Focused ultrasound treatment of abscesses induced by methicillin resistant \textit{Staphylococcus aureus}: Feasibility study in a mouse model

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\textbf{Purpose:} To study the therapeutic effect of focused ultrasound on abscesses induced by methicillin-resistant \textit{Staphylococcus aureus} (MRSA). MRSA is a major nosocomial pathogen where immunocompromised patients are prone to develop infections that are less and less responsive to regular treatments. Because of its capability to induce a rise of temperature at a very precise location, the use of focused ultrasound represents a considerable opportunity for therapy of localized MRSA-related infections.

\textbf{Methods:} 50 \( \mu \text{l} \) of MRSA strain USA400 bacteria suspension at a concentration of 1.32 ± 0.5 \times 10^5 colony forming units (cfu)/\( \mu \text{l} \) was injected subcutaneously in the left flank of BALB/c mice. An abscess of 6 ± 2 mm in diameter formed after 48 h. A transducer operating at 3 MHz with a focal length of 50 mm and diameter of 32 mm was used to treat the abscess. The focal point was positioned 2 mm under the skin at the abscess center. Forty-eight hours after injection four ultrasound exposures of 9 s each were applied to each abscess under magnetic resonance imaging guidance. Each exposure was followed by a 1 min pause. These parameters were based on preliminary experiments to ensure repetitive accurate heating of the abscess. Real-time estimation of change of temperature was done using water-proton resonance frequency and a communication toolbox (matMRI) developed inhouse. Three experimental groups of animals each were tested: control, moderate temperature (MT), and high temperature (HT). MT and HT groups reached, respectively, 52.3 ± 5.1 and 63.8 ± 7.5 \(^\circ\)C at the end of exposure. Effectiveness of the treatment was assessed by evaluating the bacterial amount of the treated abscess 1 and 4 days after treatment. Myeloperoxidase (MPO) assay evaluating the neutrophil amount was performed to assess the local neutrophil recruitment and the white blood cell count was used to evaluate the systemic inflammatory response after focused ultrasound treatment.

\textbf{Results:} Macroscopic evaluation of treated abscess indicated a diminution of external size of abscess 1 day after treatment. Treatment did not cause open wounds. The median (lower to upper quartile) bacterial count 1 day after treatment was 6.18 × 10^3 (0.76 × 10^3–11.18 × 10^3), 2.86 × 10^3 (1.22 × 10^3–7.07 × 10^3), and 3.52 × 10^3 (1.18 × 10^3–6.72 × 10^3) cfu/100 \( \mu \text{l} \) for control, MT and HT groups, respectively; for the 4-day end point, the count was 1.37 × 10^3 (0.67 × 10^3–2.89 × 10^3), 1.35 × 10^3 (0.09 × 10^3–2.96 × 10^3), and 0.07 × 10^3 (0.03 × 10^3–0.36 × 10^3) cfu/100 \( \mu \text{l} \) for control, MT and HT, showing a significant reduction (\( p = 0.002 \)) on the bacterial load four days after focused ultrasound treatment when treating at high temperature (HT). The MPO amount remained unchanged between groups and days, indicating no change on local neutrophil recruitment in the abscess caused by the treatment. The white blood cell count remained unchanged between groups and days indicating that no systemic inflammatory response was caused by the treatment.
1. INTRODUCTION

Abscesses are confined bacterial infections that consist of a localized lesion with an accumulation of pus, surrounded by a surrounding capsule built by fibroblasts and inflammation. It is formed by the interaction of the immune system of an individual with the bacteria. The initiation and purpose of the capsule formation is ambiguous, it has been suggested that it is initiated by the host to keep the infection localized but there are indications that it could be driven by the microorganism to enable undisturbed replication of the bacteria and to inhibit the immune defense. In addition to inhibiting the clearance of the bacteria by immune cells, the capsule makes it difficult for antibiotics to reach the bacteria and treatment success is unlikely if only antibiotics are used. Incision and drainage, with or without additional antibiotic therapy is the standard therapy for abscesses.

However, there are delicate or hard accessible areas that would profit from an alternative treatment, not causing scars or involving extensive surgery. Abscesses that would profit from noninvasive therapy include those appearing in locations such as groin and axillary region caused by hidradenitis suppurativa or perianal region caused by Crohn’s disease. Hard accessible areas include liver, kidney, brain, breast abscesses, and osteomyelitis.

Skin and soft tissue abscesses are mainly caused by *Staphylococcus aureus* (*S. aureus*). Those caused by methicillin resistant *S. aureus* (MRSA) are in particular a growing health concern in hospitalized and chronically ill patients, and are becoming more common in healthy population.

Focused ultrasound (FUS) can potentially be used for the treatment of these localized abscesses. Treatment can be provided by focusing the acoustic waves and inducing localized heating without causing damage to surrounding tissue. It has been used in diverse applications for the treatment of malignant and benign tumors. Real-time monitoring of the temperature can be provided by magnetic resonance imaging in order to obtain a precise and controlled treatment.

The concept using heat as a tool for disinfection or reduction of bacteria amounts is not new and for *S. aureus* this has been used for safe food handling. In the context of treating and preventing infections, heat application via noncontact radiant heat pads has been used in studies for topical treatment of pressure sores and intraoperatively to decrease surgical site infection. Focused ultrasound can become a tool to obtain this therapeutic effect in a very controlled and defined way, with the ability to reach deep tissues.

To establish a model that is of relevance to current bacteria evolution, we focused on treating abscesses caused by MRSA, in particular by a strain that is present in hospital and community settings known as USA-400/CMRSA-7. This strain along with USA-3000/CMRSA-10 are two of the major community-associated MRSA strains that often cause skin and soft tissue infections in Canada and USA.

To evaluate the therapeutic effect of focused ultrasound treatment, we studied two main responses to the treatment. We verified that the treatment could effectively reduce the amount of bacteria within an abscess and we investigated the early immunological response to the treatment by indirectly quantifying the neutrophil amount in the abscess and surrounding tissue.

Neutrophils are early responders to bacterial infection. Representing phagocytic cells, neutrophils engulf microorganisms and expose them to microbicidal products. One of the enzymes that contribute to the production of microbidental substances is myeloperoxidase (MPO). MPO represents 5% of all proteins within a neutrophil and since it correlates with the amount of neutrophils, MPO assays can be used to evaluate the amount of neutrophils.

In the present study, we validated the therapeutic response after using focused ultrasound on an *in vivo* animal subcutaneous MRSA abscess model caused by a common community acquired bacteria. A MR guided focused ultrasound animal system was used to treat and control the exposures to the abscess. The efficacy of two different temperature treatments on reducing the amount of bacteria and inducing the recruitment of neutrophils was evaluated.

2. MATERIAL AND METHODS

2.A. Animals

Fifty-five female BALB/c mice (Mus musculus, Balb/cAnNCrl, Charles River, Wilmington, MA), aged 7–12 weeks were used. The mice were housed at controlled environmental conditions with unrestricted food and water under an approved protocol according to institutional and Canadian Council of Animal Care guidelines (Lakehead University Animal Care Committee).

2.B. *S. aureus* strain and culture

Community-acquired methicillin resistant *Staphylococcus aureus*, strain USA-400 (C04-8830, pulse field type USA-400/CMRSA7), provided by Dr. Zhang, University of Calgary, AB, Canada, was used to induce the abscess. The bacterium was cultivated on tryptic soy agar (Becton, Dickinson and Company, Franklin Lakes, NJ), at 37 °C. The heavy streak
of a single colony was transferred into Brain and Heart Infusion Broth (BHI) (Becton, Dickinson and Company, Franklin Lakes, NJ) and cultivated overnight at 37 °C at 225 rpm. The bacteria suspension was then diluted 1:1000 in BHI and cultivated at 37 °C and 225 rpm to obtain an optical density at 600 nm (OD600) of 0.4. This density value was determined in preliminary animal studies to lead to successfully induce subcutaneous abscesses that were contained and non-necrotic with the USA-400 strain. The bacteria suspension was washed three times with sterile 0.9% saline and centrifuged at 5000g for 10 min. Before filling the syringes for injection, the concentration of the bacteria suspension was adjusted to an OD600 of 0.03. This was established in preliminary experiments to lead to concentrations around 2.4 × 10^6 colony forming units (cfu)/ml causing contained, non-necrotic subcutaneous abscesses with a diameter between 4 and 7 mm 2 days after injection.

2.C. Abscess model

Mice were anesthetized with 2% isoflurane (Baxter International Inc., Deerfield, IL) and the left flank and back were shaved and depilated with Nair® cream (Church & Dwight Co., Princeton, NJ). The depilated area was disinfected with ethanol and 50 μl of bacterial suspension containing in average 1.32 ± 0.5 × 10^5 cfu was injected subcutaneously. Animals received oral pain medication 24 h before bacterial injection (1.6 mg/ml acetaminophen on drinking water, Mead Johnson & Company, Glenview, IL).

2.D. FUS treatment

After bacterial injection the abscess presence was verified and the skin area around and above the abscess was depilated. Animals were anesthetized using 2% isoflurane, placed on a focused ultrasound animal gantry (FUS Instruments, Toronto, Canada) and imaged with a 3T MRI (Achieva, Philips Healthcare) using a small flex coil (Philips Healthcare). Figure 1 shows a setup for an experiment with the animal placed on the gantry on top of a water tank where the FUS transducer is embedded and directed toward the animal. MR imaging was performed to visualize the transducer and mouse using sagittal and transverse T1-weighted images (GRE, FOV = 120 × 120 × 48 mm, Voxel size = 0.5 mm, slice thickness = 2 mm, TE/TR = 2.5/4.9 ms, flip angle = 35°, acquisition matrix = 120 × 100, reconstruction matrix = 240 × 240, 2 NEX). Localization of the abscess was done using coronal T1 images (GRE, FOV = 80 × 80 × 10 mm, Voxel size = 0.33 mm, slice thickness = 0.5 mm, TE/TR = 28/56 ms, flip angle = 10°, acquisition matrix = 88 × 87, reconstruction matrix = 240 × 240, 4 NEX).

A single-element FUS transducer (model 10-09-11TBHC, FUS Instruments, Toronto, Canada) operating at 3 MHz with a focal length of 50 mm and diameter of 32 mm was used to treat the abscess. Figure 2 shows the ultrasound lateral and acoustical axis beam profiles for the transducer. The focal point was positioned 2 mm under the skin at the abscess center and four ultrasound exposures of 9 s each were applied in a square grid of 1 × 1 mm and a pause of 1 min between exposures. Real-time estimation of change of temperature was done using a communication toolbox (matMRI) developed in our laboratory using water-proton resonance frequency (PRF). Temperature maps were calculated on a coronal plane (FOV = 80 mm, Voxel size = 1 mm, slice thickness = 3 mm, TE/TR = 16/23 ms, flip angle = 19°, acquisition matrix = 68 × 63, reconstruction matrix = 80 × 80, ETL

FIG. 1. Setup for animal focused ultrasound treatment of abscess under MRI guidance.

FIG. 2. Normalized beam profiles: (a) acoustical and (b) lateral axis of the transducer operated at a 3 MHz central frequency where 0 is the geometrical focus.
The exposure parameters were defined based on preliminary experiments in order to obtain the set temperature when heating the abscess. After 9 s the temperature reached the set point and the waiting time of 1 min between exposures was used to ensure that tissues had returned to the baseline body temperature in order to reliably use PRF thermometry maps. An example of the interface used for the targeting and control of the exposures is shown in Fig. 3. On the first three images the user could locate the transducer focus and target abscess, and after the FUS exposure was started the real time temperature mapping confirmed the appearance and value of the temperature reached for each exposure.

Three experimental groups were treated: moderate temperature (MT) (n = 19), high temperature (HT) (n = 18), and control (n = 18). A calibration on a phantom was performed to obtain and set the power to reach a MT of 52 °C and a HT of 64 °C at the end of exposure. The control group received no treatment, but underwent the same preparation and imaging and stayed anaesthetized in the MRI for the same duration than the treated animals. The animals were sacrificed at day 1 (n = 29) and day 4 (n = 26) after treatment. All animals received premedication with 0.05 mg/kg buprenorphine hydrochloride (Schering-Plough, Kenilworth, NJ) immediately before focused ultrasound treatment and every 12 h afterwards.

2.E. Histology and tissue processing

Animals were euthanized by exsanguinations via cardiac puncture to collect blood using sodium heparin (Sandoz, Holzkirchen, Germany) as anticoagulant. Red blood cells were lysed with Unopette™ (Becton, Dickinson and Company, Franklin Lakes, NJ) and white blood cell count was performed using a hemocytometer (Hausser Scientific, Horsham, PA).

The abscess, an area of 4 cm² of skin around the abscess and spleen were then removed on sterile conditions. Half the skin and half of the abscess were inserted into 10% buffered formalin (Fisher Scientific, Hampton, NH) for fixation. After fixation in formalin, the samples were processed, underwent an ethanol series and were embedded in paraffin, cut, and mounted on glass. Haematoxylin and eosin (H.E.), gram and Masson’s trichrome strains were obtained.

The remaining half of the abscess was divided in two parts for bacterial count and MPO evaluation.

2.F. Bacterial count

The abscess section used for bacterial count was weighted and added to 100 μl sterile 0.9% saline and kept on ice while it was homogenized with a sterile hand held tissue grinder (Pellet Pestle® by Kimble-Kontes, Vineland, NJ). A dilution series was prepared and the resulting bacterial suspension was spread on Tryptic Soy Agar plates. The plates were cultured for 24 h at 37 °C, after which single colonies were counted. The same procedure was used for bacterial count on the spleen.

Because the process to obtain the bacterial count is destructive it was not possible to compare the bacterial count before and after treatment. To provide a bacterial count baseline, a control group with abscess but no treatment at each sacrifice time point was used. In order to ensure that any change was caused by the treatment and not by animal manipulation, the control animals were subjected to the same imaging, handling, and treatment time with the FUS transducer turned off.

2.G. Myeloperoxidase and protein assays

The skin and abscess used for MPO evaluation were weighted and snap frozen in liquid nitrogen upon collection. They were then removed from the liquid nitrogen and inserted into ice cooled 0.5% hexadecyltrimethylammonium bromide (HTAB) (Fisher Scientific, Hampton, NH) in 50 mM phosphate buffer, pH 6.0. The dilution factor of tissue to HTAB
buffer was 1:60. The tissue sample was constantly kept on ice and minced in the HTAB buffer for 1.5 min. Afterward, the tissue was homogenized with a PowerGen homogenizer (Fisher Scientific, Hampton, NH) for ten times 5 s at the highest speed. After homogenization, the samples were sonicated (Q500, QSonica, Newtown, CT) at 20% amplitude for seven times 10 s and centrifuged at 6000g for 20 min at 4 °C. The supernatant was then pipetted off and passed through a 27 G needle before dividing into 50 μl aliquots that were kept at −80 °C for MPO assays and −20 °C for protein assays.

We performed a MPO assay using human myeloperoxidase (EC 1.11.2.2, EMD Biosciences, Billerica, MA) as the standard. A series dilution of the prepared samples and standard was obtained using sodium acetate buffer pH 6.0 (100 mM NaCl, 50 mM sodium acetate). Wells were then filled with 7 μl of diluted sample or standard and topped up with 200 μl of a 0.167 mg/ml o-dianisidine and 0.0005% hydrogen peroxide mix diluted in 50 mM phosphate buffer (Sigma Aldrich, St. Louis, MO). The change in absorbance was read at 450 nm every 30 s for 90 s with a plate reader (Synergy™ 4, BioTek Instruments, Inc., Winooski, VT) using the program Gen 5™ v1.10.8 (BioTek Instruments, Inc., Winooski, VT) for analysis. MPO content for the samples was evaluated based on the maximal velocity (Vmax) using a linear regression of MPO concentration as a function of the absorbance obtained from the EC 1.11.2.2 MPO standard.

Protein was quantified using a modified Bradford assay using bovine serum albumin as the standard. Grids of 1.5 cm² were drawn on Whatman™ filter paper Grade 3 (Whatman plc, Maidstone, UK) and 10 μl of sample and standard were pipetted onto the filter paper. The paper was dried, and then rinsed in methanol (Thermo Fisher Scientific, Waltham, MA) for 40 s. After thorough drying, the filter paper was stained with 0.5% Coomassie Blue-G (Sigma Aldrich, St. Louis, MO) in 7% acetic acid (Sigma Aldrich, St. Louis, MO) for 30 min on a Belly Dancer® shaker (Stovall Life Sciences, Poesta, IA). The filter paper was destained for 1.5 h in 7% acetic acid on the belly dancer shaker and then dried. Single squares of 1.5 cm² were inserted into tubes and 1 ml extraction buffer was added (66% methanol, 33% water, 1% ammonium hydroxide), mixed and centrifuged at 5000 rpm for 2 min. Then, 300 μl were placed in 96 well plates and the absorbance was read at 600 nm (PowerWave™ XS, BioTek Instruments, Inc., Winooski, VT) using the program KCJunior™ (BioTek Instruments, Inc., Winooski, VT) for analysis.

### 2.H. Statistics

Data analysis was performed using R (Ref. 24) and SigmaPlot™ 12 (Systat Software Inc., San Jose, CA). Data were tested for normality and homogeneity of variances using a Shapiro-Wilks test and a Bartlett’s test, respectively. A resulting p-value of less than 0.05 was considered to be significant. Parametric data sets on natural logarithmic scale were analyzed using one-way ANOVAs, followed by post hoc Tukey HSD.

### 3. RESULTS

The abscess formation as well as the focused ultrasound procedure was well tolerated by all animals. Immediately after focused ultrasound the treated area could be easily depicted since the heating was performed at a shallow location and a burn was observed 24 h after the procedure on the skin region where ultrasound was concentrated (Fig. 1). During dissection no secondary lesions were observed on any of the animals and the heating was contained to the skin and abscess, with no lesion observed on muscle beneath the abscess. No symptoms of distress were observed in either group. The average maximum reached temperature was 52.3 ± 5.1 and 63.8 ± 7.5 °C for the MT and HT groups, respectively. Figure 4 shows the temperature as a function of time averaged for the different experimental groups.

Figure 5 shows examples of treated mice end points 1 and 4 days after procedure. The superficial treated region could be depicted for MT and HT groups as a red area with no blisters.
limited to the target region. This region was larger in animals that underwent HT treatment.

3.A. Bacterial count

The average weight of the abscess sections used for bacteria count was $2.16 \pm 1.38$ mg. The bacterial count was reported as cfu on a $100 \mu l$ homogenized sample. Figure 6 shows the bacterial colony forming units as a function of the experimental group and analysis day. At the 1-day end point, the median (lower to upper quartile) bacterial count was $6.18 \times 10^3 (0.76 \times 10^3–11.18 \times 10^3$ IQR $10.43 \times 10^3)$, $2.86 \times 10^3 (1.22 \times 10^3–7.07 \times 10^3$ IQR $5.85 \times 10^3)$, and $3.52 \times 10^3 (1.18 \times 10^3–6.72 \times 10^3$ IQR $5.53 \times 10^3)$ cfu/100 $\mu l$ for control, MT, and HT groups, respectively, with no significant difference between the groups. At the 4-day end point, the median (lower to upper quartile) bacterial count was $1.37 \times 10^3 (0.67 \times 10^3–2.89 \times 10^3$ IQR $2.22 \times 10^3)$, $1.35 \times 10^3 (0.09 \times 10^3–2.96 \times 10^3$ IQR $1.83 \times 10^3)$, and $0.07 \times 10^3 (0.03 \times 10^3–0.36 \times 10^3$ IQR $0.26 \times 10^3)$ cfu/100 $\mu l$ for control, MT, and HT groups, respectively. The difference between HT and the other groups was significant 4 days after focused ultrasound ($p = 0.002$). Except for one case, spleen samples did not grow any bacteria. One of the mice undergoing HT was found to have 34 cfu/100 $\mu l$ of bacteria in the complete spleen sample (endpoint day 1). The affected mouse had a WBC count within the normal range and did not show any fever, signs of distress or acute illness in the days preceding the euthanasia. There was a significant decrease of bacterial count from day 1 to day 4 for the HT group ($p = 0.001$), whereas the other groups did not show a significant decrease between endpoint days.

3.B. Myeloperoxidase assay and white blood cell count

The MPO levels were normalized to the amount of protein to account for differences on abscess weight between samples. The normalized MPO levels as a function of the experimental group and endpoint day can be seen in Fig. 7(a). The relative MPO levels were used as a measurement of the presence of neutrophils and it showed no significant difference between the experimental groups. This suggests that there is no additional infiltration of neutrophils that can be detected by MPO levels. The WBC count was used as a measurement to assess a systemic inflammatory response and it showed no significant difference between the experimental groups with an overall average of $8600 \pm 4500$ WBC/mm$^3$ [Fig. 7(b)]. The MPO levels were also not significantly different between experimental groups for animals without an abscess, but these animals did have a significantly lower MPO level ($p < 0.0001$) compared to animals with an abscess (Fig. 8). This suggests that the FUS treatment does not cause a
significant increase on neutrophil recruitment and the changes on MPO levels are due mainly to the host response to the bacteria.

3.C. Histology

The histology slides of the treatment area clearly showed heat damage and inflammation in the skin, as well as in subcutaneous muscle layer and subcutaneous fat tissue for both MT and HT treatment at all endpoints. Figure 9 shows pictures of an abscess from each group at day 1 after treatment where the typical structure of the abscess can be observed. The slides are taken at different locations of the abscess where the skin and muscle damage (d) caused by the FUS treatment could be better observed. The aspect of the abscess was very uniform for all control animals but it varied between treated animals depending on the temperature and the proximity to the treatment region. For some cases, abscess-like tissue was visible but the structure was disrupted, a border was not clearly present, and the shape was not as regular as the control abscess [Figs. 10(a) and 10(b)]. In cases where the abscess underneath the treated area was still differentiated with no apparent structural change observed on HE histology, evidence of capsule disruption was depicted on Masson’s trichrome stains as loss of the green dense collagen inner layer around the abscess [Fig. 10(c)]. The disruption of the abscess structure was observed at endpoint day 4 after HT treatment. Even though the burnt region in the treatment area could be observed for all treatments and endpoint days, the extent of this region was reduced for MT and by day 4. Gram staining confirmed the presence of MRSA (gram positive) bacteria within the abscess which appears as darker purple structures (Fig. 11).

4. DISCUSSION AND CONCLUSION

Focused ultrasound induced a therapeutic effect in abscesses induced by MRSA. This effect was observed as a reduction of the bacterial concentration in abscesses heated at 64 °C. These initial results indicate that focused ultrasound can represent a viable option for the treatment of MRSA-related infections.

The therapeutic effects were observed 4 days after the FUS procedure, suggesting that a more complex process than immediate thermal destruction of the bacteria is present. It is possible that a delayed response to the heat shock was the cause for the reduction in bacterial count since it has been reported that bacteria can respond to heat by changing their metabolism and finally dying days after the heat has been applied.

Fig. 11. (a) Gram staining for a control abscess with no focused ultrasound treatment and (b) closeup of the dashed area where the darker gram positive areas (signaling presence of MRSA bacteria) are visible.
applied. It is also possible that the focused ultrasound heat caused a disruption of the capsule of the abscess that allowed the immunological response to be effective against the bacteria.

The bacterial count baseline used compared animals with and without FUS treatment at each sacrifice day. This design was chosen to avoid reported variations of immunological markers caused by host response to the infection at different days during abcess development. This design made the results independent from bacteria multiplication and host response effects at different observation days. However, it does not allow direct comparison of a reduction of bacteria before and after treatment but gives information of a combined result caused by the FUS treatment and the host immune response.

The proposed treatment did not cover the entire abscess and therefore it could not achieve eradication of bacteria. This was done in order to obtain histological data on the effects of the heating of the abscess as well as bacterial counts. The animal model also limited the area that could be heated. Due to the subcutaneous abscess superficial location, only a small area could be treated without causing large burns. The results establish an initial feasibility reflected on a reduced bacterial count induced by a high temperature (64°C) treatment. In order to demonstrate clinical utility, the next steps will include the treatment of whole abscesses in larger models, as well as studying the effect of repetitive heating treatments on bacteria.

To establish a relationship of treatment with immune response to the abscess, the amount of neutrophils in abscesses and surrounding tissue was indirectly assessed by measuring the amount of MPO. Because there was a significant difference between the MPO levels of animals with and without abscess, while no difference was observed between experimental groups, we concluded that the changes on MPO levels are mainly due to the presence of bacteria.

Since there was no difference on the MPO levels between experimental groups, this could mean that there was no difference in the amount of recruited neutrophils. It is therefore likely that the reduction on bacterial count was directly related to heat shock to bacteria combined with a disrupted capsule. There is however another possibility since it has been reported that MPO can be inactivated when interacting with bacteria, leading to a 40% decrease of MPO per neutrophil. Since MPO is inactivated by reaction with hydrogen peroxide, some inactivation of MPO can occur during an inflammatory response given that neutrophils engulfing bacteria produce hydrogen peroxide. It is possible that a direct bacteria-neutrophil interaction occurred and the engulfing of bacteria followed by production of bactericidal product caused a partial inactivation of MPO. Having comparable level of MPO in control and treatment groups does not necessarily mean there was no difference in the neutrophil recruitment, but that the increased recruitment of neutrophils was not assessable via MPO assay. This hypothesis would explain why we did not see a difference in MPO in the treated animals, despite publications stating that neutrophils are recruited and MPO levels increase postburn. The MPO assay alone might not be sufficient for the evaluation of neutrophil recruitment in the context of abscess treatment by heat. Further experiments will involve other methods to assess neutrophil involvement.

In order to account for variations of the abscess weights we decided to normalize the MPO results to the amount of protein. This was particularly important since the treated groups usually presented a reduced size caused by the heating and a lack of normalization could have misled the results.

To establish a relationship of treatment with systemic inflammatory response in the mice, the WBC count was assessed from full blood. There was no difference on the WBC count between the experimental groups. We therefore could not observe any difference in the systemic inflammatory response in mice undergoing focused ultrasound treatment compared to the control animals.

Bacteria counts of spleen tissue were performed to investigate possible bacteremia and except for one case (out of 55) no bacteria was found in the spleen samples. It is possible that the bacteria found in this case was due to contamination since the count was very low, the WBC count of this mouse was within the normal range and it did not show any signs of fever, distress, or acute illness. The low bacterial count on the spleen makes it then less likely for an abscess to have formed in the spleen, and we concluded that contamination was the most likely explanation. It is still possible that bacteremia was induced and not cleared by the innate immune system, leading to a settlement of bacteria in the spleen. Other possibilities for the bacterial count on the spleen are previous bacteremia, error on the injection or the treatment. It is important to further investigate this since S. aureus is a bacterium with tendency to cause bacteremia leading to complications in form of secondary infections. Future studies will include longer timeframes and animal numbers to establish if treatment with focused ultrasound can lead to bacteremia. Many medical interventions can potentially lead to bacteremia, including dentistry and surgical procedures, and periprocedural administration of antibiotics is usually prescribed to reduce the risk factors for spread of infections.30

We observed that some of the slides of the abscesses in the treatment groups seemed to show a disruption of the capsule. This change in structure could have been induced by the treatment and the abscesses in treated animals indeed appeared smaller, irregularly shaped, and had different consistence than the abscesses of the control animals. However, it is also possible that differences in consistency and size caused disparities in the histology processing.

The irregular shape that was frequently observed in the histology slides in treated animals was attributed to the treatment with focused ultrasound. Even though the induction procedure can lead to irregular shapes of abscesses, when animals showed these irregularly shaped abscesses they were not treated or used as controls for the study.

Localized burns were observed when treating the animals, which were caused by the need to treat shallow targets. This was minimized by working at a high frequency in order to treat a small focal area.

We decided to target an area of 1 × 1 mm regardless of the size of the abscess. The abscesses presented overall a similar
size and aspect with an average abscess diameter of 6 ± 2 mm and round shape. The final treated area was therefore similar for all animals but not necessarily covering the whole abscess. This was necessary because of the difficulty to ensure complete coverage without causing burns out of the intended area for such small size on a superficial target. A complete coverage could provide a better therapeutic response, and for larger animal models and the final clinical application this would be the goal.

In conclusion, the study indicates that a focused ultrasound treatment causing heating at 64°C on subcutaneous abscesses is capable of significantly reducing bacterial load four days posttreatment. This demonstrates that focused ultrasound produces a therapeutic effect when treating localized abscesses. Further work will be pursued in order to apply this treatment clinically as well as understanding the mechanism by which the therapeutic effect is obtained.

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