Received: 10 July 2019 | Accepted: 3 November 2019

DOI: 10.1002/jor.24565

Journal of Orthopaedic Research®

RESEARCH ARTICLE

Focused high-energy extracorporeal shockwaves as supplemental treatment in a rabbit model of fracture-related infection

Jan Puetzler[1] | Alexander Milstrey[2] | Jens Everding[1] | Michael Raschke[1] | Daniel Arens[2] | Stephan Zeiter[2] | Robert Geoff Richards[2] | Thomas Fintan Moriarty[2]

[1]Department of Trauma Surgery, University Hospital of Muenster, Muenster, Germany

[2]AO Research Institute Davos, Davos, Switzerland

Correspondence
Thomas Fintan Moriarty, AO Research Institute Davos, Clavadelerstrasse 8, 7270 Davos, Switzerland.
Email: fintan.moriarty@aofoundation.org

Funding information
AOTrauma as part of the clinical priority program bone infection

Abstract

Focused high-energy extracorporeal shockwave therapy (fhESWT) is used to improve fracture healing in cases of nonunion. In addition, it has been shown to have direct antibacterial effects. We evaluated fhESWT as an adjunct to conventional treatment in a clinically relevant rabbit model of fracture-related infection (FRI). A humeral osteotomy in 31 rabbits was fixed with a seven-hole locking compression plate. FRI was established with a clinical *Staphylococcus aureus* isolate. After 2 weeks, a revision surgery was performed with debridement, irrigation, and implant retention. Rabbits then received: no further treatment (controls); shockwaves (4000 impulses with 23 kV at days 2 and 6 after revision); systemic antibiotics (rifampin and nafcillin); or the combination of antibiotics and shockwaves. Treatments were applied over 1 week. Blood cultures were taken before and after shockwave sessions. After another week without treatment, rabbits were euthanized and quantitative bacteriology was performed on implants and tissues to determine infection burden. Indicator organs (brain, heart, liver, lungs, kidneys, and spleen) were cultured to assess possible bacteremia. All the rabbits were infected at revision surgery as determined by the bacteriological culture of debrided materials. fhESWT in combination with antibiotic treatment lowered the bacterial burden 100-fold compared with antibiotic treatment alone in all samples ($P = .38$). This effect was most prevalent for the implant sample ($P = .08$). No significant effect was seen for fhESWT alone compared with untreated controls. No signs of bacteremia occurred in blood cultures and organs. fhESWT appears safe and could be a helpful adjunct to conventional treatment in certain difficult-to-treat FRIs.

KEYWORDS

extracorporeal shockwaves, fracture-related infection, osteomyelitis, rabbit model, supplemental treatment

1 | INTRODUCTION

Fracture-related infection (FRI) is one of the most feared complications in orthopedic trauma surgery, which can lead to failure to heal and

Jan Puetzler and Alexander Milstrey contributed equally to this study.

nonunion of the fracture. Despite best practice in prophylactic antibiotic therapy, infection rates still reach up to 5% in closed fractures and 30% in open fractures.[1-6] Modern prophylactic approaches such as anti-infective coatings on implants might decrease the infection rate in high-risk situations such as open fractures.[7]

However, once FRI is established, conventional treatment comprising of surgical debridement and prolonged systemic antibiotic therapy fails in approximately 10% to 30% of patients.[8,9] Additional strategies are, therefore, needed to improve treatment.

Focused high-energy extracorporeal shockwave therapy (fhESWT) has been shown to enhance bone healing due to osteo- and angio-inductive effects in animal models[10-12] and in human clinical studies for treating cases of fracture nonunion with success rates ranging from 70% to 90% after 6 months.[13-17] At the present time, acute FRI is regarded as contraindication for fhESWT due to fear of inducing bacteremia.[13,18-20]

fhESWT has been shown to have antibacterial effects in vitro[21,22] and in simple rat models of osteomyelitis.[23,24] The mechanism behind this phenomenon is still unknown, yet bacterial membrane disruption was excluded as an explanation.[22] Any direct antibacterial effect of fhESWT could be beneficial as an adjunct to debridement and systemic antibiotics in the treatment of FRI. Moreover, fhESWT has been shown in a rabbit model by Wang et al[11] to improve vascularity, and this could facilitate antibiotic penetration into infected tissue, and in addition, improve bone healing. Therefore, contrary to current clinical concerns, fhESWT may act as an adjunctive treatment alongside conventional antibiotic treatment in acute FRI by increasing antibiotic penetration to the site of infection and through direct antibacterial activity.

In the present study, our aim was to study the effect of fhESWT in a well-established rabbit model of FRI. fhESWT was evaluated as a stand-alone treatment, as well as in combination with systemic antibiotic therapy. Control groups received either systemic antibiotics alone or no treatment after debridement. The primary outcome measure was infection burden at euthanasia. The secondary outcome measure was bacteriaemia after shockwave therapy.

2 | METHODS

The study was approved by the ethical committee of the canton of Grisons in Switzerland (approval number GR 14_2018). All procedures were performed in an Association for Assessment and Accreditation of Laboratory Animal Care International approved facility and according to the Swiss animal protection law and regulations.

2.1 | Animal model and study design

The animal model applied was a modification of the contaminated plate model by Arens et al.[25] It comprises a mid-diaphyseal osteotomy of the rabbit humerus, created with a 0.44-mm Gigly saw (RISystem, Switzerland). Humeral fixation was achieved with a seven-hole locking compression plate (LCP) and six 2-mm locking screws. The osteotomy was located directly underneath the unused central combi-hole. Inoculation was then performed by pipetting three separate 34 μL injections of a freshly prepared bacterial suspension of a clinical strain of *Staphylococcus aureus* onto the central screw hole overlying the osteotomy and to the adjacent proximal and distal screw holes (prepared as described below). The number of bacteria was chosen based on previous studies where it was found to result in a 100% infection rate.[25] Postoperative radiographs of the operated limb were taken in anteroposterior and lateral view to assure adequate fixation and further radiographs taken once a week thereafter for the remainder of the study.

In a modification to the original model,[25] we performed a revision surgery after 2 weeks of observation, during which time the infection developed without any intervention. Revision comprised of debridement and irrigation with implant retention. Each layer was debrided systematically down to the level of the bone (Figure S1). Visible necrotic tissue was removed, and only viable tissue remained. Viable muscle tissue was assessed using the classic 4Cs: red color, consistency, capillary circulation, and contractility. Irrigation was then performed with 100 mL of standard saline solution NaCl 0.9% and low pressure (bulb syringe, pressure < 15 psi, 103.4 kPa). Debrided tissue (subcutaneous tissue, muscle/fascia, and bone) and irrigation fluid were collected separately for quantitative microbiology in order to quantify the bacterial burden and to confirm all rabbits were indeed infected prior to commencement of any treatment.

Rabbits were randomly assigned to four groups after revision surgery (see Figure 1): group 1: control group (no further treatment, n = 7), group 2: fhESWT group (two sessions at days 2 and 6 after revision, n = 8), group 3: antibiotics group (systemic nafcillin and rifampin for 1 week after revision, n = 8), group 4: combined group (fhESWT at days 2 and 6 + systemic nafcillin and rifampin for 1 week; n = 8). The control group was formed by seven rabbits compared with eight rabbits in the intervention groups. This was accepted because results in the control group were consistent. The rabbits were euthanized 2 weeks after revision (4 weeks after initial surgery and inoculation), allowing for 1 week of antibiotic washout.

2.2 | FhESWT settings and application

FhESWT was applied on days 2 and 6 after revision surgery. Rabbits were sedated, placed in supine position, and received earplugs (rolled sterile gauze swabs). The fur was shortened with a clipper and the plate was palpated through the skin. The location of the plate and the central combi-hole (location of osteotomy) was marked with a surgical pen. Ultrasound gel was applied to allow coupling. The fhESWT machine (LithoSpace Ortho; Jena Medtech) was placed next to the operation table with the flexible therapy head directed onto the fractured limb in anteroposterior direction (Figure 2). The water cushion of the therapy head was then filled so that the focus of the shockwaves lied directly in the osteotomy area. Treatment was

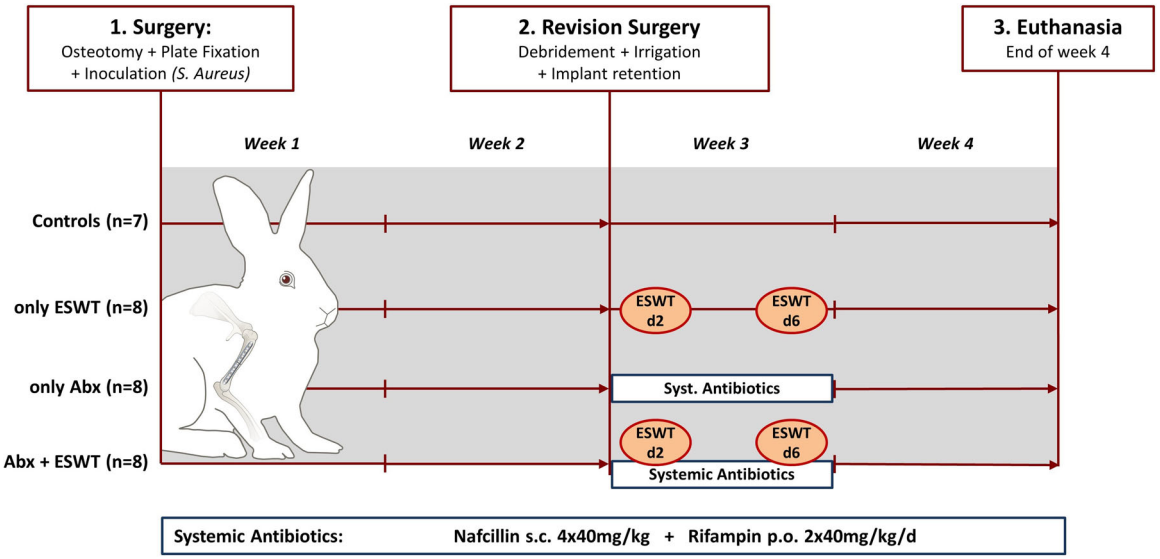

FIGURE 1 Outline schematic of study design. Four groups of rabbits receive an initial surgery with humeral osteotomy, plate fixation and inoculation of *Staphylococcus aureus*. All the rabbits (n = 31) develop infection over 2 weeks. After a revision surgery (debridement, irrigation, and implant retention) systemic antibiotics and focused high-energy shockwave therapy are tested either alone or in combination against a control group that receives no further treatment. ESWT, extracorporeal shockwave therapy [Color figure can be viewed at wileyonlinelibrary.com]

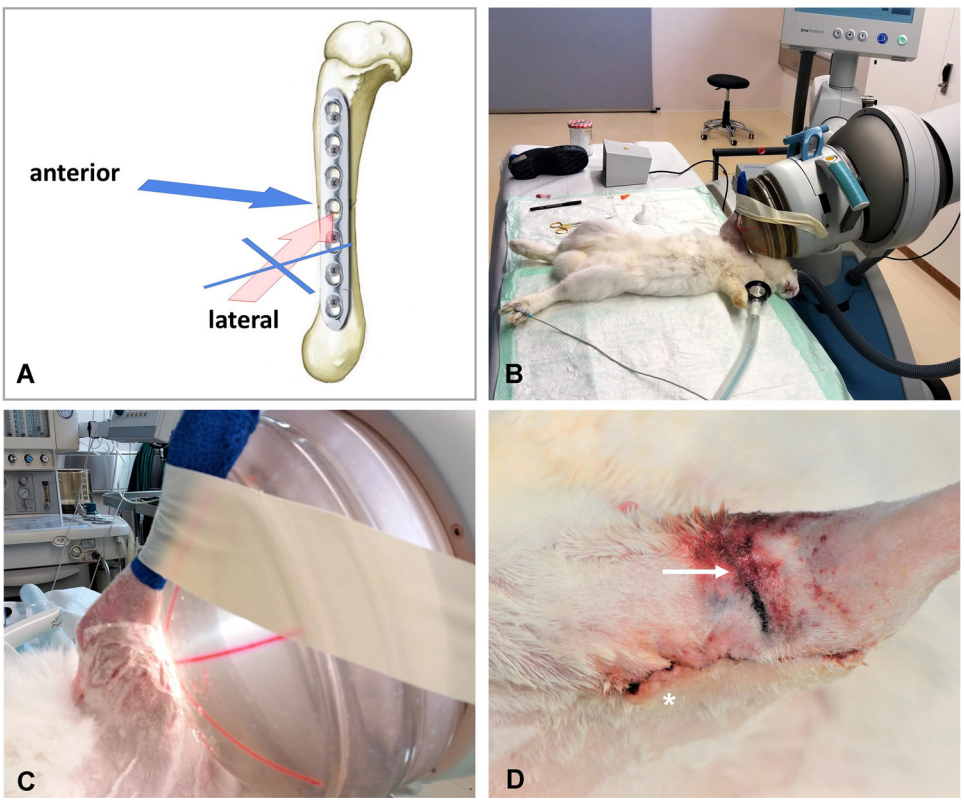

FIGURE 2 Experimental setup during fhESWT. A, Shockwaves applied in anteroposterior direction in order to reach the osteotomy gap, as the plate shields the area underneath. B, Rabbit in sedation is placed in supine position. fhESWT machine (LithoSpace Ortho; Jena Medtech) stands next to the operation table with therapy head aiming at the osteotomy. C, Application of 4000 impulses at 4 Hz with 23 kV. D, Petechia at the target area after fhESWT application (arrow). *, incision from previous surgery; fhESWT, focused high-energy extracorporeal shockwave therapy [Color figure can be viewed at wileyonlinelibrary.com]

performed with 4000 impulses perpendicular to the skin from anterior to posterior, so that shockwaves reached the osteotomy (Figure 2). The settings were: energy flux density 0.4 mJ/mm^2, 23 kV, and 4 Hz.

2.3 | Blood cultures

Blood cultures were taken 30 minutes before and after the shockwave session and before euthanasia. The skin was prepared with alcoholic disinfectant and then 0.5 to 1 mL of full blood was taken from the ear vein directly into preautoclaved and labeled Eppendorf vials. The resulting blood clot was transferred into a Falcon tube with 15 mL of sterile TSB (Oxoid Ltd, Basingstoke, England) and incubated for 24 and 48 hours. Then, 200 μL were plated onto blood agar and selective mannitol salt agar plates for quantitative bacteriology.

2.4 | Antibiotic administration

Antibiotics were administered for seven full postoperative days, starting on day 1 postrevision surgery with Nafcillin (SC 4 × 40 mg/kg/d) and Rifampin (po 2 × 40 mg/kg/d). Rifampin was administered orally mixed with food supplement (Critical Care).

2.5 | Animals

In total, 31 skeletally mature specific-pathogen-free female New Zealand White rabbits (Charles River, Germany) between 28 and 39 weeks of age and a mean bodyweight of 4.11 ± 0.74 kg were included. All animals were screened prior to entry into the study and found to be healthy after standard clinical examination. Approved animals were then allowed to acclimatize to their surroundings for 2 weeks prior to the start of the study. During this time, they were group-housed with a 12-hour dark/light cycle, fed with hay, lettuce, and supplemental feed for rabbits (Biomill, Switzerland). Rabbits were randomized into four treatment groups and surgeons were blinded during the whole study period in order to minimize the effects of subjective bias. After surgery, the animals were single-housed until the end of the observation period.

2.6 | Implants

The seven-hole LCP and locking screws were made of electropolished stainless steel and are commercially available for human medicine (plate: 52 mm long, straight, seven-hole, 2.0 mm LCPs, catalog number: 247.347; screws: 2.0 mm diameter, catalog number: 201.360.97-201.364.97; DepuySynthes, Somerville, NJ). All LCPs and screws were steam autoclaved before surgery.

2.7 | Exclusion criteria and euthanasia

Exclusion criteria were set as described by Arens et al[25] at a weight loss exceeding 15% of the initial body weight within 2 weeks, local infection with severe lameness, persistent swelling, and discharge or signs of systemic infection such as fever, depression, and anorexia. In addition, postoperative peri-implant fracture of the operated bone, and infection-free status at revision were additional exclusion criteria. After the observation period, all animals were humanely euthanized using intravenously administered pentobarbital (Esconarkon; Streuli Pharma AG, Switzerland).

2.8 | Quantitative bacteriology

A clinical *S. aureus* strain (JAR060131), isolated from a patient with an infected hip prosthesis, was used in the present study.[26] The strain is broadly antibiotic susceptible (including nafcillin and rifampin) except for resistance to penicillin. It is available at the Swiss Culture Collection, with accession number CCOS 890. The bacterial inocula were individually prepared in phosphate-buffered saline solution (PBS; Sigma-Aldrich, Switzerland) for each surgery as previously described.[27] The quantitative culture of each inoculum was performed immediately after preparation to check the accuracy of the prepared inoculum. The target average colony-forming unit (CFU) count was 2.0×10^6, with an acceptable range of 9.0×10^5 to 3.0×10^6.

Postmortem quantitative bacterial cultures were performed in all animals for the soft tissue adjacent to the plate, the implants and the humerus in three separate assessments according to the protocol previously described.[25] In addition, any abscesses that were found in soft tissue not directly adjacent to the plate were also separately collected and assessed for quantitative bacteriology. Bacterial growth was checked to determine if it was *S. aureus* by latex agglutination test (Staphaurex; Thermo Fisher Scientific).

2.9 | Organ harvesting

Rabbits were dissected with sterile instruments. Brain, heart, lung, liver, kidney, and spleen were harvested separately, and samples were transferred into glass vials containing sterile PBS. After homogenization of the organs, 200 μL of the sample was placed on blood agar plates and incubated at 37°C for 24 hours. *S. aureus* growth was confirmed with latex agglutination test (Staphaurex Plus; Remel Inc, Lenexa, KS).

2.10 | Statistical analysis

Results are presented as means of each group with the standard error of the mean. Normally distributed data were analyzed by a one-way analysis of variance, followed by Tukey's range test. In the case of

nonnormal distribution Kruskal-Wallis test and Dunn's correction were performed. In all cases, significance was set at $P < .05$. Prism 7 software was used for all statistical tests (GraphPad Software Inc, La Jolla, CA).

3 | RESULTS

3.1 | Animal welfare

All 31 rabbits included in this study tolerated the surgeries, the antibiotic administration, and the fhESWT sessions. No rabbits had to be excluded from the study. Rabbits receiving fhESWT showed no sign of pain or discomfort during or after the application. Small petechia appeared as expected and confirmed the application of the shockwaves at the correct target area (Figure 2). There was no sign of wound breakage or delayed wound healing in these rabbits.

All rabbits experienced weight loss, with a maximum of 15% after 2 weeks, although differences between the groups were not significant. The body temperature in all rabbits was within the normal range throughout the study (38.3°C-39.5°C) (data not shown).

3.2 | Microbiology

Inocula ranged from 1.45×10^6 CFU to 3.74×10^6 CFU (mean 2.15×10^6 CFU). As expected from previous studies,[27] infection with *S. aureus* was established in all animals with this inoculum. At the revision surgery after 2 weeks (prior to any treatment), all debrided material and irrigation fluid from the groups revealed high bacterial counts that were confirmed as *S. aureus* with no differences in burden between the groups (Figure 2).

At euthanasia, all animals from the control group were still infected and almost all samples had high bacterial counts. Rabbits receiving fhESWT were also all infected and displayed similar high bacterial burden (total CFU: control: 2.60×10^7 vs fhESWT: 1.87×10^7 CFU/mL).

Both the group receiving systemic antibiotics alone and the group receiving antibiotics in combination with fhESWT, showed a significant decrease of total bacterial load ($P = .0008$ each), and a reduced infection rate compared with the control group, although there were no differences between these two treatments (Figure 3). Treatment with systemic antibiotic therapy over the course of 1 week achieved a significant reduction of bacteria in our model in all investigated samples but did not result in complete eradication in all animals. The total CFU count of all samples was lower when antibiotics and fhESWT were combined, compared with antibiotics only (4.44×10^4 vs 1.09×10^3 CFU/mL). However, this difference was not significant ($P = .38$). With regard to the different samples bacterial burden was lower in every sample when fhESWT was combined with antibiotics, with the greatest impact on the implant sample (implant: 1.4×10^3 vs 13 CFU/mL, $P = .08$; soft tissue: 3.6×10^2 vs 0 CFU/mL, $P = .3$; bone: 4.2×10^4 vs 1.08×10^3 CFU/mL, $P = .4$) (Figure 3). A comparison of bacterial load between groups in the bone, soft tissue andimplant is shown in Figure 4.

3.3 | Blood cultures and organ harvesting

All blood cultures taken before and after the shockwave sessions were culture negative for *S. aureus*. Harvested organs resulted in

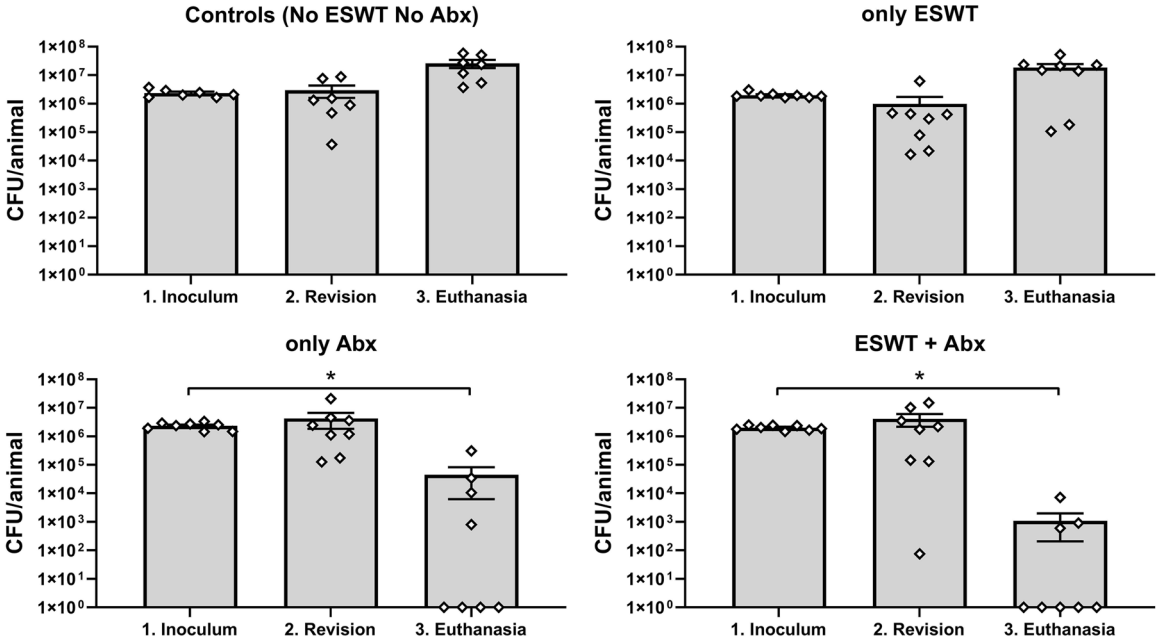

FIGURE 3 Colony-forming units (CFU) of *Staphylococcus aureus* in the four study groups at three different time points: first bar: CFU in the initial inoculum that rabbits receive after osteotomy and fixation, second bar, CFU in the debrided material and irrigation fluid retrieved during revision surgery and finally in the third bar, postmortem CFU in the soft tissue, bone, and on the implants. Data are expressed as means and standard error of the mean. *$P < .05$. ESWT, extracorporeal shockwave therapy

bacterial growth in 36% of the samples. Especially in the lung, a heterogenous bacterial spectrum was detected. However, all colonies were found to be negative with the *S. aureus* latex agglutination test.

4 | DISCUSSION

FRI is one of the most feared complications in orthopedic trauma surgery as it can lead to fracture nonunion, significant bone loss and protracted treatment protocols. FRI requires stability,[28] meaning implants often cannot be removed completely in revision surgery but have to be retained or at least exchanged by temporary implants. The implant, however, facilitates biofilm formation, thus impeding curative antibiotic treatment.[28] In fact, conventional treatment often fails, with relapsing infections in 10% to 30%,[8,9] with biofilm believed to be largely responsible for this. fhESWT was shown to have direct antibacterial effects[20,29] and osteo- and angioinductive effects.[13-17] These properties could facilitate antibiotic penetration into infected tissue and allow earlier implant removal after bone healing, making this a promising candidate for supplementary treatment. However, at the present time, acute infection is regarded as a contraindication for ESWT since tissue damage and microlesions might be a risk for bacteremia and potentially sepsis.[13,20]

In our model, the combination of fhESWT and systemic antibiotics (rifampin and nafcillin) resulted in an average 100-fold reduction of total CFU compared with antibiotic treatment alone. Although not statistically significant, a bacterial reduction was found across all three different samples (soft tissue, bone, implant). The reduction of bacteria on the implants was most significant (1.4×10^3 vs 13 CFU/mL, $P = .08$). This effect on the implant is of special interest as it suggests that shockwaves might facilitate in situ eradication of biofilm on foreign bodies. This aspect warrants further investigation, particularly for cases where implant retention is required.

In our model, we did not find bacterial spreading into the blood due to the application of shockwave directly into the infected area. All blood cultures and harvested indicator organs were culture negative for *S. aureus* despite the high intensity and amount of impulses. This finding is consistent with Gollwitzer et al,[19] who also could not find any sign of bacterial spreading due to fhESWT in a rabbit model of chronic osteomyelitis. However, in that model, the energy of the shockwave was lower (0.3 vs 0.4 mJ/mm^2) with less impulses (1500 vs 8000) and it lacked the creation of an osteotomy. Therefore, our new data further extend the indication that shockwave therapy may not be a risk for bacteremia, even when given with higher energy/impulses.

The timing of shockwaves at days 2 and 6 was based on the clinical situation, since human patients are commonly hospitalized for at least 10 days to receive IV antibiotics. Taking into consideration that fhESWT sessions require general anesthesia, it appears feasible to organize two sessions (45 minutes each) and not more within this timeframe. Additional treatments could, in theory, increase the impact fhESWT may have on treating an infection, but such further applications may not be clinically achievable. Previous in vitro studies by Gerdesmeyer et al. demonstrated exponential increase in bacterial killing with increasing impulses and energy level.[22] The application of 4000 impulses at 0.59 mJ/mm^2 significantly reduced CFU count of *S. aureus* to 60% under growth-promoting conditions. In our in vivo model fhESWT alone, although applied twice (8000 impulses in total) had no effect on bacterial growth without additional antibiotics as measured at euthanasia. Only in the "soft tissue" and "implant" sample were CFU counts lowered on average by factor 100 and 10, respectively (Figure 4). However, there was a huge variation across results and these differences were not significant. Antibacterial effects due to fhESWT alone without antibiotics appear to have no relevant effect in our model.

Our finding supports previous work from Inanmaz et al,[23] who also showed in an experimental rat model of implant-related osteomyelitis that fhESWT alone was not able to reduce CFU counts. In that study, the combination of fhESWT with teicoplanin significantly lowered bacterial burden compared with animals that received teicoplanin only. However, in that study, the femoral implant (K-wire) was removed before the treatment began, rendering this model not ideal to resemble the clinical case of acute FRI, or cases where the implant must be retained.

Preclinical infection models that have an osteotomy, bone defect or fracture are much more difficult to treat. Possible reasons are a lack of stability, damaged and necrotic tissue, and impaired

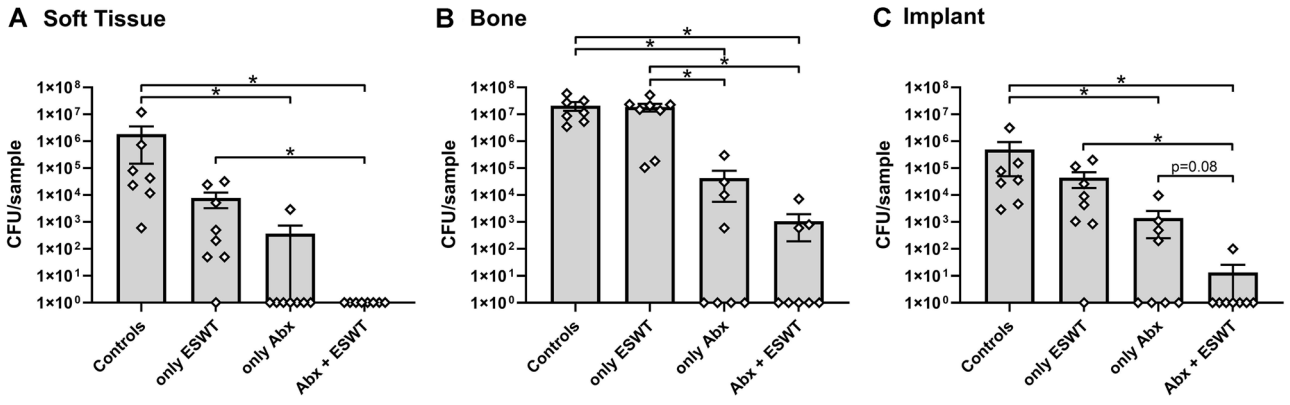

FIGURE 4 Colony-forming units (CFU) of *S. aureus* in the four study groups after euthanasia in three different samples. A, Soft tissue. B, Bone. C, Implant (plate and screws). Data are expressed as means and standard error of the mean. *$P < .05$. ESWT, extracorporeal shockwave therapy

vascularity at the bone defect.[28] Thus, therapies that eradicate infection in simpler models can fail when a bone defect is present.

In our model, the application of a clinically relevant antibiotic regimen (teicoplanin is used as reserve antibiotic) and using a model with implant retention, offers greater clinical relevance to the present data.

Another study by Qi et al[24] demonstrates that the combination of gentamicin and fhESWT in a rat model is more effective than the use of both treatments alone. The implant is in fact retained during fhESWT in this model and 4 weeks of peritoneal gentamicin are administered, but the model lacks the creation of a complete osteotomy and sufficient stable osteosynthesis. The stabilization of the fracture is of utmost importance since instability of the fracture promotes infection.[28,30]

Implant retention in this model could be a reason for failed treatment success, as *S. aureus* rapidly forms biofilm on foreign bodies and could potentially evade the immune system and conventional treatment.[31] However, the size of the rabbit humerus does not allow exchange of the plates and screws without risking the creation of additional fractures. Furthermore, from the clinical perspective, 2 weeks after fracture fixation this would be deemed as an "early" FRI, where biofilm is regarded to be immature and suitable for debridement and systemic antibiotic treatment.[32]

Compared with the striking effect, the antibiotic treatment had alone on lowering the bacterial burden, the potential additional effect of fhESWT was not as pronounced. The clinical benefit of fhESWT in the treatment of FRI could, in any case, extend beyond infection control and influence bone healing. This was not an outcome measure in this study, as we first wanted to determine the effect soon after treatment, rather than let the osteotomy progress for many weeks without treatment where the immediate effects could be lost. In order to determine if fhESWT can have an influence on fracture healing after FRI needs a different study design.

5 | CONCLUSION

FhESWT displayed a consistent trend of reducing bacterial numbers in all tested tissues and on the implant but was not sufficient to be statistically significant in this in vivo rabbit model. In certain difficult-to-treat infections, the addition of fhESWT might be beneficial, particularly in cases where the implant needs to be retained since fhESWT seemed to have the greatest effect against biofilm on the surface of the implant. Importantly, fhESWT appears to be safe in this model as no signs of bacteremia occurred. These experimental results cannot be directly generalized to human medicine, but they can give an idea of what effect size one can expect with this intervention.

ACKNOWLEDGMENTS

This work was funded by AOTrauma as part of the Clinical Priority Program Bone Infection. We thank Jena Medtech (Storz Medical) for providing us with the LithoSpace Ortho. Iris Keller, Tanja Schmid, Willemijn Boot, Valentina Stenger, and Tim Buchholz, all from AO Research Institute Davos, are acknowledged for their expert assistance in the design, performance, and discussion of the presented work.

AUTHOR CONTRIBUTIONS

JP designed the study, performed surgeries, and fhESWT sessions, did a relevant part of the quantitative microbiology and writing of the final manuscript. AM performed surgeries and fhESWT sessions and did a relevant amount of quantitative microbiology and writing of the final manuscript. JE helped in developing the idea, the study plan, and final discussion of the results. MR and RGR supervised the study and provided expert assistance in the performance of the study. DA and SZ were veterinarians in charge of the animal surgeries, anesthesia protocols, antibiotic administrations, and supervised animal caretaking and assured standards of the Assessment and Accreditation of Laboratory Animal Care International and Swiss animal protection law. TFM provided expert assistance during all steps of the study, supervised and performed laboratory work, discussion of the results, and writing of the final manuscript. All the authors have read and approved the final submitted manuscript.

ORCID

Thomas Fintan Moriarty http://orcid.org/0000-0003-2307-0397

REFERENCES

1. O'Brien CL, Menon M, Jomha NM. Controversies in the management of open fractures. *Open Orthop J*. 2014;8:178-184.
2. Ryan SP, Pugliano V. Controversies in initial management of open fractures. *Scand J Surg*. 2014;103:132-137.
3. Craig J, Fuchs T, Jenks M, et al. Systematic review and meta-analysis of the additional benefit of local prophylactic antibiotic therapy for infection rates in open tibia fractures treated with intramedullary nailing. *Int Orthop*. 2014;38:1025-1030.
4. Darouiche RO. Treatment of infections associated with surgical implants. *N Engl J Med*. 2004;350:1422-1429.
5. Trampuz A, Zimmerli W. Diagnosis and treatment of infections associated with fracture-fixation devices. *Injury*. 2006;37(suppl 2):S59-S66.
6. Papakostidis C, Kanakaris NK, Pretel J, Faour O, Morell DJ, Giannoudis PV. Prevalence of complications of open tibial shaft fractures stratified as per the Gustilo-Anderson classification. *Injury*. 2011;42:1408-1415.
7. Schmidmaier G, Kerstan M, Schwabe P, Südkamp N, Raschke M. Clinical experiences in the use of a gentamicin-coated titanium nail in tibia fractures. *Injury*. 2017;48:2235-2241.
8. Berkes M, Obremskey WT, Scannell B, Ellington JK, Hymes RA, Bosse M. Maintenance of hardware after early postoperative infection following fracture internal fixation. *J Bone Joint Surg Am*. 2010;92:823-828.
9. Tschudin-Sutter S, Frei R, Dangel M, et al. Validation of a treatment algorithm for orthopaedic implant-related infections with device-retention-results from a prospective observational cohort study. *Clin Microbiol Infect*. 2016;22(457):e451-e459.
10. Koolen MKE, Kruyt MC, Zadpoor AA, Öner FC, Weinans H, van der Jagt OP. Optimization of screw fixation in rat bone with extracorporeal shock waves. *J Orthop Res*. 2018;36:76-84.

11. Wang CJ, Wang FS, Yang KD, et al. Shock wave therapy induces neovascularization at the tendon-bone junction. A study in rabbits. *J Orthop Res*. 2003;21:984-989.
12. Lama A, Santoro A, Corrado B, et al. Extracorporeal shock waves alone or combined with raloxifene promote bone formation and suppress resorption in ovariectomized rats. *PLoS One*. 2017;12:e0171276.
13. Everding J, Freistühler M, Stolberg-Stolberg J, Raschke MJ, Garcia P. Extrakorporale fokussierte Stoßwellentherapie zur Behandlung von Pseudarthrosen. *Der Unfallchirurg*. 2017;120:969-978.
14. Cacchio A, Giordano L, Colafarina O, et al. Extracorporeal shock-wave therapy compared with surgery for hypertrophic long-bone nonunions. *J Bone Joint Surg Am*. 2009;91:2589-2597.
15. Furia JP, Juliano PJ, Wade AM, Schaden W, Mittermayr R. Shock wave therapy compared with intramedullary screw fixation for nonunion of proximal fifth metatarsal metaphyseal-diaphyseal fractures. *J Bone Joint Surg Am*. 2010;92:846-854.
16. Notarnicola A, Moretti L, Tafuri S, et al. Extracorporeal shockwaves versus surgery in the treatment of pseudoarthrosis of the carpal scaphoid. *Ultrasound Med Biol*. 2010;36:1306-1313.
17. Raschke M, Rosslenbroich S, Everding J. Pseudarthrosen: Immer 6 Monate warten oder muss früher etwas passieren? *Trauma und Berufskrankheit*. 2017;19:255-259.
18. Sistermann R, Katthagen BD. [Complications, side-effects and contra-indications in the use of medium and high-energy extracorporeal shock waves in orthopedics]. *Z Orthop Ihre Grenzgeb*. 1998;136:175-181.
19. Gollwitzer H, Roessner M, Langer R, et al. Safety and effectiveness of extracorporeal shockwave therapy: results of a rabbit model of chronic osteomyelitis. *Ultrasound Med Biol*. 2009;35:595-602.
20. Horn C, Gerdesmeyer L, von Eiff C, Gradinger R, Gollwitzer H. Energy-dependent stimulatory and inhibitory effects of extracorporeal shock waves on bacteria and on gentamicin activity. *Med Sci Monit*. 2009;15:MT77-MT83.
21. Gerdesmeyer L, von Eiff C, Horn C, et al. Antibacterial effects of extracorporeal shock waves. *Ultrasound Med Biol*. 2005;31:115-119.
22. Horn C, Mengele K, Gerdesmeyer L, Gradinger R, Gollwitzer H. The effect of antibacterial acting extracorporeal shockwaves on bacterial cell integrity. *Med Sci Monit*. 2009;15:BR364-BR369.
23. Inanmaz ME, Uslu M, Isik C, Kaya E, Tas T, Bayram R. Extracorporeal shockwave increases the effectiveness of systemic antibiotic treatment in implant-related chronic osteomyelitis: experimental study in a rat model. *J Orthop Res*. 2014;32:752-756.
24. Qi X, Zhao Y, Zhang J, et al. Increased effects of extracorporeal shock waves combined with Gentamicin against *Staphylococcus aureus*biofilms in vitro and in vivo. *Ultrasound Med Biol*. 2016;42:2245-2252.
25. Arens D, Wilke M, Calabro L, et al. A rabbit humerus model of plating and nailing osteosynthesis with and without *Staphylococcus aureus* osteomyelitis. *Eur Cell Mater*. 2015;30:148-161.
26. Campoccia D, Montanaro L, Moriarty TF, Richards RG, Ravaioli S, Arciola C. The selection of appropriate bacterial strains in preclinical evaluation of infection-resistant biomaterials. *Int J Artif Organs*. 2008;31:841-847.
27. Moriarty TF, Campoccia D, Nees SK, Boure LP, Richards RG. In vivo evaluation of the effect of intramedullary nail microtopography on the development of local infection in rabbits. *Int J Artif Organs*. 2010;33:667-675.
28. Schmidt AH, Swiontkowski MF. Pathophysiology of infections after internal fixation of fractures. *J Am Acad Orthop Surg*. 2000;8:285-291.
29. Horn C, Mengele K, Gerdesmeyer L, Gradinger R, Gollwitzer H. The effect of antibacterial acting extracorporeal shockwaves on bacterial cell integrity. *Med Sci Monit*. 2009;15:BR364-BR369.
30. Sabaté Brescó M, O'Mahony L, Zeiter S, et al. Influence of fracture stability on *Staphylococcus epidermidis* and *Staphylococcus aureus* infection in a murine femoral fracture model. *Eur Cell Mater*. 2017;34:321-340.
31. Moormeier DE, Bose JL, Horswill AR, Bayles KW. Temporal and stochastic control of *Staphylococcus aureus* biofilm development. *mBio*. 2014;5:e01341-14.
32. Metsemakers WJ, Kuehl R, Moriarty TF, et al. Infection after fracture fixation: current surgical and microbiological concepts. *Injury*. 2018;49:511-522.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Puetzler J, Milstrey A, Everding J, et al. Focused high-energy extracorporeal shockwaves as supplemental treatment in a rabbit model of fracture-related infection. *J Orthop Res*. 2020;38:1351–1358. https://doi.org/10.1002/jor.24565