu et al²² and Ricker and Nuxoll²³ used heat conduction rather than induction heating.

Regarding hyperthermia treatment alone, Pavlovsky et al²⁴ have shown a 2 log reduction in CFUs/cm² for *S. epidermidis* biofilm after 60 minutes exposure to 60°C. Our 4.4 log reduction for 60°C is significantly higher than the 2 log reduction in CFUs/cm² observed by Pavlovsky et al.²⁴ Also our heating time of 3.5 minutes was less than the 60 minutes of heating conducted by Pavlovsky et al.²⁴ Thus with considerably lower thermal dose we achieved higher reduction (4.4 log vs 2 log). A reason may be the applied PEMF. The PEMF together with eddy currents at the implant (titanium coupon) surface may interfere with the transport of charged molecules within the bacteria, possibly making them more susceptible to thermal shock.

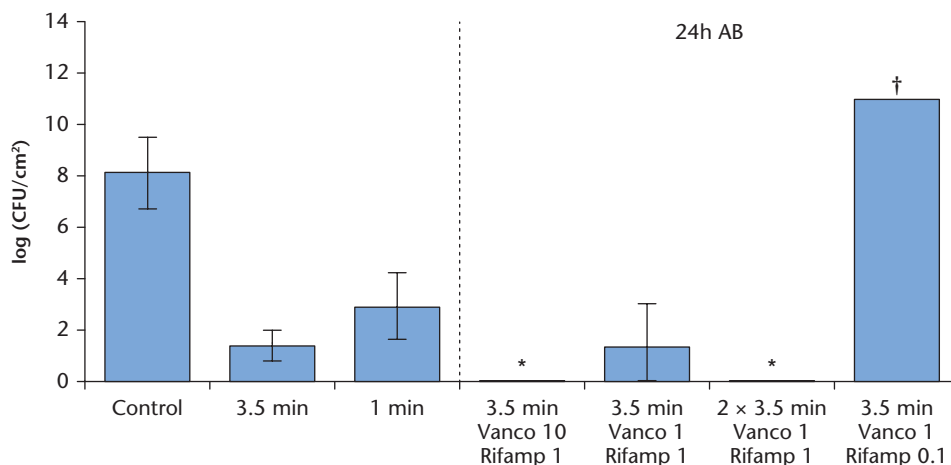


Fig. 6

Graph showing the log colony forming units (CFUs) per cm² for seven day *Staphylococcus epidermidis* biofilms. Data are presented as means and corresponding 95% confidence intervals of at least four experiments per group (except n = 3 for the group: two times 3.5-minute heating, vancomycin 1 mg/l, and rifampicin 1 mg/l). *Full eradication. †CFUs could not be counted due to very large numbers. AB, vancomycin from 1 mg/l to 10 mg/l and rifampicin from 0.1 mg/l to 1 mg/l for 24 hours after thermal shock from induction heater; 3.5 min, thermal shock of 60°C for 3.5 minutes; 1 min, thermal shock of 60°C for one minute; 2 x 3.5 min, thermal shock of 60°C for 3.5 minutes, followed by 24 hours of antibiotics and subsequently followed by a second thermal shock of 60°C for 3.5 minutes (plating was performed directly after second thermal shock); Vanco 10, vancomycin 10 mg/l; Vanco 1, vancomycin 1 mg/l; Rifamp 1, rifampicin 1 mg/l; Rifamp 0.1, rifampicin 0.1 mg/l.

Furthermore the direction of heat is different: with induction heating the heat originates at the biofilm-implant interface and then travels into the biofilm, whereas with externally applied heat (such as by Pavlovsky et al),²⁴ the heat travels into the biofilm starting at the outer border of the biofilm and ending at the biofilm-implant interface.

While a 5 log reduction can be achieved on a mature biofilm with a thermal dose of 60°C for one minute, full eradication necessitates a thermal dose of 60°C to 65°C for 3.5 minutes. Although this may seem high, such a thermal dose is not uncommon in orthopaedic surgery, for example with cementing, drilling, and using diathermia.^{25–27} There are also animal experiments that confirm the lack of clinically relevant necrosis after induction heating up to 60°C to 65°C. Müller et al²⁸ heated a nickel-titanium shape memory rod in the femur of rats at 40°C to 60°C using induction heating and found no evidence of necrosis of the surrounding bone and tissues. They also heated an osteosynthesis plate in a rabbit model with induction heating and noted that all osteotomies underneath the plate healed.²⁹ Chopra et al⁹ have shown in a mouse model that thermal damage is confined to a localized region (< 2 mm) around the implant. Fang et al³⁰ heated metal implants in a rat model to 75°C without any significant thermal damage on the surrounding tissue. These studies are in agreement with Samara et al,³¹ who have shown that bone cement achieves durable fixation despite the temperature reaching 80°C for more than ten minutes, caused by the curing process of the cement. Furthermore special heating techniques, such as segmental induction heating, can be used to apply localized heating of a segment of an implant using the remainder of the implant as heat sink and avoiding damage to vital areas of the implant necessary to maintain fixation.¹¹

We should note some limitations. Regarding non-contact temperature control, an IR sensor such as the one used in our experiments cannot be readily used in clinical situations because of the absence of a direct line of site: there is tissue and bone between the implant and the IR sensor. However, there already exist noninvasive temperature safety systems relying on different mechanisms. Cheng et al⁸ developed remote acoustic sensing. This method uses remote acoustic sensors to detect sounds associated with boiling on the implant surface.⁸

Also, our experiments are in vitro work and may not translate entirely to in vivo situations. Physiological and molecular effects of hyperthermia are not accounted for. Localized hyperthermia has been shown to increase the blood flow and vessel permeability, activate the immune system, and increase the fluidity and permeability of membranes, all of which are helpful in combatting PJI.³²

The results of our study show that there are two ways to eradicate mature *S. epidermidis* biofilms from Ti6Al4V surface in vitro. First, thermal shock of 60°C followed by antibiotics with concentrations relatively high for bone

(vancomycin 10 mg/l and rifampicin 1 mg/l). In clinical practice this could be achieved by high local levels of antibiotics released by the implant coating or other carriers triggered by, for example, the thermal shock. Second, thermal shock of 60°C followed by antibiotics at concentrations achieved in bone followed by a second thermal shock of 60°C. In clinical practice this could be achieved by intravenous or oral administration of antibiotics during or directly after noninvasive induction heating, followed by multiple thermal shocks by induction heating if required or by using heat-triggered carriers.³³ Since neither induction heat nor antibiotics were able to achieve full eradication on their own, a multimodality treatment of PJI would likely be most successful. Thermo-microbiology and thermo-infectiology are emerging fields and induction heating may have an important future role in the multimodality treatment of PJI together with, for example, antibiotics, surgery, peptides, phage therapy, surface therapies, and local radiation.^{34–39}

In conclusion, induction heating of Ti6Al4V coupons is effective in reducing bacterial load in vitro for *S. epidermidis* biofilms. Induction heating and antibiotics have a synergistic effect (for 60°C and 65°C) resulting in total eradication of the biofilm at 60°C or higher for clinically relevant concentrations of vancomycin and rifampicin.

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Ethical review statement

- This study did not require ethical approval.

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