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Betreff: Rückfrage zur Abrechnung der Personalkosten im Rahmen unserer Biofilm-Studie

Sehr geehrte Frau Holtemeyer,

Sehr geehrte Damen und Herren,

wir hätten eine Bitte hinsichtlich der Förderung unserer Biofilm-Studie, da es zu einer internen Misskommunikation mit unserer Drittmittelstelle bezüglich der Abrechnung der Personalkosten gekommen ist. Gern möchte ich den Sachverhalt kurz schildern:

Von den insgesamt ca. 6.500 € für die veranschlagten Personalkosten habe ich über die ProImplant Foundation meine Kollegin Frau Svetlana Karbysheva als supervidierende Kollegin für unseren Studenten eingebunden. Zu diesem Zeitpunkt war Frau Karbysheva nicht an der Charité angestellt, sondern wurde im Rahmen der Supervision und mit ihrem fachlichen Know-how über die ProImplant Foundation eingebunden.

Da unsere studentische Hilfskraft ein Vollstipendium erhält, Frau Karbysheva jedoch einen erheblichen zeitlichen Aufwand neben ihrer eigentlichen Tätigkeit hatte, wurde diese Lösung gewählt. Nach Rücksprache mit Ihnen war es dabei unerheblich, ob die Personalmittel für den Studenten oder für Frau Karbysheva verwendet werden – entscheidend sei lediglich, dass es sich um Personalkosten handelt.

Nun möchten wir die entsprechende Rechnung der ProImplant Foundation für Frau Karbysheva (siehe Anhang) begleichen. Unsere Drittmittelstelle vertritt jedoch die Auffassung, dass es sich

hierbei nicht um Personalkosten handele, da kein Arbeitsvertrag mit der Charité besteht und die Abrechnung über die Foundation erfolgt. Daher soll die Ausgabe intern als Sachkosten verbucht werden – was nach meinem Kenntnisstand in der Abrechnung mit der Stiftung zu Problemen führen könnte. Leider wurde ich im Vorfeld von der zuständigen Kollegin der Drittmittelverwaltung nicht auf diesen Punkt hingewiesen, sonst hätten wir das Problem proaktiv thematisiert oder eine Alternativlösung angestrebt.

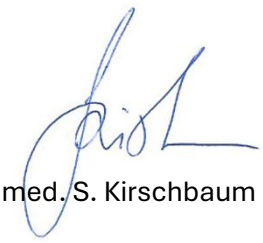
Die Thematik wurde bereits mit Prof. Beckmann besprochen, der in diesem Zusammenhang empfahl, die Position gegebenenfalls als Sachkosten umzudeklarieren, falls dies aus Sicht der Stiftung erforderlich oder zweckmäßiger wäre.

Daher meine Rückfrage: Ist es für die Stiftung problematisch, wenn eine Rechnung der ProImplant Foundation mit ausgewiesenen „Personalkosten“ eingereicht wird, auch wenn unsere Drittmittelstelle diese intern anders verbucht?

Falls ja – wäre eine nachträgliche Umdeklarierung in „Sachkosten“ unter den genannten Umständen möglich?

Ich danke Ihnen herzlich für Ihre Unterstützung und Ihre Rückmeldung. Wir stehen für Rückfragen jederzeit gerne unter *stephanie.kirschbaum@charite.de* oder 0176 47 037 644 zur Verfügung und verbleiben

Mit freundlichen Grüßen,



Dr. med. S. Kirschbaum

Bone & Joint Research

Biofilm Under Attack: Ultrasound Toothbrushing as a Potential Intraoperative Biofilm-Removal Strategy

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Keywords:	biofilm eradication, periprosthetic joint infection, ultrasound

1. Abstract

Aims Successful debridement, antibiotics, and implant retention (DAIR) requires near-complete biofilm removal from retained components to prevent infection recurrence. This study evaluated the efficacy of chemical and mechanical treatment options Polyhexanide (PHMB), mechanical brushing, ultrasonic toothbrushing, and sonication – alone and in combination – for eradicating biofilms from orthopedic implant materials.

Methods Biofilms of *Staphylococcus epidermidis* (ATCC 35984) and *Escherichia coli* (ATCC 25922) were grown on Ti-6Al-4V, CoCrMo, and highly cross-linked polyethylene (PE) discs. Treatments included Polyhexanide (PHMB) (0.0004–0.04%), mechanical toothbrushing (MT), ultrasonic toothbrushing (UT) and sonication (Soni) applied individually or combined with PHMB to each biofilm colonized disk for 1 minute. Treatment efficacy was assessed using colony-forming unit (CFU) enumeration, isothermal microcalorimetry (IMC) and scanning electron microscopy (SEM).

Results PHMB at 0.02–0.04% completely suppressed metabolic activity of *S. epidermidis* and *E. coli* biofilms on Ti64 and CoCrMo, and significantly delayed regrowth on PE. Lower concentrations (0.004–0.0004%) exhibited dose-dependent inhibition. CFU analysis detected no viable bacteria in any PHMB-treated samples. All combination therapies (PHMB + Soni, PHMB + MT, PHMB + UT) achieved complete metabolic suppression and eradication of biofilms on all tested materials. SEM confirmed surface clearance and biofilm disruption.

Conclusion The combination of PHMB with an ultrasonic toothbrush represents a rapid, cost-effective and intraoperatively compatible mechanical–chemical approach, offering a promising and practical decontamination strategy for eradication of *S. epidermidis* and *E. coli* biofilms from orthopedic implant materials.

Keywords: polyhexanide; biofilm eradication, periprosthetic joint infection; ultrasonic toothbrushing; DAIR

Article summary

Article focus: The aim of this in vitro study was to evaluate the efficacy of different potential treatment options of typical DAIR procedure for the eradication of *Staphylococcus epidermidis* and *E.coli* biofilms relevant arthroplasty biomaterials (highly cross-linked polyethylene (PE), titanium alloy (Ti64), and cobalt–chromium–molybdenum alloy (CoCrMo)). The findings are expected to contribute to improving in vivo biofilm removal and, ultimately, to enhance the success rate of DAIR procedures in the future.

Key messages: Effective biofilm eradication on orthopedic materials requires a dual chemical-mechanical approach. PHMB provides strong antiseptic activity against detached bacteria but limited biofilm penetration, especially on PE, whereas ultrasonic brushing enhances mechanical disruption and adds bactericidal effects.

Strengths and limitations of this study: A limitation is the in vitro design of the study. Strength the testing of two different microbiological strains as well as the feasibility to transfer these in vitro treatments in intraoperative setting.

2. Introduction

Infection persistence after treatment of Periprosthetic Joint Infection (PJI) is largely driven by resilient biofilms on implant surfaces, which shield pathogens from immune defenses and markedly reduce antimicrobial susceptibility, making eradication difficult even with thorough debridement and irrigation.[1] Especially the success rate of the "debridement, antibiotics, and implant retention" (DAIR) procedure for acute PJI varies widely in the literature (11–88%), depending on symptom duration (reflecting biofilm maturity) and pathogen.[2-4] *Staphylococcus* species, the most common pathogens in PJI, are associated with a particularly high DAIR failure rate, up to three-fold higher than infections caused by *Streptococcus spp.* or *E. coli.*[5-8] Moreover, exchange of mobile components seems to increase success rate by improving surgical access for debridement and reduction of the intra-articular bacterial load as highly cross-linked polyethylene in particular exhibits rapid and extensive bacterial adhesion within 4 - 48 hours.[9-13] In clinical practice, however, DAIR is often performed as emergency procedure and compatible components may not always be immediately available. Moreover, biofilms on retained metallic components remain vulnerable to persistent adherent biofilm. For those reasons, intraoperative strategies such as sonication or antiseptic irrigation have been explored but are limited in efficacy, safety or intraoperative practicality by now.[14-16] Polyhexanide (PHMB) is a broad-spectrum antimicrobial with antibiofilm activity and lower cytotoxicity than povidone-iodine or

hypochlorite, already widely used in wound care, yet its intraoperative potential for biofilm elimination remains unknown.[14] Translational approaches adapted from dental hygiene have gained attention: mechanical brushing has reduced reoperation rates and accelerated normalization of inflammatory markers in spinal implant infections and ultrasonic toothbrushing (UT), combining mechanical and cavitational forces, showed better outcome concerning dental biofilm removal.[17, 18] Nevertheless, UT has not yet been evaluated in arthroplasty or PJI contexts.

Therefore, the aim of this in vitro study was to evaluate the efficacy of PHMB, mechanical brushing (MT), ultrasonic toothbrushing and sonication for the eradication of *Staphylococcus epidermidis* and *E.coli* biofilms relevant arthroplasty biomaterials (highly cross-linked polyethylene (PE), titanium alloy (Ti64), and cobalt–chromium–molybdenum alloy (CoCrMo)). The findings are expected to contribute to improving in vivo biofilm removal and, ultimately, to enhance the success rate of DAIR procedures in the future.

3. Materials and Methods

Bacterial strains and biofilm formation

Biofilms were established using *Staphylococcus epidermidis* (ATCC 35984) and *Escherichia coli* (ATCC 25922). Bacteria were grown on 6-mm Ti64, CoCrMo and PE discs (Waldemar Link GmbH & Co. KG, Hamburg, Germany). Discs were incubated in 2 mL brain heart infusion broth (BHIb; Sigma-Aldrich, St. Louis, MO, USA) inoculated with 1×10^5 colony-forming units (CFU)/mL at 37 °C for 24 hours, transferred to fresh BHIb and incubated for another 72 hours to allow biofilm maturation. Following incubation, discs were washed six times with sterile phosphate-buffered saline (PBS) to remove non-adherent bacteria. Strains were preserved at - 80 °C using Microbank™ cryovials (Pro-Lab Diagnostics, Canada).

Biofilm Treatment Procedures

The following treatments were evaluated individually and in combination with polyhexanide (PHMB, SERASEPT® 2, SERAG-WIESSNER GmbH & Co. KG, Naila, Germany): sonication (Soni, BactoSonic system, BANDELIN electronic, Berlin, Germany), mechanical toothbrushing (MT; WLLHYF, China) and ultrasonic toothbrushing (UT; MEGASONEX M8, Goldspire Group Limited, PRC). All treatments were applied for 1 minute. PBS-washed discs served as negative controls. Experiments were performed in triplicate.

To simulate intraoperative dilution effects, serial PHMB concentrations (0.04%, 0.02%, 0.004%, 0.0008%, 0.0004%) were tested to determine a sub-eradicating "working concentration" for use in combination treatments. For subsequent experiments, 0.004% PHMB (Ti64, CoCrMo) and 0.04% PHMB (PE) were selected.

Sonication was performed using a modified protocol adapted from previously published methods.[15, 19] Briefly, biofilm discs were immersed in 1 mL PBS, vortexed 30 s, sonicated at 40 kHz and power density at 100% output for 1 minute using the BactoSonic (BANDELIN electronic GmbH & Co. KG, Berlin, Germany), and vortexed again for 30 s. This modified protocol yielded improved biofilm detachment compared to conventional sonication and was employed for all further experiments (data not shown).

Treatment efficacy was assessed through quantitative colony-forming unit (CFU) enumeration, isothermal microcalorimetry (IMC) and scanning electron microscopy (SEM) to provide complementary insights into bacterial viability and biofilm structure.

CFU Enumeration

CFU analysis quantified viable bacteria detached by each treatment. Serial dilutions of the post-treatment suspension (100 µL) were plated on Tryptic Soy Agar (TSA) (Sigma-Aldrich, St. Louis, MO, USA) and incubated for 24 h at 37 °C. The detection range was 0–10^8 CFU/mL.

Isothermal microcalorimetry analysis

Isothermal microcalorimetry (IMC) (TAM III, TA Instruments, New Castle, DE, USA) was used to monitor metabolic activity of bacteria remaining attached to discs after treatment.[16] Following six PBS washes, discs were placed in glass ampoules containing 3 mL BHIb and sealed. Heat flow was recorded every 2 min for 48 h. The time to maximal heat flow (t_{Max}, h) was used as a marker of delayed bacterial regrowth, indicating treatment efficacy. Untreated discs served as positive controls.

Scanning electron microscopy (SEM)

SEM was used to visualize residual *S. epidermidis* biofilm and treatment-related surface changes. PE was selected as the sole imaging material due to its low elastic modulus and higher susceptibility to abrasion and bacterial adhesion.[11] Biofilm-covered or sterile control discs were treated with PBS, PHMB, Soni, MT, UT, or their PHMB-combined variants.

After treatment, discs were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, dehydrated through graded ethanol (30-100%), vacuum-dried, sputter-coated, and imaged using a Zeiss GeminiSEM 300 (Carl Zeiss, Zeiss Oberkochen, Germany). Images were acquired at 30 µm, 10 µm and 3 µm scales.

Statistics

Data were expressed as mean ± SD or median (range). Normality and variance were tested prior to analysis. One-way ANOVA was used for normally distributed data with

equal variances; otherwise, Kruskal–Wallis and Wilcoxon rank-sum tests were applied. A p-value <0.05 was considered significant. Analyses were performed in GraphPad Prism 10 (GraphPad Software, La Jolla, CA, USA).

4. Results

Determining Optimal PHMB Working Concentrations

Microcalorimetry showed that 0.02 - 0.04% PHMB (standard out-of shelf concentration) completely suppressed metabolic activity of *S. epidermidis* biofilms on Ti64 and CoCrMo, whereas on PE these concentrations only delayed the time to peak heat flow t_{Max} ($p < 0.05$). Lower concentrations (0.004%, 0.0008%, and 0.0004%) produced a dose-dependent delay across all materials (Fig. 1). For *E. coli*, 0.02 - 0.04% PHMB eradicated biofilms on all materials, while 0.004% yielded a significant delay in t_{Max} compared to untreated control ($p < 0.05$). Based on these findings, 0.004% PHMB (Ti64, CoCrMo) and 0.04% PHMB (PE) (the highest concentrations which failed to achieve complete metabolic eradication) were selected for combination treatments.

Activity of Different Treatment Modalities on S. epidermidis and *E. coli Biofilm Eradication*

CFU counting analysis

Across all biomaterials, *S. epidermidis* biofilms exposed to Soni, MT, or UT released significantly higher CFU counts than untreated controls (Ti64: 4.7 - 5.3 vs 4.1 $\log_{10}$ CFU/mL; CoCr: 4.6 - 5.3 vs 4.1; PE: 6.2 - 6.7 vs 4.3; $p < 0.05$ for all). MT produced higher CFU counts than Soni on Ti64 ($p < 0.05$), whereas Soni and UT showed no

differences on any material. Similar trends were observed on CoCr and PE (Fig. 2, Supplemental Material Table 1). For *E. coli*, all treatments again exceeded controls across materials (Ti64: 4.2 - 5.3 vs 3.2 $\log_{10}$ CFU/mL; CoCr: 4.4 - 5.4 vs 3.9; PE: 5.1 - 5.9 vs 4.1; $p < 0.05$ for all). MT consistently yielded the highest CFU counts, while UT performed comparably to Soni and showed lower CFU release on PE ($p < 0.05$). All PHMB-containing treatments resulted in no detectable viable bacteria for both species and all materials (Fig. 2, Supplemental Material Table 1).

Isothermal Microcalorimetry Analysis

On Ti64 and CoCr, *S. epidermidis* exposed to PHMB, Soni, MT, or UT showed significantly delayed t_{Max} compared with controls (Ti64: 17.5 - 25.5 h vs 11.4; CoCr: 20.7 - 26.5 h vs 14.7; $p < 0.01$ for all). PHMB delayed t_{Max} more than MT and Soni ($p < 0.05$), with no difference from UT. On PE, all treatments similarly prolonged t_{Max} (15.0 - 17.6 h vs 10.0; $p < 0.01$). Unlike metal biomaterials, no differences were observed among the individual treatments (Fig. 3, Supplemental Material).

For *E. coli*, all monotherapies significantly delayed t_{Max} on Ti64 and PE, with no differences among treatments. On CoCr, Soni showed a significantly greater metabolic reduction than PHMB ($p < 0.05$), and UT uniquely achieved complete biofilm eradication, with no detectable heat production.

Across all materials and both species, all PHMB combination therapies (PHMB + Soni, PHMB + MT, PHMB + UT) completely suppressed heat production for 48 h, confirming

total biofilm eradication.

Scanning electron microscopy

Untreated PE discs showed dense S. epidermidis biofilm (Fig. 4A). All monotherapies reduced visible biomass, with PHMB and UT producing the greatest disruption (Fig. 4B – E). Combination treatments demonstrated near-complete biofilm elimination, with surfaces appearing largely free of bacterial clusters and matrix structures (Fig. 4F – H).

Sterile PE discs subjected to MT or UT displayed minor, localized surface elevations (Fig. 5D – E). Importantly, the topographical alterations were limited in extent, microscale in size, and did not suggest structural compromise of the material. No surface cracking, gouging, or signs of chemical degradation were observed across any treatment condition.

5. Discussion

This study provides the first evidence that neither PHMB disinfection nor mechanical treatment alone is sufficient to eradicate *S. epidermidis* or *E. coli* biofilms from PE, even at an early stage of maturation. Complete in vitro eradication was achieved only when PHMB was combined with a mechanical modality – UT, MT or Soni. These findings underscore the need for multimodal strategies to overcome the inherent recalcitrance of biofilms on implant materials.

PHMB demonstrated robust efficacy on metal surfaces but was markedly less effective on PE probably due to stronger mechanical interlocking of biofilms within PE surface microtexture and thick peptidoglycan layers of Gram-positive staphylococcal biofilms.[20] The superior susceptibility of *E. coli* compared to *S. epidermidis* across all PHMB concentrations supports this interpretation. Importantly, in clinical practice, substantial bleeding during DAIR procedures inevitably dilutes antiseptic solutions. Even modest dilution can compromise PHMB efficacy, suggesting that PHMB irrigation alone, especially on PE and potentially also on metal surfaces, may be insufficient for achieving clinically meaningful biofilm reduction. This observation aligns with clinical evidence showing improved DAIR success when modular components, particularly PE inserts, are exchanged rather than retained.[9, 10] Most studies evaluate antiseptic agents primarily in a preventive setting and rely on observational data rather than microbiological biofilm analyses.[21] As clinical outcomes depend strongly on pathogen

virulence, immunocompetence, symptom duration and surgical technique, observational data fail to identify the optimal irrigation agent.[2-4, 22] Recent ex vivo work showed that none of 14 approved irrigation fluids effectively removed *S. aureus* biofilm.[23] Complementary translational studies on orthopedic materials demonstrated that PHMB-based formulations outperformed povidone–iodine in biofilm eradication while maintaining acceptable cytocompatibility.[14] Collectively, these findings and our own data indicate that PHMB remains highly effective against planktonic or detached bacteria but – especially if diluted - insufficient against adherent biofilm without mechanical disruption. Consequently, effective eradication appears to require additional mechanical disruption of biofilm structures.

All three mechanical modalities (sonication, MT, and UT) significantly reduced biofilm on all surfaces but did not achieve complete eradication on their own. Sonication alone provided moderate detachment but left viable bacteria, consistent with reports that classify sonication primarily as a dispersal rather than bactericidal method.[15] MT and UT were more effective on structured surfaces, but only UT achieved complete *E. coli* eradication on CoCr, indicating that material properties influence treatment success. A notable and mechanistically important finding was the discrepancy between CFU enumeration and microcalorimetry when comparing UT with MT. As described, microcalorimetry showed a modestly greater delay in metabolic activity after UT, whereas CFU analysis of the detached biofilms consistently demonstrated fewer viable

bacteria. This divergence suggests that UT does more than detach biofilm: it exerts a direct bactericidal effect. By generating cavitation and microstreaming it produces high shear stress and localized pressure fluctuations that can not only disrupt bacterial membranes but also increase permeability and induce lysis – effects not achieved by simple mechanical abrasion.[18] This mechanistic difference likely explains why UT achieved complete *E. coli* eradication on CoCr surfaces whereas MT did not and highlights the potential clinical utility of UT as a biofilm-targeting tool.

The concept of applying ultrasonic brushing to orthopedic implants is novel. While manual brushing has proven beneficial in spinal implant decontamination, no previous studies have evaluated UT in a PJI context.[17] The present work thus represents the first translational adaptation of this established dental hygiene technology to orthopedic biofilm management. In dental research, ultrasonic and sonic toothbrushes are known to remove plaque more effectively than manual brushes as their microstreaming and shear forces detach biofilm both at and beyond the contact zone — also a protentional benefit in intraoperative arthroplasty settings with restricted implant access.[18] Furthermore, oral biofilm research also showed synergistic effect of sonic toothbrush and antiseptic agents.[24] Similar synergy between mechanical and chemical treatment options was observed in our study: UT combined with PHMB produced complete metabolic suppression and absence of CFU growth, outperforming either modality alone. SEM findings further supported these results. Combined treatments produced

the lowest levels of residual surface-associated bacteria.

Surface integrity is essential for implant functionality. SEM imaging revealed only minor, localized microscale surface elevations on PE after MT and UT – consistent with the material's low elastic modulus rather than significant abrasion. Despite PE's susceptibility to mechanical wear, these changes were clinically negligible and did not indicate structural compromise, supporting the material safety of the tested procedures. Recent study is limited by its *in vitro* design, and future validation in *ex vivo* or *in vivo* models is necessary to confirm safety and efficacy under clinical conditions. However, previous ex vivo studies have demonstrated acceptable cytotoxicity of PHMB in osteoblast and keratinocyte cultures, suggesting that no major safety concerns are to be expected in the aforementioned setting.[14] Additionally, only two bacterial strains were tested. Although inclusion of polymicrobial biofilms and antibiotic-resistant organisms would expand the generalizability, S. epidermidis is known for building strong biofilms which was highly beneficial in the setup of the present study.[25]

Conclusion

Effective biofilm eradication on orthopedic materials requires a dual chemical-mechanical approach. PHMB provides strong antiseptic activity against detached bacteria but limited biofilm penetration, especially on PE, whereas ultrasonic brushing enhances mechanical disruption and adds bactericidal effects. The synergy between PHMB and especially UT resulted in complete metabolic inhibition and elimination of

viable bacteria across all tested materials. This combined strategy offers a promising translational pathway for improving intraoperative implant decontamination and may enhance the success rates of DAIR procedures in acute PJI.

Abbreviations

Ti64 – Ti-6Al-4V (Titanium alloy)

CoCrMo – Cobalt–chromium–molybdenum alloy

PE – Polyethylene (highly cross-linked)

CFU – Colony-forming units

BHIb – Brain Heart Infusion broth

PBS – Phosphate-buffered saline

PHMB – Polyhexanide

TSA – Tryptic Soy Agar

MT – Mechanical Toothbrushing

UT – Ultrasonic Toothbrushing

GC – Growth Control

SEM – Scanning Electron Microscopy

SD – Standard Deviation

ANOVA – Analysis of Variance

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Figure Legend

Figure 1. Microcalorimetry analysis of PHMB performance at various concentrations against S. epidermidis biofilm formed on (A) Ti64, (B) CoCr, (C) PE surfaces and E. coli biofilm on (D) Ti64, (E) CoCrMo and (F) PE surfaces. GC, growth control; PHMB, polyhexanide. *, $p < 0.05$; **, $p < 0.01$.

Figure 2. CFU counting analysis after treatment of S. epidermidis biofilm on (A) Ti64, (B) CoCrMo, (C) PE surfaces and E. coli biofilm on Ti64 (D), CoCrMo (E), and PE (F) surfaces using different biofilm removal methods. GC, growth control; PHMB, polyhexanide; Soni, sonication; MT, mechanical toothbrush; UT, ultrasonic toothbrush; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Data is presented as mean ± SD.

Figure 3. Microcalorimetric analysis after treatment of S. epidermidis biofilm on (A) Ti64, (B) CoCrMo surfaces, (C) PE, and E. coli biofilm on (D) Ti64, (E) CoCrMo, and (F) PE surfaces using different biofilm removal methods. GC, growth control; PHMB, polyhexanide; Soni, sonication; MT, mechanical brushing; UT, ultrasonic toothbrush. * For the treatment of the S. epidermidis biofilm on PE, a PHMB concentration of 0.04% was used.

Figure 4. Scanning electron microscopy (SEM) of S. epidermidis biofilm grown on PE discs. Discs after individual treatments with PBS (control) (A), PHMB (B), Soni (C), MT (D) and UT (E), as well as after combination treatment with Soni + PHMB (F), MT + PHMB (G) and UT + PHBM (H). Scale bars: 30 µm (inserts in the images represent 3 µm).

Figure 5. Scanning electron microscopy (SEM) of PE discs without bacterial biofilms. Discs after treat-ments with PBS (control) (A), PHMB (B), Soni in PBS (C), MT with PBS (D) and UT with PBS (E). Scale bars: 30 µm (inserts in the images represent 10 µm).

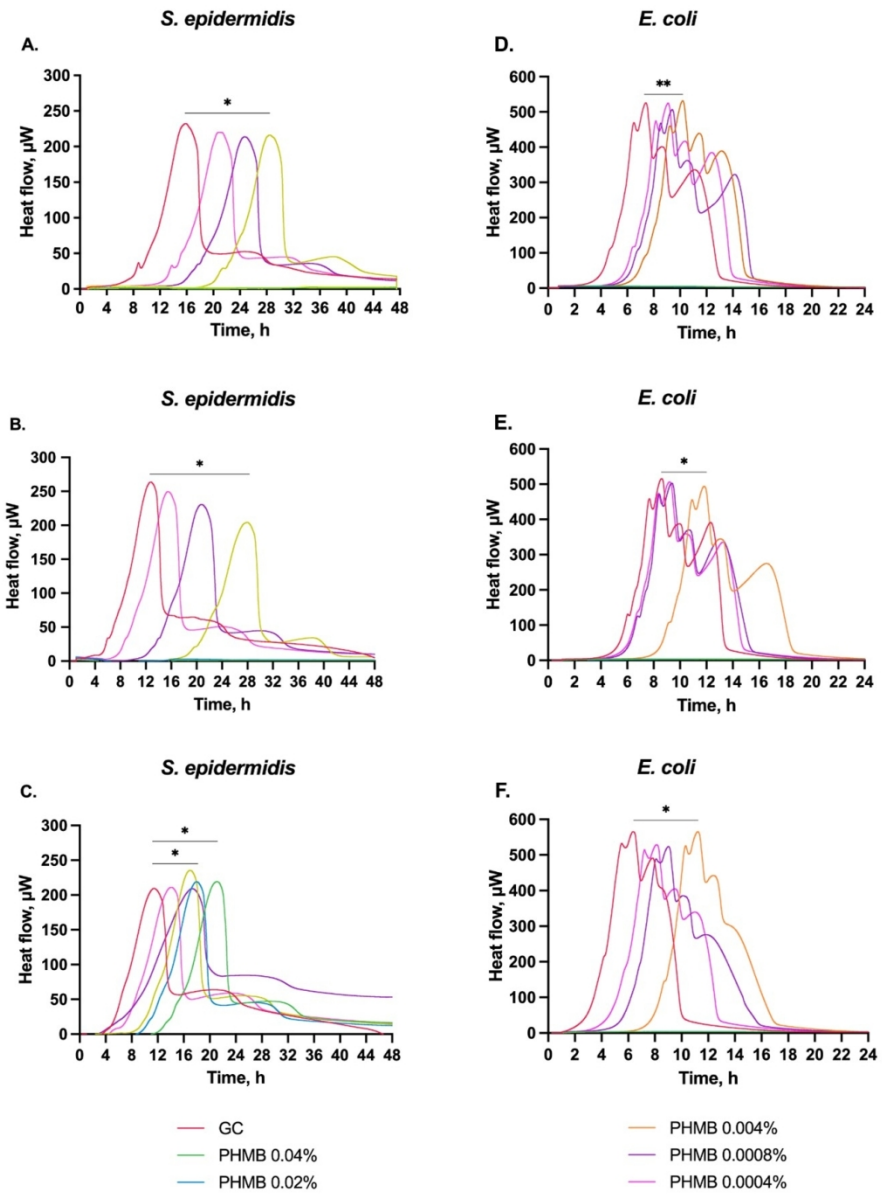

Figure 1. Microcalorimetry analysis of PHMB performance at various concentrations against S. epidermidis biofilm formed on (A) Ti64, (B) CoCr, (C) PE surfaces and E. coli biofilm on (D) Ti64, (E) CoCrMo and (F) PE surfaces. GC, growth control; PHMB, polyhexanide. *, $p < 0.05$; **, $p < 0.01$.

160x205mm (220 x 220 DPI)

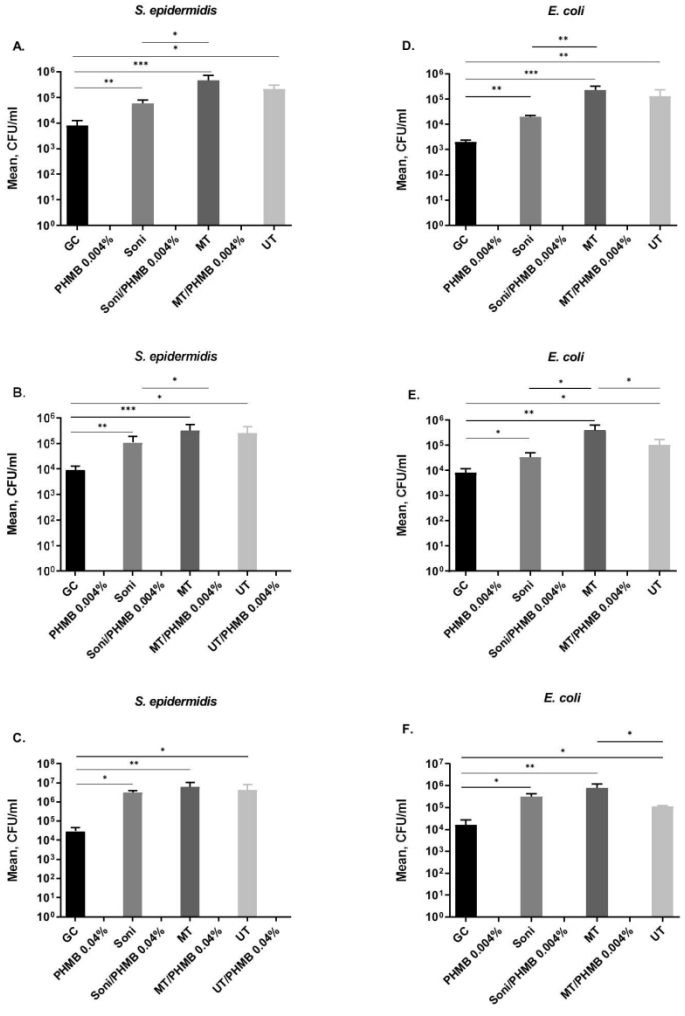

Figure 2. CFU counting analysis after treatment of S. epidermidis biofilm on (A) Ti64, (B) CoCrMo, (C) PE surfaces and E. coli biofilm on Ti64 (D), CoCrMo (E), and PE (F) surfaces using different biofilm removal methods. GC, growth control; PHMB, poly-hexanide; Soni, sonication; MT, mechanical toothbrush; UT, ultrasonic toothbrush; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Data is presented as mean ± SD.

583x825mm (72 x 72 DPI)

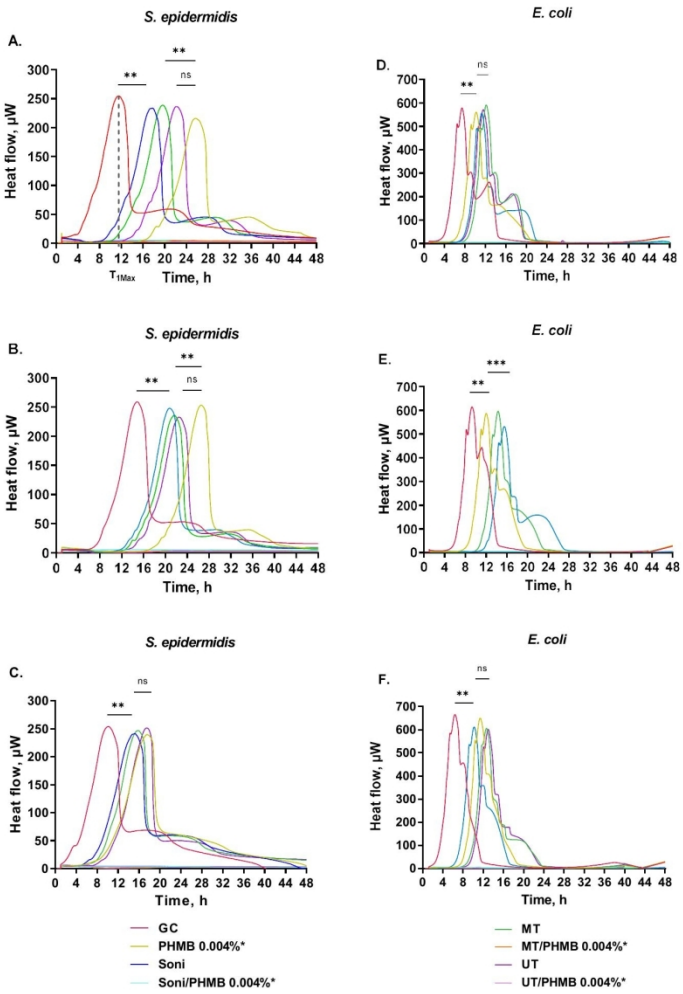

Figure 3. Microcalorimetric analysis after treatment of S. epidermidis biofilm on (A) Ti64, (B) CoCrMo surfaces, (C) PE, and E. coli biofilm on (D) Ti64, (E) CoCrMo, and (F) PE surfaces using different biofilm removal methods. GC, growth control; PHMB, poly-hexanide; Soni, sonication; MT, mechanical brushing; UT, ultrasonic toothbrush. * For the treatment of the S. epidermidis biofilm on PE, a PHMB concentration of 0.04% was used.

583x825mm (72 x 72 DPI)

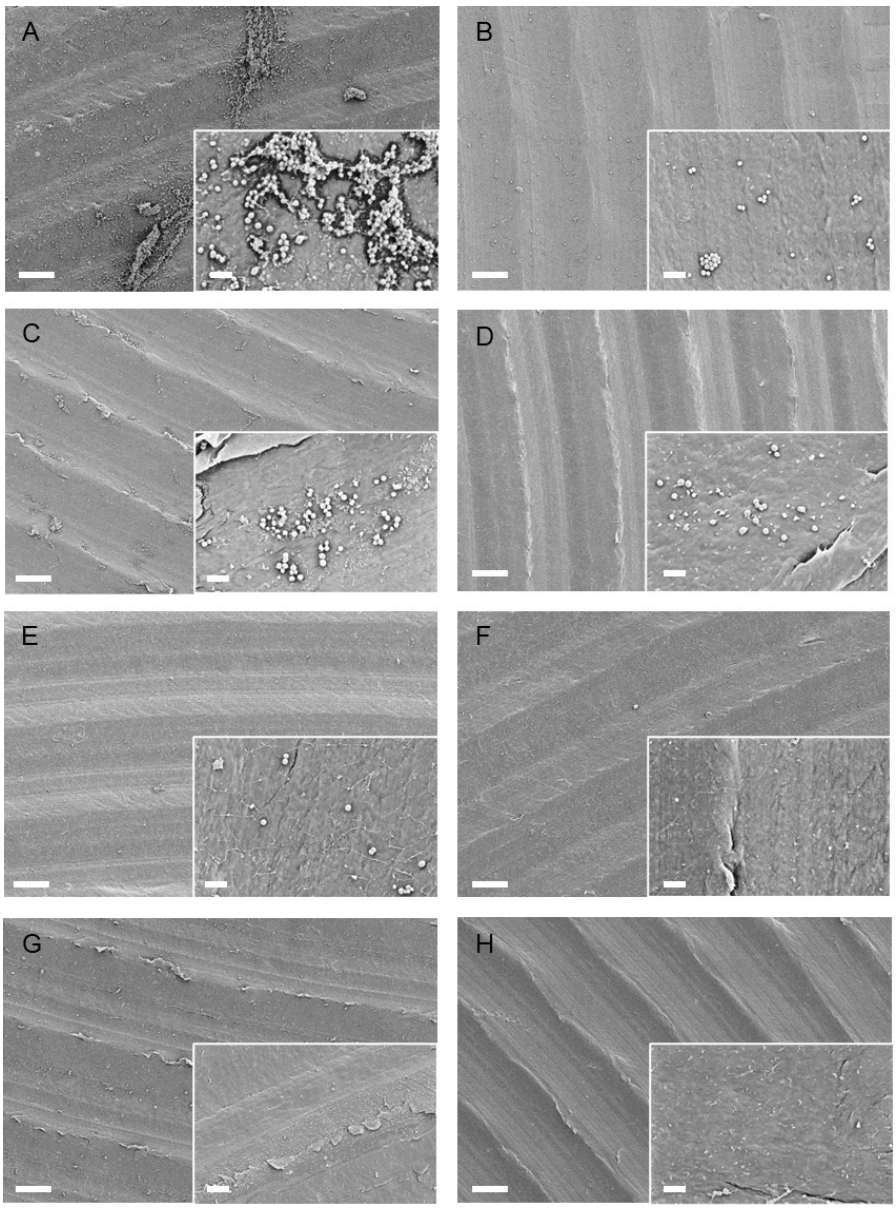

Figure 4. Scanning electron microscopy (SEM) of S. epidermidis biofilm grown on PE discs. Discs after individual treatments with PBS (control) (A), PHMB (B), Soni (C), MT (D) and UT (E), as well as after combination treatment with Soni + PHMB (F), MT + PHMB (G) and UT + PHBM (H). Scale bars: 30 µm (inserts in the images represent 3 µm).

143x192mm (150 x 150 DPI)

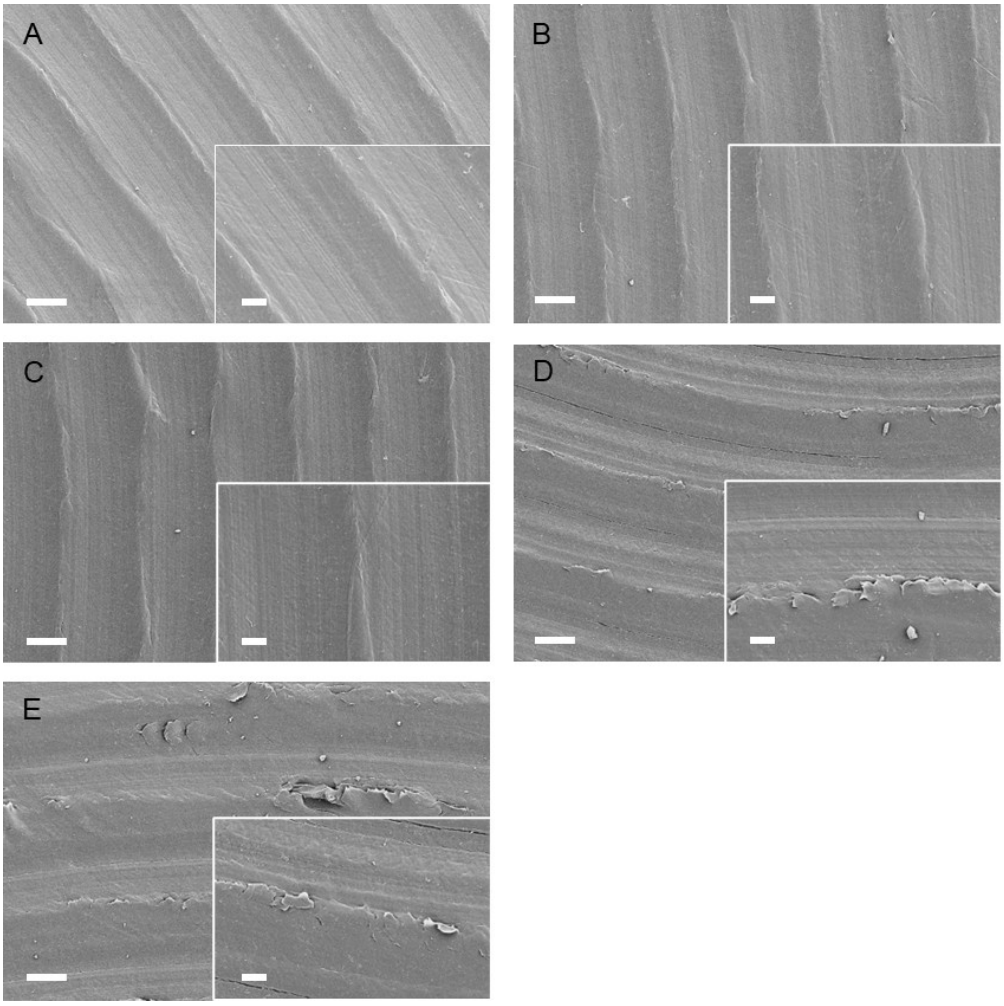

Figure 5. Scanning electron microscopy (SEM) of PE discs without bacterial biofilms. Discs after treat-ments with PBS (control) (A), PHMB (B), Soni in PBS (C), MT with PBS (D) and UT with PBS (E). Scale bars: 30 μm (inserts in the images represent 10 μm).

143x142mm (150 x 150 DPI)

Supplemental Materials

Supplementary Table 1. Microcalorimetry and CFU analysis of *S. epidermidis* and *E. coli* biofilm treated with different methods. CFU/ml are presented as median (IQR).

S. epidermidis ATCC 35984												
	Ti64				CoCrMo				PE			
Method	t_{Max} (h)	*p* value vs GC	log_{10} CFU/mL	*p* value vs GC	t_{Max} (h)	*p* value vs GC	log_{10} CFU/mL	*p* value vs GC	t_{Max} (h)	*p* value vs GC	log_{10} CFU/mL	*p* value vs GC
GC	11.4 (11.1-12.1)		4.1 (3.2-4.1)		14.7 (12.5-15.3)		4.1 (3.4-4.1)		10.0 (9.6-10.1)		4.3 (4.1-4.5)	
PHMB	25.5 (25.3-26.0)	<0.01	-		26.5 (26.0-27.4)	<0.01	-		17.6 (17.5-18.2)	<0.01	-	
Soni	17.5 (16.8-18.3)	<0.01	4.7 (4.4-4.7)	<0.01	20.7 (19.2-22.0)	<0.01	4.6 (4.6-5.2)	<0.01	15.0 (13.6-16.2)	<0.01	6.3 (6.2-6.4)	<0.05
MT	19.6 (19.1-21.4)	<0.01	5.3 (5.3-5.8)	<0.01	21.6 (20.1-22.0)	<0.01	5.2 (5.2-5.6)	<0.01	15.0 (15.3-17.5)	<0.01	6.7 (6.1-7.1)	<0.01
UT	22.1 (21.9-22.3)	<0.01	5.2 (5.1-5.3)	<0.05	22.5 (21.2-24.1)	<0.01	5.2 (5.1-5.5)	<0.05	17.5 (17.3-17.8)	<0.01	6.2 (6.2-6.9)	<0.05
Soni/PHMB	-		-		-		-		-		-	
MT/PHMB	-		-		-		-		-		-	
UT/PHMB	-		-		-		-		-		-	
E. coli ATCC 25922												

GC	7.3 (6.9-8.3	-	3.2 (3.1-3.3)	-	8.4 (7.7-9.5)	-	3.9 (3.4-4.1)	-	6.7 (6.4-7.6)	-	4.1 (3.7-4.2)	-
PHMB	10.2 (10.0-11.4)	<0.01	-	-	11.6 (10.0-12.3)	<0.01	-	-	11.3 (10.2-12.2)	<0.01	-	-
Soni	13.1 (11.7-14.0)	<0.01	4.2 (4.1-4.3)	<0.01	15.6 (14.1-17.3)	<0.01	4.4 (4.2-4.5)	<0.01	10.5 (10.0-11.5)	<0.01	5.3 (5.2-5.4)	<0.05
MT	12.3 (14.2-17.9)	<0.01	5.3 (5.1-5.3)	<0.01	15.4 (14.2-17.9)	<0.01	5.4 (5.2-5.6)	<0.01	12.6 (11.6-12.9)	<0.01	5.9 (5.3-6.1)	<0.01
UT	12.1 (11.4-12.7)	<0.01	5.1 (4.3-5.2)	<0.05	13.0 (11.9-14.6)	<0.01	4.9 (4.5-5.1)	<0.05	13.0 (11.9-14.6)	<0.01	5.1 (5.1-5.2)	<0.05
Soni/PHMB	-		-		-		-		-		-	
MT/PHMB	-		-		-		-		-		-	
UT/PHMB	-		-		-		-		-		-	

GC, growth control; PHMB, polyhexanide; Soni, sonication; MT, mechanical brushing; UT, ultrasonic toothbrush; CFU/ml, colony-forming unit per milliliter; IQR, interquartile range; −, complete biofilm eradication (no CFU or heat flow detected).