MRI detection of bacterial brain abscesses and monitoring of antibiotic treatment using bacCEST

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Purpose: To develop a new MRI method to detect and characterize brain abscesses using the CEST contrast inherently carried by bacterial cells, namely bacCEST.

Methods: Bacteria S. aureus (ATCC #49775) and F98 and 9L glioma cells were injected stereotactically in the brains of F344 rats to form abscesses and tumors. The CEST signals of brain abscesses (n = 4) and tumors (n = 7) were acquired using 2 B1 values (i.e., 1 and 3 μT) and compared. The bacCEST signal of the brain abscesses in the rats (n = 3) receiving ampicillin (intraperitoneal injection 40 mg/kg twice daily) was acquired before, 4 and 10 days after the treatment.

Results: The bacCEST signal of S. aureus was characterized in vitro as a strong and broad signal in the range of 1 to 4 ppm, with the maximum contrast occurring at 2.6 ppm. The CEST signal in S. aureus–induced brain abscesses was significantly higher than that of contralateral parenchyma (p = .003). Moreover, thanks to their different B1 independence, brain abscesses and tumors could be effectively differentiated (p = .005) using ΔCEST(2.6 ppm, 3 μT–1 μT), defined by the difference between the CEST signal (offset = 2.6 ppm) acquired using B1 = 3 μT and that of 1 μT. In treated rats, bacCEST MRI could detect the response of bacteria as early as 4 days after the antibiotic treatment (p = .035).

Conclusion: BacCEST MRI provides a new imaging method to detect, discriminate, and monitor bacterial infection in deep-seated organs. Because no contrast agent is needed, such an approach has a great translational potential for detecting and monitoring bacterial infection in deep-seated organs.

KEYWORDS
bacterial infection, brain abscess, CEST, MRI
1 | INTRODUCTION

Brain abscess is a severe central nervous system disorder and the most common focal suppurative brain infection.\(^1\)\(^,\)\(^2\) Although there has been a significantly reduced mortality in recent years, thanks to advances in modern neuroradiological diagnosis and neurosurgical and antimicrobial therapy,\(^3\)\(^-\)\(^5\) the mortality is still relatively high (19-43%) and highly dependent on etiology.\(^6\) For abscesses with an intraventricular extension, for example, mortality is up to 80%. Quick and accurate diagnosis at the earliest stage is critical to direct the appropriate treatment of brain bacterial infections. However, effective differentiation of brain abscesses from other brain disorders, such as brain tumors (cystic malignancy) and neuroinflammation, is still challenging because of their similarity in clinical symptoms, and pathologic and radiological characteristics.\(^7\) Most conventional medical imaging methods rely on either morphological changes or contrast-enhancement characteristics, which are unfortunately unable to detect brain abscesses specifically. For example, in the late stage of bacterial infection, the formation of brain abscess shows MRI manifestation as a typical rim-like enhancement, which is often similar to necrotic malignant tumors, especially glioblastoma multiforme.\(^8\) Classically, an abscess has a thin, regular enhancing capsule and a tumor is likely to have a thick or nodular capsule; however, many exceptions exist and often lead to ambiguous diagnoses, indicating that more advanced imaging methods are required.

In the past decades, tremendous efforts have been made to develop specific molecular imaging methods to detect bacterial infections using CT,\(^9\) MRI,\(^10\)\(^,\)\(^11\) fluorescence imaging,\(^12\) bioluminescence imaging,\(^13\)\(^,\)\(^14\) and positron emission tomography.\(^15\)\(^,\)\(^16\) However, these methods rely on the use of injectable contrast agents, which limit their applications in poorly perfused tissues.\(^17\) Moreover, clinical translation of new exogenous agents is challenging and may take a long time because of the approval process.\(^18\)\(^,\)\(^19\) Therefore, there is an urgent need to develop a non-contrast agent-based imaging technology that could overcome these difficulties and potentially improve the clinical management of bacterial infection in deep organs. In the clinic, several functional MRI methods have been reported to visualize pyogenic brain lesions or discriminate them from other neural disorders. For instance, DWI MRI has been suggested to differentiate brain abscess from primary, cystic, or necrotic tumors,\(^20\) based on the limited free motion of water molecules in the viscous milieu in the necrotic center of abscess cavity. Dynamic susceptibility contrast perfusion-weighted MRI was shown to discriminate tumor and abscess by their different relative cerebral blood volumes.\(^8\) Unfortunately, these MR methods are not specific to bacterial infection, and unable to differentiate different brain abscesses by etiology. Magnetic resonance spectroscopy is another potential method to detect bacterial infection, to differentiate bacterial infection, and even possibly to differentiate anaerobic brain abscesses from aerobic abscesses on the basis of metabolite patterns.\(^21\)-\(^25\) However, MRS approaches are often limited by low detection sensitivity, and resulting low spatial and temporal resolution. Because the identification of the presence of a pathogen is crucial to determine antibiotic susceptibility patterns and tail- lor antibiotic treatment, a noninvasive MRI method to more specifically detect bacterial infection and identify the pathogens is still an unmet clinical need.

Chemical exchange saturation transfer (CEST)\(^26\)-\(^29\) is a recently developed MRI technique that enables the sensitive detection of a variety of molecules with exchangeable protons through the changes in water signal, providing a new pathway for molecular MR imaging. Recently, we successfully developed a “bacterial CEST” (bacCEST) MRI approach to detect bacterial infection in a tumor directly using the endogenous CEST MRI contrast generated by bacteria.\(^30\) This method requires no exogenous imaging probes, which is particularly useful for application to deep organs like brain and bone marrow, where contrast agents are difficult to reach. In the current study, we aimed to expand the utility of bacCEST to the diagnosis and treatment monitoring of brain abscess formed by Staphylococcus aureus (S. aureus), one of the most common causes of brain abscesses and other life-threatening infections.\(^31\) Using a rat brain abscess model, we show that bacCEST can detect bacterial abscesses and demonstrate the ability to differentiate brain abscesses from brain tumor. Moreover, we show the feasibility of using bacCEST MRI to monitor the bacterial response to treatment.

2 | METHODS

2.1 | Cell culture

Both rat F98 and 9L glioma cells were cultured in DMEM media (Life Technologies, Rockville, MD) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin.

Cultures of pathologic S. aureus (ATCC, no. 49775) were prepared by overnight growing at 37°C in 12 mL of ATCC® Medium 1806 culture broth. The bacteria were pelleted by centrifugation at 3000 g for 15 minutes, washed twice in sterile phosphate buffered saline (PBS), and resuspended in PBS to the desired concentrations.

2.2 | Animals

All animal experimental protocols are approved by Johns Hopkins Institutional Animal Care and Use Committee. A total of 15 rats was used, as listed in Table 1.
About 12 days after the tumor inoculation, crystalline (ampicillin) or saline was intraperitoneally injected into the right frontal lobe located 3 mm lateral and 2 mm anterior of Bregma. To directly compare the endogenous CEST contrast between tumors and bacterial abscess, 2 of F98 tumor-bearing brains were also induced with S. aureus packaged as agarose beads. Magnetic resonance imaging was performed approximately 9 days after the implantation, when the bacterial abscess was well circumscribed from the surrounding brain parenchyma, and the extent of edema was mild.

### 2.2.1 Brain abscess model

F344 Fisher rats (6 weeks old, female, 100-150 g, National Cancer Institute, Bethesda, MD) were anesthetized using a previously published procedure and injected stereotactically (left frontal lobe, 3 mm lateral and 2 mm anterior of Bregma, 1.0 μL/minute injection rate, injection volume = 2 μL) with 6 x 10⁶ S. aureus packaged as agarose beads. Magnetic resonance imaging was performed approximately 9 days after the implantation, when the bacterial abscess was well circumscribed from the surrounding brain parenchyma, and the extent of edema was mild.

### 2.2.2 Antibiotic treatment

Six rats were selected randomly into 2 groups to receive intraperitoneal injection of ampicillin (40 mg/kg) or saline twice daily for 10 days. The CEST MRI was carried out before, 4 and 10 days after the treatment.

### 2.2.3 Brain tumor model

Using the same procedure described previously, F344 Fisher rats were anesthetized and stereotactically implanted with 5 x 10⁴ F98 cells (n = 4) or 5 x 10⁵ 9L cells (n = 3) in the right frontal lobe located 3 mm lateral and 2 mm anterior to the Bregma. To directly compare the endogenous CEST contrast between tumors and bacterial abscess, 2 of F98 tumor-bearing brains were also induced with S. aureus on the contralateral side. Magnetic resonance imaging was performed approximately 12 days after the tumor inoculation.

### 2.3 Magnetic resonance

All MR images were acquired on an 11.7T Bruker Biospec horizontal bore scanner (Bruker Biosciences, Billerica, MA) equipped with a rat brain surface array RF coil (receiver) and a 72-mm-volume coil (T11232V3, transmitter). For each animal, T₂-weighted images were first acquired to assess the formation and extent of bacteria abscesses using a RRE (rapid acquisition with refocused echoes) sequence with a RARE factor of 20 (TR/TE = 3000/100 ms), slice thickness = 1 mm, acquisition matrix size = 256 x 256, FOV = 25 x 25 mm, and number of acquisitions = 2 (total acquisition time = 2 minutes and 40 seconds). Z-spectral (saturation spectra as a function of irradiation frequency) images were acquired using a modified RARE sequence (TR = 5.0 seconds, effective TE = 5 ms, RARE factor = 10, slice thickness = 1 mm, FOV = 20 x 20 mm, matrix size = 128 x 64, and number of acquisitions = 2), including a magnetization transfer module with saturation time (tₛₐₜ) of 3 seconds and RF field strengths (B₁) of 1 and 3 μT with offsets swept from −5 ppm to + 5 ppm (step = 0.2 ppm). The B₀ inhomogeneity maps were acquired using a modified water saturation shift reference method with the same parameters as used for CEST imaging, except that TR = 1.5 seconds, tₛₐₜ = 500 ms, B₁ = 0.5 μT, and offset range of −1 to 1 ppm (0.1-ppm steps). The total acquisition time was 30 minutes. ¹H MR spectra of the volume of interest (2 x 2 x 2 mm³) were obtained using a single-voxel STEAM (stimulated echo acquisition mode spectroscopy) method with VAPOR (variable-power radiofrequency pulses with optimized relaxation delays) water suppression and outer volume suppression schemes, with the following parameters; TR = 3.0 seconds, TE = 8 ms, mixing time = 7 ms, and 256 acquisitions (total acquisition time = 12 minutes and 54 seconds). Before each spectroscopic measurement, the shimming of the volume of interest was optimized using the field map–based automatic shimming implementation method.

### 2.4 Data processing

Data processing was performed using custom-written scripts in MATLAB (MathWorks, Natick, MA). Regions of interest were drawn manually based on the T₂-weighted images. Mean Z-spectra, which display the ratio of the MRI water signal intensity during RF saturation (S₀) as a function of saturation frequency offset (Δ₀), were calculated after B₀ correction on a per-voxel basis, and CEST signals were quantified by asymmetric magnetization transfer ratio MTRₐ₅ₚₐₓₚₚ = (S₁₋Δ₀ − S₁+Δ₀)/S₀. ¹H MR spectra were manually phased, baseline-corrected, and referenced to N-acetylaspartate at 2.0 ppm in Topspin software (version 3, Bruker, Germany). A line-broadening function of 20 Hz was applied before Fourier transformation. Spectral peaks were assigned according to the literature.

### 2.5 Histology

Hematoxylin/eosin and Gram staining were performed as described previously.

<table>
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<tr>
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<td>Treatment (saline)</td>
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<td>Tumor</td>
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2.6 | Statistics

All data are shown as mean ± SD. Student’s t-tests were used for statistical analyses, and differences were considered significant when p was less than .05.

3 | RESULTS

3.1 | Endogenous CEST contrast of pathogenic Staphylococcus

We first performed in vitro studies to confirm the CEST MRI detectability of the pathogenic bacterium *S. aureus*. As shown in Figure 1, *S. aureus* (~2 × 10⁸ cells/mL in pH 7.4 PBS solution) exhibited CEST characteristics similar to *Clostridium novyi-NT* (*C. novyi-NT*, ~2 × 10⁷ cells/mL in pH 7.4 PBS solution), an anaerobic gram-positive bacterial strain that has been studied previously. Despite the slightly different shapes of z-spectra, both bacterial strains showed a strong and broad CEST signal in the range of 1 to 4 ppm, with the maximum CEST *MTRasym* contrast occurring at a frequency of approximately 2.6 ppm. Quantitatively, based on the number of cells/mL, *S. aureus* showed almost 10 times less CEST contrast (*MTRasym*) than *C. novyi-NT*. In particular, the CEST contrasts were determined to be 0.2% and 2.6% per million cells for *S. aureus* and *C. novyi-NT*, respectively. Furthermore, the CEST MRI signal of *S. aureus* at different concentrations were measured (Supporting Information Figure S1), confirming the bacCEST MRI signal is dependent on viable bacteria.

3.2 | Noninvasive detection of brain abscess in vivo using CEST MRI

To investigate the CEST MRI detectability of brain abscess in vivo, we performed CEST studies on a rat brain abscess model. The formation of abscesses was confirmed by anatomical T₂-weighted MR images (Figure 2A) at a time point of 12 days after implantation of *S. aureus*. All lesions appeared either isointense or hypo-intense at the rim, likely caused by the formation of a collagen capsule. The formation of bacterial abscess was further confirmed by hematoxylin/eosin staining (Figure 2B), which shows the formation of capsules surrounded by inflammatory cell infiltration, and the presence of bacterial cells in the center of abscess was revealed by Gram staining (Figure 2C). Strongly elevated lactate (1.33 ppm) and cytosolic amino acids/lipids (0.9 ppm) were observed on MRS (Figure 2D), confirming the bacterial infection in these areas. The CEST parametric map (*MTRasym* at 2.6 ppm, Figure 2E) showed a significantly elevated CEST signal within the area of bacterial infection. Figure 2F shows the corresponding region of interest–based analysis for the abscess and contralateral brain. In this rat, the mean *MTRasym* (2.6 ppm) of brain abscesses (4.20 ± 0.86%) was significantly higher than that of the contralateral brain (1.75 ± 0.47%) with a p less than .001 (n = 8, Student t-test, 2-tailed, paired). As shown in Supporting Information Figure S2, empty agarose beads completely degraded after 9 days and generated no detectable CEST signal.

3.3 | Differentiation of infection and brain tumor by B₁-dependent CEST signal

As shown in Figure 3A, conventional T₂-weighted imaging is able to delineate brain abscesses and tumors, but unable to discriminate them. It shown in Figure 3B,C, both brain abscess and tumor exhibit markedly higher CEST signal than normal brain parenchyma at the B₁ values of both 1 and 3 μT, in good agreement with the hyperintense areas in the T₂-weighted image. Although brain abscess and tumor cannot be differentiated directly using the CEST MRI signal.
acquired with a single B1 strength, we found that they can be differentiated in a statistically significant manner by their different B1-dependent CEST when acquired using B1 values of 1 and 3 μT. For the representative rat shown in Figure 3, the mean MTR asym increased from -0.67 and -1.77% to 5.14 to 4.82% for the brain abscess and tumor, respectively (Figure 3E,F), leading to net MTR asym increases of 5.81 and 6.59%, respectively. To highlight the brain abscess, we calculated the $D_{\text{MTR asym}}$ as $D_{\text{MTR asym}}$ (2.6 ppm, 3 μT) - $D_{\text{MTR asym}}$ (2.6 ppm, 1 μT). As shown in Figure 3D, after this simple one-step processing, CEST MRI can differentiate brain abscess from tumor. The same effect was observed in multiple animals (Figure 3G) and generated a significant difference between the groups of brain abscess and brain tumors (Figure 3H, $p = .0013$, $n = 3$, Student t-test, 2-tailed, unpaired). Only 2 rats were implanted with both tumor cells and $S$. aureus, whereas the other 4 rats were implanted either with tumor cells or $S$. aureus to generate only 1 lesion. The purpose of double implantation was to display the CEST contrast in both lesions, whereas the purpose of single implantation was to avoid the potential effect of 1 type lesion on the other. Our data show that the double implantation has negligible effects on CEST contrast as compared with single implantation.

### 3.4 Monitoring the response of bacteria to antibiotic treatment

Because the intensity of bacCEST is proportional to cell numbers, it can potentially be used to monitor the inhibition
of a treatment on the targeted bacterial cells. To demonstrate this, we performed a longitudinal CEST MRI study on a group of brain abscess–bearing rats ($n=3$) before and after antibiotic treatment. As reflected by the area of the hyperintense region in T2-weighted images (Figure 4A), the size of abscess decreased slightly on day 4 (from $20.4 \pm 4.1\%$ to
16.4 ± 3.8%, p = .2624), followed by a striking reduction to 12.7 ± 1.6% on day 10 (p = .035). In contrast, the CEST map showed not only shrinking of hyperintense areas but also a marked decrease in CEST signal in these areas starting on the fourth day after the treatment. For example, using the regions of interest drawn based on T2-weighted hyperintense areas, the mean MTRasm (2.6 ppm, 3 μT) dropped from 4.69 ± 0.74% (day 0) to 3.95 ± 0.74% (day 4) and 3.83 ± 1.06% (day 10), respectively. As shown in Figure 4B, the statistical analysis clearly showed a significant decrease of CEST signal on day 4 and day 10 (i.e., p = .035 and .016, n = 3, Student t-test, 2-tailed, paired), respectively. In contrast, the CEST MRI of abscesses in animals receiving no treatment remained mostly unchanged (Figure 4C), confirming that the decreased CEST MRI signal in the treatment group was caused by the response to treatment. To compare the longitudinal bacCEST signal changes between the treated and untreated groups, the bacCEST signals of each rat at different time points were normalized by its signal on day 0 (before treatment). As shown in Figure 4E, there was a significant difference between the bacCEST signals of treated and untreated animals on day 10 (p = .039, Student t-test, 2-tailed, unpaired) but not on day 4 (p = .1776, Student t-test, 2-tailed, unpaired). Furthermore, Gram-stained brain sections from cohorts with and without antibiotic treatment (Supporting Information Figure S4) confirm the absence of any viable bacteria at the end of antibiotic treatment compared with the untreated animals, where abundant viable bacteria were visualized. The quantitative analysis depends highly on the choice of region of interest, because a clear inhomogeneity was present on all CEST maps.

4 | DISCUSSION

In the current study, we successfully expanded the principle of bacCEST, an endogenous CEST contrast for detecting the bacterial strain C. novyi-NT,30 to detect the pathogenic bacterium S. aureus. Our in vitro results showed that, similar to C. novyi-NT, S. aureus also has a broad CEST contrast over a frequency range from 0.5 to 4 ppm, with the maximum CEST contrast in terms of MTRasm occurring at approximately 2.6 ppm, indicating that bacCEST strategy can potentially be used for detecting different types of bacteria. We consider the apparent endogenous CEST contrast as the sum of CEST signals of all proton-exchangeable components of bacteria, such as cellular carbohydrates, peptides and proteins, and metabolites.30 Thus, the maximum in the MTRasm spectrum is only an apparent maximum and may vary in vivo because the magnetization transfer and relaxation properties are significantly different in different tissues,41 which can affect the shape of Z-spectra significantly. It is interesting that S. aureus has a CEST pattern similar to C. novyi-NT, but approximately 10 times less CEST contrast, tentatively attributed to the difference in the cell shape and biochemical components between these 2 strains. For example, S. aureus is rod-shaped bacterium with the diameter of approximately 1 μm,32 whereas C. novyi is rod-shaped with cell dimensions of 0.5 to 1.6 by 1.6 to 18 μm,43 indicating the cell volume of the later could be up to 70 times bigger than the former. The difference in CEST signal may allow the differentiation of different bacterial strains based on the relative CEST signal, which requires further investigation. Nevertheless, the endogenous bacCEST signals of S. aureus allow the direct detection of its bacterial abscesses formed in the rat brain without the need for any exogenous contrast agents.

Compared with those agent-based molecular imaging approaches, bacCEST is a non-agent-based contrast method, which can greatly improve the applicability in poorly perfused lesions such as brain abscesses in middle and later stages. The results of this study suggest that bacCEST MRI might be used to differentiate S. aureus abscesses from brain tumors without the need for injecting any imaging agents. Although both S. aureus and tumor exhibited strongly elevated CEST contrast, they had different B1-dependent CEST properties, attributed to the difference in the combined effect of their constituent protons with multiple exchange rates. It can be reasoned from our data that pathogens with different overall exchange rates could be differentiated by the same approach. However, such claims would need to be established for individual bacterial and tumor types. In the future, more sophisticated CEST methods44,45 may be used to improve the ability to differentiate different pathogens based on the differences in their CEST properties, including offsets and exchange rates.

Another potential clinical utility of the bacCEST is to monitor the effect of bacterial treatment response. Early detection of the response of brain abscess to an antibiotic treatment is crucial for treatment planning and adjusting. Very often, surgical excision of the abscess foci is immediately required when the antibiotic treatment is ineffective and neurologic deterioration occurs.46 Conventional MRI is unable to directly detect the treatment response, and especially abscesses may enlarge initially when the antibiotic treatment is effective.47 The bacCEST MRI has the potential to provide a new means to monitor the treatment response before any morphological changes can be observed. Our results show that the relative bacCEST contrast decreases significantly after the rats were treated with antibiotics for 4 days, earlier than the changes observed in T2-weighted image, which did not show noticeable morphological changes until day 10. Our study demonstrates that the relative changes in the bacCEST contrast has potential as an imaging biomarker for the inhibition and clearance of bacteria from the infection sites after the administration of treatment in deeply seated organs such as brain, and in predicting the therapeutic outcomes. Although our study was
demonstrated using an 11.7 T high-field small animal scanner, the bacCEST contrast at 2.6 ppm is expected to be translatable to clinical MRI scanner (i.e., 3 T). It has been demonstrated recently that most metabolites except the amine protons of glutamic acid, aspartic acid, and γ-aminobutyric acid could be detected at 3 T. Moreover, it has been shown that CEST technologies can be quickly translated from high-field small animal scanners (e.g., 4.7 T, 9.4 T, or 11.7 T) to clinical scanners (i.e., 3 T) and from rodent models to patients. Hence, it is expected to have potential for quick translation to the clinic, especially when no imaging agent is required.

5 | CONCLUSION

In this study, we exploited the endogenous CEST contrast from bacteria cells to noninvasively detect bacterial infection by *S. aureus* abscesses in the brain and to differentiate them from brain tumors by their different CEST-B₁ dependence. Moreover, we demonstrated its potential application in monitoring the responses of bacteria to antibiotic treatments. The present technology does not require the injection of imaging probes, making it highly clinically translatable, especially suitable for monitoring the effectiveness of an antibiotic treatment in deeply seated organs.

ACKNOWLEDGMENT

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REFERENCES


[32] Flaris NA, Hickey WF. Development and characterization of an atomic force micros-}

[33] **FIGURE S1** Additional Supporting Information may be found in the supporting information tab for this article.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the supporting information tab for this article.

**FIGURE S1** Chemical exchange saturation transfer MRI signal of *S. aureus* at different concentrations using both B1 strengths. Z-spectra (A) and MTR_asym (B) plots of *S. aureus* (2 × 10^8 cells/mL in PBS, pH 7.4) acquired using
B$_1$ of 1.2 and 3.6 $\mu$T. C, Chemical exchange saturation transfer MRI signal at 2.6 ppm of \textit{S. aureus} at different concentrations ranging from $2 \times 10^6$ to $2 \times 10^9$ cells/mL in PBS (pH 7.4). The CEST MRI was performed on a 9.4T Bruker vertical bore scanner using a 3-second-long continuous-wave RF pulse at 37°C.

**FIGURE S2.** Chemical exchange saturation transfer MRI of rat brains implanted with both bacteria and empty agarose beads. A, left: T$_2$-weighted image showing the bacterial abscess and needle track as indicated by arrows; middle: MTR$_{\text{asym}}$ map at 2.6 ppm acquired using B$_1$ = 1 $\mu$T; right: MTR$_{\text{asym}}$ map at 2.6 ppm acquired using B$_1$ = 3 $\mu$T. B, MTR$_{\text{asym}}$ plots (mean ± SD) of regions of interest encompassing the brain abscess, agarose beads (the area along needle track), and contralateral normal brain tissue (n = 3)

**FIGURE S3.** Chemical exchange saturation transfer MRI of 9-L brain tumors. A, T$_2$-weighted image showing the tumor as indicated by the arrow. B, Chemical exchange saturation transfer map at 2.6 ppm acquired using B$_1$ = 3 $\mu$T. C, MTR$_{\text{asym}}$ map at 2.6 ppm acquired using B$_1$ = 1 $\mu$T. D, ΔCEST map calculated from MTR$_{\text{asym}}$ (B$_1$ = 3 $\mu$T) − MTR$_{\text{asym}}$ (B$_1$ = 1 $\mu$T). Region of interest-based MTR$_{\text{asym}}$ plots of abscess, tumor, and brain using B$_1$ = 3 $\mu$T (E) and B$_1$ = 1 $\mu$T (F). G, B$_1$ dependence of CEST signal of tumors and normal brain at 2.6 ppm offset (n = 3)

**FIGURE S4** Gram stain of brain abscesses from a representative ampicillin-treated rat (A, C) and a control rat (B, D). No viable bacteria could be found in the abscesses in the treated animal, confirming the effectiveness of the ampicillin treatment