Received: 23 December 2022 | Revised: 21 March 2023 | Accepted: 8 May 2023

DOI: 10.1002/jor.25599

RESEARCH ARTICLE

Journal of Orthopaedic Research®

Piezoelectric ultrasonic debridement as new tool for biofilm removal from orthopedic implants: A study in vitro

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Funding information
Mectron S.p.A. (Carasco, Italy)

Abstract

Pulse lavage (PL) debridement is the standard treatment used in Debridement, Antibiotics and Implant Retention (DAIR) for bacterial biofilm removal during acute and early postoperative cases of periprosthetic joint infection (PJI). The failure rate of DAIR is still high due to the inadequacy of PL in removing the biofilm. Ultrasound-based techniques are a well-established tool for PJI diagnosis due to their ability to completely eradicate the biofilm from implant surfaces. Hence, this study investigates the efficiency of a piezoelectric ultrasonic scalpel (PUS) in removing bacterial biofilm from different orthopedic implant materials in vitro and compares the results with PL. Biofilms of methicillin-resistant *Staphylococcus aureus* strains were grown on titanium alloy (Ti6Al4V ELI), stainless steel (AISI 316L), and ultrahigh molecular weight polyethylene (UHMWPE) disks for 24 h. The disks of each material were divided into three groups: (i) a control group (no lavage/debridement), (ii) a group treated with PL, (iii) a group treated with PUS. The disks were then sonicated for viable cell count to measure the residual biofilm content. Compared to the initial cell count (10^5 CFU/mL for each material), PL showed a two-log reduction of CFU/mL ($p < 0.001$ for each material), while for PUS a four-log reduction was found ($p < 0.001$ for each material). The comparison between the two lavage/debridement displayed a two-log reduction of CFU/mL ($p < 0.001$ for each material) of PUS compared with PL. Its increased efficiency compared with PL promotes the use of PUS in removing bacterial biofilm from orthopedic implants, suggesting its implementation to improve the success rate of DAIR.

KEYWORDS
biofilm, debridement, periprosthetic joint infection, piezoelectric surgery, pulse lavage

1 | INTRODUCTION

Periprosthetic joint infection (PJI) is one of the most dramatic complications in joint arthroplasty, especially in total hip arthroplasty (THA) and total knee arthroplasty (TKA).[1] Despite the incidence is below 2%,[2] PJI is the leading cause of revision failure and early primary failure for both THA and TKA,[3] causing pain for the patient, prolonged hospitalization, other implant-associated infections, multiple surgeries, functional incapacitation, and even death.[4]

The classification of PJI is controversial, due to the difficulties in differentiating implant-associated infections.[5] One of the most used classification differentiates PJI in early postoperative, acute hematogenous, and chronic,[6] as described in Table 1.

Staphylococcus aureus (*S. aureus*) is the most commonly isolated bacterium in acute PJI case, and one of the most common in all three categories of PJI[7]; infections from this bacterium are characterized by a rapid biofilm formation.[8,9] Infections due to methicillin-resistant *S. aureus* (MRSA) could worsen the situation because of its ability to resist to many antibiotic classes.[10]

For acute and early postoperative infections, when the implant is stable and there is sufficient soft tissue, surgeons avoid the implant replacement and choose more conservative solutions, namely retaining the implant.[11] Debridement, Antibiotics and Implant Retention (DAIR) is a technique that is increasingly used in the operating room: it includes the removal of all infected and necrotic tissues, the debridement of all prosthetic components, the exchange of the modular components and the extensive irrigation, and the application of local antibiotics through antibiotics loaded bone cement or beads.[12–15] DAIR is less invasive for the patient and less expensive compared to revision surgery, but its failure rate is still high.[16] Although many studies have been made about the incidence of the type of antibiotics,[17] the timing of the procedure,[18] or the change of the modular components,[19] there are no substantial work for improving the lavage/debridement method. Therefore, the high failure rate of DAIR could be ascribed to the inadequacy of the lavage/debridement type for the biofilm removal, usually performed by pulse lavage (PL) gun.[20] Despite its ability to mechanically remove the biofilm from bone, soft tissues, and prosthetic implants, several in vitro studies have highlighted the inadequacy of PL alone in the complete biofilm removal,[21–24] suggesting the implementation of other technologies to improve the outcome.

Currently, bacteria isolation and identification for diagnosing PJI is performed by means of cultures of synovial fluid and intraoperative periprosthetic tissues; however, diagnosis often fails due to the low sensitivity of this method[25] (about 60%).[26,27] This fact could be due to the protection that the biofilm offers to the pathogens with respect to detection and elimination.[28] In this context, some studies have shown that the sonication of removed implants and the culture of the sonication fluid culture shows a higher sensitivity (about 90%),[26,27] improving the diagnosis of PJI. The sonication technique relies on two effects of the ultrasound: cavitation and microstreaming. Cavitation is the formation of microbubbles and their implosion, while microstreaming is the generation of vibrations due to the shockwaves caused by the cavitation.[29] This synergistic effect disrupt the bacteria cell membrane and dislodge completely the biofilm from the implant surface,[30] suggesting the exploitation of the same technique during lavage/debridement operation for DAIR procedures. Recent in vitro studies have demonstrated the complete biofilm removal from metallic implants by a direct-contact low-frequency ultrasound device, operated in tandem with PL.[24,31]

Furthermore, a method that involves a piezoelectric ultrasonic device is widely used in oral surgery: this method exploits the microvibrations of the tip of the device at ultrasonic frequency (>20 kHz) to perform efficient and safe osteotomies.[32] This device combined with a plastic tip is also used to remove the plaque from dental implants, as an efficient debridement method.[33–35]

The rationale behind the preliminary study present here is based on the combination of the effects described above, namely the cavitation, the microstreaming, the vibration of the tip at ultrasonic frequency, and the mechanical action of the operator moving the device with the tip in contact with the implant: this synergetic combination can be applied in PJI for the removal of biofilm from orthopedic implants, as better alternative for lavage/debridement in DAIR procedures. Therefore in this study, we test the efficacy in vitro of a piezoelectric ultrasonic scalpel (PUS) with a polyether ether ketone (PEEK) tip in removing MRSA biofilms from three prototypical

TABLE 1 Classification of PJIs.

Type of PJI	Duration/period	Etiology	Symptoms
Early postoperative	Manifests within 1 month from the surgery	Exogenously acquired during implantation or in the early postoperative period when the drains are still not removed	Pain and redness in surgical site
Acute hematogenous	Lasts less than 3 weeks after postoperative period	Seeding from the bloodstream	Sepsis, skin and soft-tissue infection, pneumonia, enterocolitis, new on-set joint pain
Late chronic	Lasts more than 3 weeks after the postoperative period	Exogenously or hematogenously acquired	Low-grade infections (delay in diagnosis), joint effusion, local erythema and hyperthermia, sinus tracts, pain

Abbreviation: PJI, periprosthetic joint infection.

implant materials surfaces, namely a titanium alloy, a stainless steel, and ultrahigh molecular weight polyethylene (UHMWPE).[36] The hypothesis of this study is that PUS lavage/debridement is more effective than PL in removing the biofilm from prosthetic components, with the purpose of increasing the probability of success of DAIR.

2 | MATERIALS AND METHODS

2.1 | Surfaces

The surfaces tested were provided by Citieffe S.r.l. (Calderara di Reno, Italy), which specifically treats biomedical devices for research in traumatology and orthopedics and are commercially available. They were delivered as individually packaged (i.e., separated from each other) disks, all of the same size (diameter 20 mm, thickness 2 mm). The materials treated were a titanium alloy (Ti6Al4V ELI, ASTM F136), stainless steel (AISI 316L, ASTM F138), and UHMWPE (ASTM F648). The machining produces the characteristic circular lay[37] and controlled roughness (R_a) of surfaces; in particular, $R_a \approx 0.376$ µm for Ti6Al4V ELI, $R_a \approx 0.345$ µm for AISI 316L, and $R_a \approx 0.726$ µm for UHMWPE. To avoid the presence of contamination layer due to greasing of surface during the manufacturer's machining, the three separate groups were cleaned by 10 min sonication in a degreasing aqueous solution; after rinsing, 10 min sonication in ethanol and drying in N_2 flow followed.

2.2 | Bacterial strain

In this study, an MRSA strain originally isolated from PJI during microbiological routine investigations was used. The clinical isolate was resistant, in vitro, to beta-lactam antibiotics, erythromycin, quinolones, and clindamycin, while the susceptibility to aminoglycosides, glycopeptides, tetracyclines, and sulfamethoxazole–trimethoprim was preserved. The initial inoculum was prepared from an overnight culture of *S. aureus* added to 3 mL of growth media (Brain Heart Infusion broth) to achieve the final cell concentration of approximately 10^8 colony-forming units (CFU)/mL.

2.3 | Biofilm formation

Before biofilm formation, the disks were sterilized in an autoclave FOB3 (Fedegari, Albuzzano, Italy).

Biofilms were grown for 24 h on the orthopedic prosthesis materials disks soaked with bacterial suspension in 6-well plastic plates (Figure 1). Biofilm growth after 24 h was previously compared with biofilm formed after 48 and 72 h by crystal violet (CV) staining analysis described below. We found that after 24 h the maturation of biofilm was appropriate for the purpose of our study, since no qualitative differences were found after 48 and 72 h (data not shown).

For each type of implant material (Ti6Al4V ELI, AISI 316L, and UHMWPE), 18 disks were processed.

2.4 | Lavage/debridement

At the end of the incubation period, bacterial suspension was removed and the disks underwent to lavage/debridement process. Disks of each material were divided in three groups as follows:

- Control (six disks for each material), no lavage/debridement applied.
- PL (six disks for each material) by PULSAVAC® PLUS technology (Zimmer Biomet, Warsaw, IN), delivering the sterile saline solution an approximate flow-rate $Q = 1000$ mL/min at an approximate peak pulse force $F = 300$ mN. The device was used at high-pressure setting and the nozzle was kept upright with about 5 cm distance from the surface of the disks as in surgical conditions.
- PUS (six disks for each material) by PIEZOSURGERY® technology (Mectron, Carasco, Italy), at working vibrating frequency $f = 24$–36 kHz and sterile saline solution irrigation at flow-rate $Q =$ 65 mL/min. The device has a cone-shaped PEEK tip, 12 mm long,

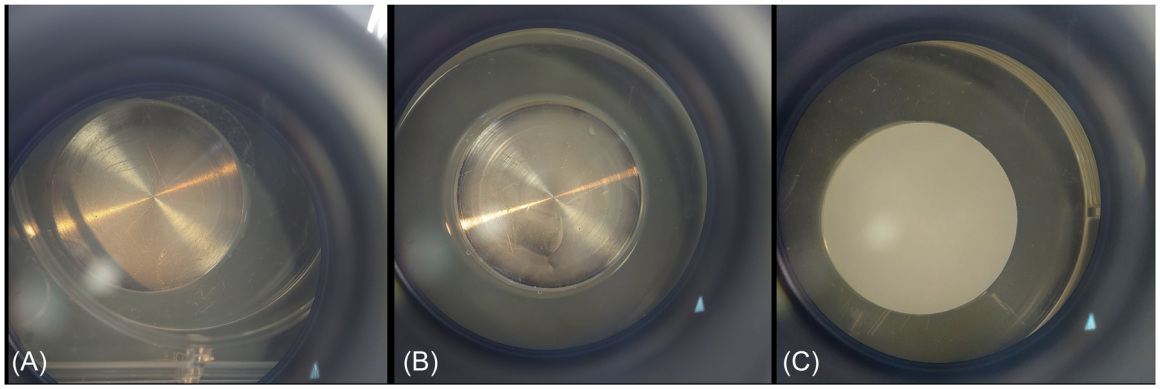

FIGURE 1 Disks with bacterial biofilm grown on surfaces before lavage/debridement: (A) Ti6Al4V ELI, (B) AISI 316L, (C) UHMWPE.

4 mm in diameter, and 0.8 mm apex width. The surgeon moved the tip tangentially with respect to the disks surface in circular movements and in contact with it, but without applying a considerable pressure, since the tip would stop vibrating (Figure 2).

The lavage/debridement of each disk was performed under a laminar flow hood by an orthopedic surgeon, cleaning all the sides (top side, bottom side and edges) of the disks for 3 min, in operation room conditions.

2.5 | Viable cell count

After the lavage/debridement step, each disk was rinsed with 0.9% NaCl solution for three times. Subsequently, we performed sonication treatment using an ultrasonic sonicator BANDELIN SONOPULS HD 2070 (ProfiLab24, Berlin, Germany) with frequency of 20 kHz for 10 s into 10 mL of sterile saline in a plastic container to detach the remaining bacterial cells from disk surface. Afterward, 10 µL of each suspension was plated in appropriate dilutions onto 5% horse blood agar plates (Vacutest Kima, Padova, Italy). Plates were incubated for 24 h at 37°C; then, the number of CFU was visually counted, expressed as CFU/mL and $\log_{10}$ transformed. The measurement for each disk was assayed in triplicate.

FIGURE 2 Operator while using piezoelectric ultrasonic scalpel (PUS) to remove biofilm from a disk under the laminar flow hood.

2.6 | CV stain

CV staining analysis were performed on the same disks as in the control group as in the treatment groups after lavage/debridement, to conform to the CFU data. The bacterial biofilms on disks surface were primary observed and photograph using a modular stereomicroscope (Leica, Wetzlar, Germany) and then stained with CV dye following the protocol described by other authors,[38] with minor modifications. Briefly, adherent biofilm was fixed to disks surfaces with 3 mL of 99% ethanol for 15 min and then stained with 2 mL of 2% (w/V) CV in 99% ethanol for 2 min (Figure 3). Each disk was washed with water to remove residual dye and then air-dried at room temperature. The CV bound to the adherent biofilm was solubilized in 3 mL of 33% (V/V) ethanol and 200 µL of the solution were distributed in a 96-well plate; the absorbance (*A*) was measured at 550 nm with an Enspire multiplate reader (PerkinElmer, Shelton, CT): this instrument emits light at a specific wavelength and has a detector on the other side of the well that determines the amount of light absorbed by the sample. CV has the ability to bind the polysaccharide matrix of the biofilm preventing the passage of light. Consequently, a high value of *A* may be correlated with the presence of bacterial biofilm. The measurement for each disk was assayed in triplicate.

2.7 | Statistical analysis

Statistical analyses of the results for both the viable cell count and the CV staining analysis were performed with nonparametric one-way analysis of variance Kruskal–Wallis test followed by a *post hoc* Mann–Whitney multiple comparison with a Bonferroni correction. The analyses were performed using MATLAB R2019b (MathWorks, Natick, MA). Statistical significance was determined if $p < 0.05$ for all

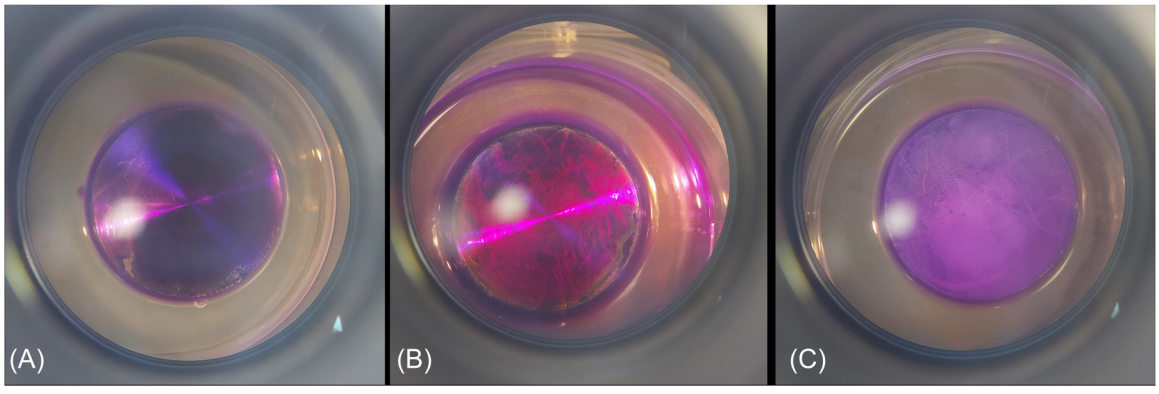

FIGURE 3 Disks after crystal violet (CV) staining on bacterial biofilm: (A) Ti6Al4V ELI, (B) AISI 316L, (C) UHMWPE.

analysis. The graphs of the means and standard deviations were made with Excel software (Microsoft, Redmond, MA).

3 | RESULTS

3.1 | Viable cell count

After incubation for 24 h at 37°C the number of CFU of the bacterial suspensions of MRSA were counted. Normalizing the values in CFU/mL and transforming them into $\log_{10}$, the following results were obtained, represented with means and standard deviations in Figure 4.

In the Control groups of each material, the biofilms had grown to approximately 10^5 CFU/mL ($10^{5.23 \pm 0.05}$ for Ti6Al4V ELI, $10^{5.38 \pm 0.07}$ for AISI 316L, $10^{5.30 \pm 0.14}$ for UHMWPE). In the treatment groups, PL lavage/debridement showed an approximate two-log reduction in CFU/mL ($10^{2.97 \pm 0.93}$ for Ti6Al4V ELI, $10^{2.52 \pm 1.34}$ for AISI 316L, $10^{2.82 \pm 1.34}$ for UHMWPE) for each material ($p < 0.001$), while PUS lavage/debridement showed an approximate four-log reduction in CFU/mL ($10^{1.45 \pm 1.29}$ for Ti6Al4V ELI, $10^{0.68 \pm 1.14}$ for AISI 316 L, $10^{0.78 \pm 1.14}$ for UHMWPE) for each material ($p < 0.001$). The comparison between PL lavage/debridement and PUS lavage/debridement shows an approximate two-log reduction in CFU/mL of PUS group for each material ($p < 0.001$).

3.2 | CV stain

The analysis of *A* at 550 nm through CV dye of the biofilm shows the values of mean and standard deviation in Figure 5.

The Control groups show $A = 0.329 \pm 0.124$ for Ti6Al4V ELI, $A = 0.343 \pm 0.099$ for AISI 316L, $A = 0.344 \pm 0.073$ for UHMWPE; a reduction of one order of magnitude both in PL + Sonication groups ($A = 0.045 \pm 0.007$ for Ti6Al4V ELI, $A = 0.049 \pm 0.006$ for AISI 316L, $A = 0.058 \pm 0.005$ for UHMWPE) ($p < 0.001$ for all materials) and in PUS + Sonication groups ($A = 0.050 \pm 0.004$ for Ti6Al4V ELI, $A = 0.054 \pm 0.004$ for AISI 316L, $A = 0.063 \pm 0.010$ for UHMWPE) ($p = 0.006$ for Ti6Al4V ELI, $p = 0.003$ for AISI 316L, $p < 0.001$ for UHMWPE) was found. No statistical differences were found between the PL + Sonication groups and PUS + Sonication groups of each material ($p = 0.10$ for Ti6Al4V ELI, $p = 0.24$ for AISI 316L, $p = 1$ for UHMWPE).

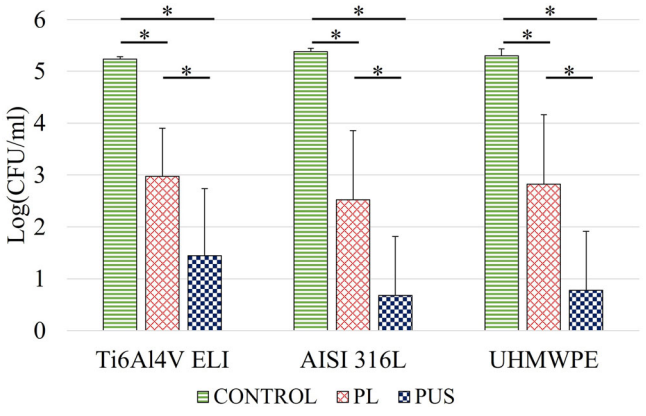

FIGURE 4 Means and standard deviations of colony-forming units (CFU)/mL with log10 transformation for Control groups, pulse lavage (PL) groups, and piezoelectric ultrasonic scalpel (PUS) groups for all three materials (Ti6Al4V ELI, AISI 316L, and UHMWPE). *$p < 0.05$.

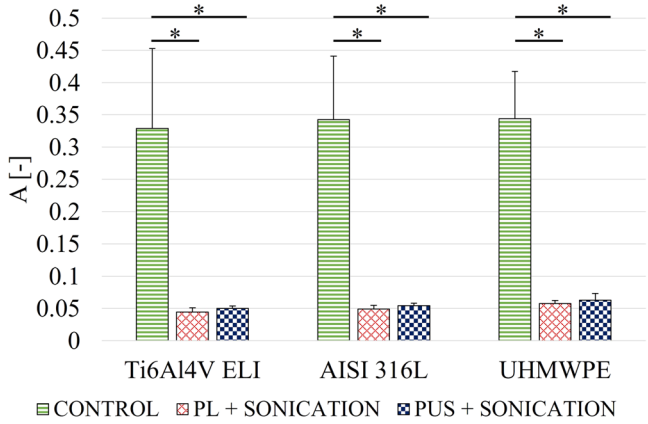

FIGURE 5 Means and standard deviations of A at 550 nm for Control groups, pulse lavage (PL) + Sonication groups and piezoelectric ultrasonic scalpel (PUS) + Sonication groups for all three materials (Ti6Al4V ELI, AISI 316L, and UHMWPE). *$p < 0.05$.

4 | DISCUSSION

The formation of biofilms and bacterial proliferation cause infections on implants and on peri-implant tissues. PL is a common technique used for lavage/debridement, and in the present study, we compared its efficacy on biofilm removal from different implant materials (Ti6Al4V ELI, AISI 316L, UHMWPE) with PUS with a PEEK tip.

Our results showed PL had approximately a two-log reduction of CFU/mL in all materials compared to the control and this is consistent with previous studies that reported the same reduction.[21,23,24] These studies assess that PL removes a large part of biofilm, but some authors have pointed out that residual bacteria on disks after PL are sufficient to restore a biofilm.[39] Although it is not known which is the minimum bacterial density for an implant to become infected, it is crucial to reduce as much as possible this value. Therefore, a new lavage/debridement method to dislodge the bacterial biofilm is needed.

The ultrasound technology is starting to get widely used for diagnosing PJI,[27] since low-frequency ultrasound devices are able to eradicate the biofilm from metallic implants (titanium alloy and stainless steel)[24,31] due to cavitation and microstreaming effects.[40] These principles can be exploited with PUS with a PEEK tip vibrating at low-ultrasonic frequency (24–36 kHz). In our study, PUS showed approximately a four-log reduction of CFU/mL in all materials compared to the control.

PUS does not eradicate completely the biofilm from the disks, but is capable to reduce the cell count of two orders of magnitude

compared to PL, increasing the success probability of DAIR. It is noted that possible roughening of the UHMWPE surface during PUS lavage/debridement is irrelevant as polymeric components are generally used only for modular parts, therefore, subjected to replacing during DAIR procedure.

The results of CV staining show that the combination of lavage/debridement and sonication for each material leads to a significantly reduction of *A* with respect to the control groups. These findings validate the viable cell count results, since they prove that sonication completely eradicated all residual bacterial cells on treated disks surfaces. This is confirmed by the results of other studies, which show that the combination of PL and sonication is able to completely dislodge the biofilm.[24,31] Moreover, the values of absorbance that we obtained for PL + Sonication groups and PUS + Sonication groups of each material are not statistically different, therefore, the sonication used in our study is able to dislodge the biofilm regardless which lavage/debridement has been used.

To the best of our knowledge, this is the first in vitro study that investigates the effects of PUS with a PEEK tip against MRSA biofilm on three different orthopedic implant materials. Reductions of approximately two Log(CFU/mL) and four Log(CFU/mL), respectively, were observed with PL and PUS. It seems likely that the higher efficiency of PUS is due to the synergistic combination of the cavitation and the microstreaming of ultrasound, the vibration of the tip at ultrasonic frequency and the mechanical action carried out by the operator moving the tip of the device on the surface of the disks.

There were some limitations in our study. First, we did not analyze the surface modifications of the disks, such as the roughness after the two treatments, which may affect the implant stability. Second, we used only one type of bacteria: although MRSA is one of most problematic bacteria for PJI,[41] the same experimental set up could be applied also for other type of bacteria. Furthermore, to assess the efficiency of PUS with a view to a future clinical trial, this in vitro experimental model could be applied also to bone and soft tissues, to have a complete view of both implant materials and tissues.

5 | CONCLUSIONS

In the present study, we investigate the efficiency PUS with a PEEK tip, compared to PL, against MRSA biofilms grown on different implant material disks (Ti6Al4V ELI, AISI 316L, UHMWPE). Our experimental evidence indicates that PUS is more efficient than the PL in removing the biofilm, significantly reducing the presence of biofilm on the disks. Therefore, this result suggests that PUS can be used as an optimal lavage/debridement method, increasing the success rate in DAIR procedures for acute and early postoperative PJI cases.

AUTHOR CONTRIBUTIONS

Conceptualization: Alessandro Russo. *Data curation*: Alessandro Gatti, Silvia Felici. *Formal analysis*: Alessandro Gatti, Alessandro Gambardella. *Funding acquisition*: Alessandro Russo, Stefano Zaffagnini, Milena Fini, Tiziana Lazzarotto. *Investigation*: Alessandro Russo, Alessandro Gatti, Silvia Felici. *Methodology*: Alessandro Russo, Silvia Felici, Maria Pia Neri, Tiziana Lazzarotto. *Resources*: Alessandro Russo, Stefano Zaffagnini, Tiziana Lazzarotto. *Supervision*: Alessandro Russo, Stefano Zaffagnini, Tiziana Lazzarotto. *Validation*: Alessandro Russo, Alessandro Gatti, Silvia Felici. *Visualization*: Alessandro Russo, Alessandro Gambardella, Maria Pia Neri, Stefano Zaffagnini, Tiziana Lazzarotto. *Writing—original draft*: Alessandro Gatti, Silvia Felici, Alessandro Gambardella. *Writing—review and editing*: Alessandro Russo, Alessandro Gatti, Silvia Felici, Alessandro Gambardella, Tiziana Lazzarotto. All authors have read and approved the final submitted manuscript.

ACKNOWLEDGMENT

This work was supported by Mectron S.p.A. (Carasco, Italy). Open access funding provided by BIBLIOSAN.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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How to cite this article: Russo A, Gatti A, Felici S, et al. Piezoelectric ultrasonic debridement as new tool for biofilm removal from orthopedic implants: a study in vitro. *J Orthop Res*. 2023;41:2749-2755. doi:10.1002/jor.25599