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A compound magnetic field generating system for targeted killing of *Staphylococcus aureus* by magnetotactic bacteria in a microfluidic chip

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ABSTRACT

A compound magnetic field generating system was built to kill *Staphylococcus aureus* (*S. aureus*) by magnetotactic bacteria (MTB) in a microfluidic chip in this paper. The system was consisted of coil pairs, a switch circuit, a control program and controllable electrical sources. It could produce a guiding magnetic field (gMF) of ± 1 mT along arbitrary direction in the horizontal plane, a rotating magnetic field (rMF) and a swing magnetic field (sMF, 2 Hz, 10 mT) by controlling the currents. The gMF was used to guide MTB swimming to the *S. aureus* pool in the microfluidic chip, and then the rMF enhanced the mixture of *S. aureus* and MTB cells, therefore beneficial to the attachments of them. Finally, the sMF was used to induce the death of *S. aureus* via MTB. The results showed that MTB could be navigated by the gMF and that 47.1% of *S. aureus* were killed when exposed to the sMF. It provides a new solution for the targeted treatment of infected diseases and even cancers.

1. Introduction

Staphylococcus aureus (*S. aureus*) is the major cause of severe infections worldwide, especially methicillin-resistant *S. aureus* which accounts for about 70% of healthcare-associated infections in Asian countries [1–4]. Critical challenges including antibiotic-resistance, biofilms and its mechanisms of immune evasion make *S. aureus* one of the most intractable pathogenic bacteria, which lead to an urgency to find other treatments for these infections [5–9].

The approaches without using antibiotics were presented recent years. Kim MH et al. [10] developed the magnetic hyperthermia based on magnetic nanoparticles (MNPs) as an alternative therapy and found that MNPs could deliver high specific loss power to kill *S. aureus* under a high-frequency, high-amplitude alternating magnetic field (AMF). In our previous work, C. Chen et al. [11] demonstrated that magnetic hyperthermia mediated by magnetotactic bacteria (MTB) could also affect the viability of *S. aureus*, especially when *S. aureus* cells were attached to MTB cells. The results suggested the potential of magnetic hyperthermia mediated by MTB for the treatment of some *S. aureus* infections.

MTB is a kind of bacteria that is capable of synthesizing the endogenous MNPs named magnetosomes. The magnetosomes are membrane-bounded crystals of magnetite (Fe_3O_4) or greigite (Fe_3S_4), ranging from 40 to 120 nm in size [12,13]. Magnetosomes are the single domain particles with relatively large magnetic moment [14], thus enabling MTB to respond quickly to the changes of the applied magnetic field known as magnetotaxis. Therefore, MTB has promising potentials in biomedical areas of magnetic separation, targeting delivery and targeted therapy [15–19]. Moreover, the specific loss power of magnetosomes in the AMF leads to its application in magnetic hyperthermia [11,20].

During the last few years, we focused on the killing of *S. aureus* by using magnetic bacteria MO-1, which was isolated from the Mediterranean Sea [21], with ovoid shape, a chain of magnetosomes and two bundles of lateral flagella. The killing of *S. aureus* under AMF [11] is due to the heating effect because of the high frequency of the magnetic fields. But in clinical applications, the hyperthermia may cause side-effect of damaging the surrounding healthy cells. Therefore, it is necessary to detect and regulate the real time temperature during the therapeutic process, which is just a difficulty for hyperthermia, and makes the system and operations greatly more complicated.

Here, we developed a system to kill *S. aureus* under a compound

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magnetic field mediated by MTB MO-1. The killing process under the compound magnetic field produced low heat, avoiding the request of temperature measurement in vivo. Besides, the compound magnetic field also realized the targeting of MO-1 cells and the enhancement of the mixture of the two bacteria to promote the killing effect. All the processes took place in a microfluidic chip which simulated the human microvessel. The effects of the compound magnetic field on targeting of MO-1 and killing of *S. aureus* in the environment without any antibiotic were evaluated in this paper.

2. Methods

2.1. Design and construction of compound magnetic field system

The compound magnetic field system was designed for the guiding of MO-1 and the killing of *S. aureus* in the same device under an optical microscope (OLYMPUS IX70). The system was composed of four parts: magnetic field generating coils, a peripheral circuit, a PC control program and two adjustable direct current (DC) supplies.

The coil pairs, divided into two groups, were assembled perpendicularly to generate magnetic fields respectively along the X-/Y-dimensions of the horizontal plane. The coils were embedded in the stage of a microscope, and a microfluidic chip was placed at the center plane enclosed by coil pairs. Coils in each group were wound in the same direction by 1 mm enameled copper wire. The magnetic field was induced by currents in coils, which were calculated using Maxwell (Ansoft Inc., PA, USA) based on coil structures adapted for the microscope platform. The coil frameworks were epoxy resins, designed using Solidworks (Dassault Systèmes S. A., MA, USA).

The peripheral circuit included several switches used to change the directions of currents in all coils, a joystick and a data acquisition (DAQ) card. The other important part of the system was the control program which managed the DAQ to set appropriate states for switches and get the information of the joystick. Meanwhile, the program transferred commands to set outputs of two power supplies through the serial ports.

The compound magnetic field generated by the system comprised guiding magnetic field (gMF), rotating magnetic field (rMF) and swing magnetic field (sMF). The gMF was the static magnetic field induced by stable DCs, whose direction was controlled by a joystick. Through precise controlling by the control program or by a manual joystick, the currents of two group coil pairs could generate the magnetic field with continuously changing orientation clockwise or counterclockwise, which was a rMF. The sMF was a kind of field whose direction swapped quickly in the two orientations between which the angle was 90 degrees. The direction swap of sMF was at a low frequency with sharp rising and falling edges. In the duration of plateau, the intensity and direction of the magnetic field would keep invariant. After the transient swap, the intensity of the magnetic field would be the same as before but the direction would rotate 90 degrees.

2.2. Bacterial culture

Magnetotactic ovoid strain MO-1, incubated in EMS2 medium at 24–26 °C [21]. MO-1 cells were enriched by a magnetic block when growing exponentially, and their concentration was determined using a bacterial counter.

S. aureus (ATCC25923) were gifted from the 306th Hospital of People's Liberation Army and grown on blood LB agar plates (Land Bridge Technology Co. Ltd., Beijing, China). The conventional plate counting method was then used to determine the bacterial concentration.

2.3. The navigation of MTB and killing of S. aureus

Magnetotactic bacteria MO-1 were first conjugated with rabbit anti-MO-1 polyclonal antibody. Briefly, 1×10^5 MO-1 cells were incubated with 5 μl polyclonal antibody at room temperature for 30 min. Then the antibody-conjugated MO-1 cells were enriched by using a magnetic block.

A microfluidic chip fabricated previously was used to simulate the blood capillary. Antibody-conjugated MO-1 cells and *S. aureus* were injected into the two micropores of the chip. After the experimental chip was put in the center of the coils, the set of coils was placed in the microscope which was used to observe the movement of antibody-conjugated MO-1 cells.

Basing on the magnetotaxis, the movement of antibody-conjugated MO-1 cells was guided by an external gMF produced by the system. The joystick was used to control the direction of the magnetic field according to the bacterial movement and the shape of the chip. A rMF was applied by rotating the handle of the joystick to induce the rotation of antibody-conjugated MO-1 cells. The mixture in the *S. aureus* pool underwent a standing for 30 min to promote the attachment of the antibody-conjugated MO-1 bacteria to *S. aureus*. The attachment was based on the fact that Protein A was expressed naturally on *S. aureus* surface, which can bind highly to the antibody. After the attachment, an sMF with the flux density of 10 mT and a frequency of 2 Hz was applied for 40 min to kill *S. aureus*. During sMF exposure, the temperature rise was also measured by using a fiber optic sensor (FooxTek Co., Ltd., Shenzhen, China).

2.4. Evaluation of the killing effect

The killing effect on *S. aureus* by antibody-conjugated MO-1 cells were evaluated by using a conventional plate counting method. The mixture solutions in the *S. aureus* pool were suctioned and diluted 10^3 times. Then a 50 μl suspension was tiled to blood LB agar plates using an inoculating stick. The plates were incubated for 12 h at 37 °C. The number of CFUs was counted to evaluate the killing effect on *S. aureus*. The *S. aureus* viability in the control group of *S. aureus* alone was also tested.

2.5. Statistical analysis

Student's *t*-test was applied to compare the difference between the experimental and the control groups. A *P*-value of <0.05 was considered significant in the statistical test.

3. Results and discussion

3.1. The compound system and magnetic fields

The compound magnetic field system was fabricated (Fig. 1). The system includes coils (Fig. 1b, c) embedded in the microscope platform, a PC control program shown in the screen (Fig. 1a), two power supplies under the computer and a peripheral circuit partly enclosed in an aluminum case (Fig. 1a) with a joystick connected to a DAQ card outside (Fig. 1d). The coils were assembled by coil pairs with a 36 mm×40 mm square room with a hole in the center prepared for the light path. As shown in Fig. 1b, the X/Y arrows defined the positive directions of magnetic fields generated by coils.

The distributions of static magnetic fields in two orientations were measured by a Gauss/Tesla meter (F. W. Bell Model 7010, Bell Technologies, Inc., USA) (Fig. 2). The magnetic flux density in each direction could be up to 10 mT, and hence, the range of the magnetic field was both −10 to 10 mT by controlling the currents in coils and the states of switches. The uniform area was about 12 mm×10 mm with 10% shift, enough for the microfluidic chip (12 mm×4 mm). The flux density of gMF and rMF was 1 mT, which were enough to guide the movement of MO-1, as MO-1 is sensitive to the geomagnetic field with the flux density of 0.5 Gauss (i.e. 0.05 mT) [13,21,22].

The swing magnetic field was measured by a Gauss/Tesla meter and

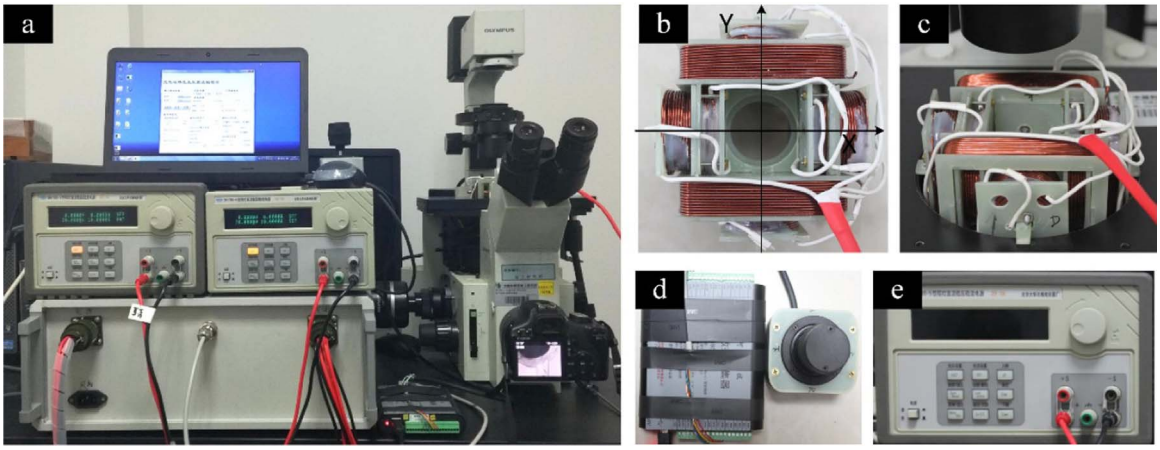

Fig. 1. The compound magnetic field system. (a) the compound system, (b) the coils, (c) coils device embedded into microscope platform, (d) the handle joystick connected with a DAQ device, (e) the power supply panel.

an infiniium oscilloscope (54810A, Agilent, USA) (Fig. 3). On the one hand, the magnetic field should be strong enough to induce an effect on the viability of *S. aureus*; on the other hand, the amplitude should not be too high to harm normal tissue and limit their clinic applications in the future. Based on above considerations, the flux density of 10 mT was chosen for the sMF. However, it could be found that there were rise and fall times when sMF changed due to the inductors of the coils, which resulted in a restricted frequency of 2 Hz. In the future, we will improve the system and further study the higher frequency effect.

Besides the magnetic fields mentioned above, the system could generate other modes of magnetic fields. The two groups of the coils were connected respectively with two adjustable power supplies, so the currents in two group of the coils could be controlled by the program and peripheral circuit independently. Different values of X-/Y- components could generate different output magnetic field not limited to the fields in these paper. Therefore, the system could be used in other researches offering various magnetic fields.

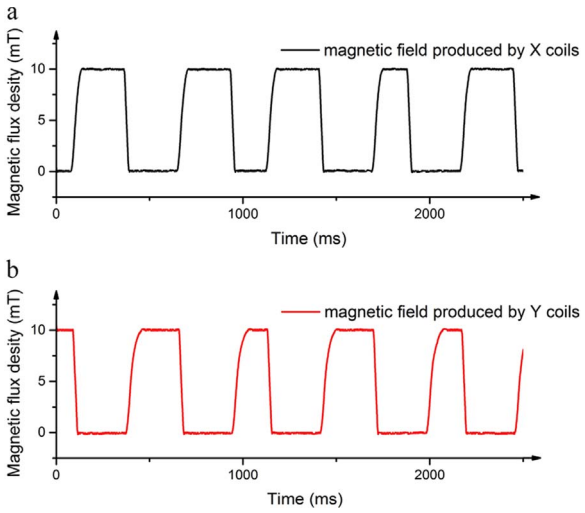

Fig. 3. The waveform of the swing magnetic field. (a) the magnetic flux density along the X-direction, (b) the magnetic flux density along Y-direction. The rise time is about 100 ms and fall time about 60 ms.

3.2. The targeting movement of antibody-conjugated MO-1 cells

The microfluidic chip was used to simulate the blood capillary (Fig. 4a). After being injected into the pool, antibody-conjugated MO-1 cells swam in the microfluidic channel under the gMF (Fig. 4b). When antibody-conjugated MO-1 cells arrived at a turn (Fig. 4c–e), the handle was used to change the direction of gMF which then controlled the MO-1 cells to swim continuously. Finally, the antibody-conjugated MO-1 cells successfully swam across the channel and arrived at the *S. aureus* pool (Fig. 4f). In the *S. aureus* pool, *S. aureus* was pre-injected; and a rMF was applied to trigger homogenization, which can increase the contact of MO-1 cells with *S. aureus* (Fig. 4g). These results demonstrated that the system was able to control the movement of MTB, which displayed the potential in clinical applications to navigate MTB by an external magnetic field to target deep areas. In addition, MTB MO-1 were auto-propelled and did not need the extra power supplies, which was an advantage for the targeted therapy.

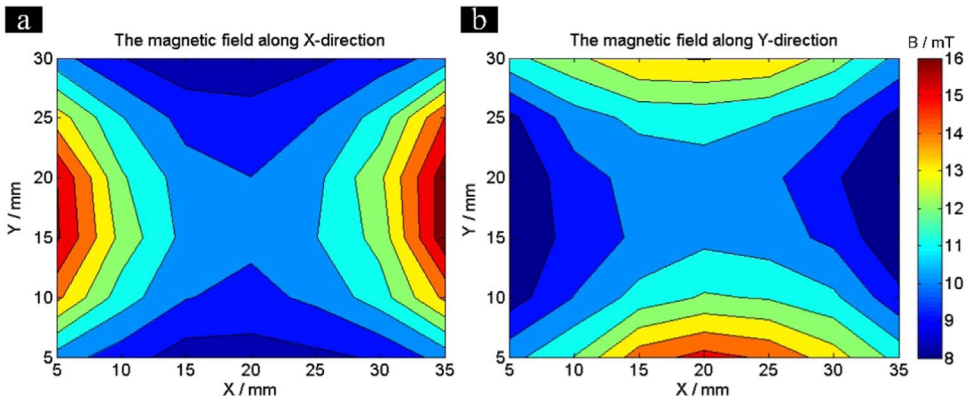

Fig. 2. The distribution of the static magnetic field flux density in each orientation. (a) the measurement of magnetic field along X-direction, and (b) the measurement value along Y-direction. The uniform area is about 12 mm×10 mm with the error less than 10%.

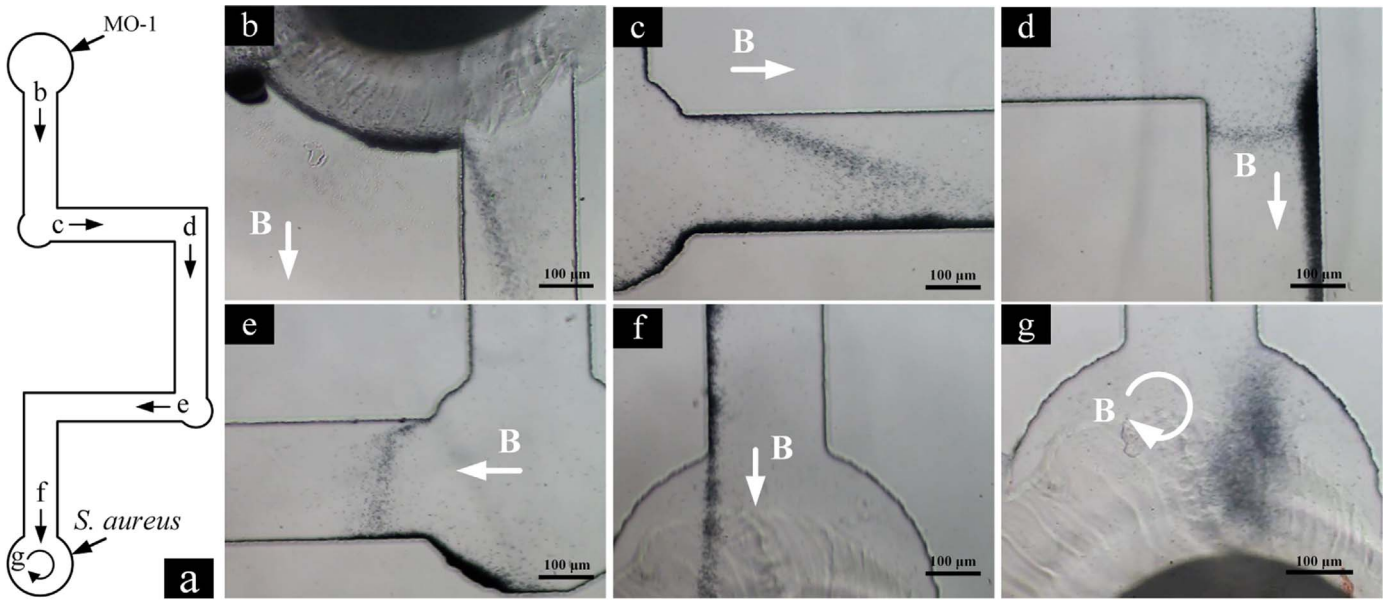

Fig. 4. The movement of MO-1 under applied magnetic fields in a microfluidic chip. (a) the microfluidic chip. (b)–(f) illustrate the targeted move of MO-1 under gMF, swimming along the magnetic field direction and turning around as the direction changed, and (g) presents the rotation of MO-1 under rMF.

3.3. *The swinging motion of MO-1 and* S. aureus

The sMF was applied to the *S. aureus* pool in the microfluidic chip. The MO-1 swung when magnetic field changed (Fig. 5). In the meantime, the displacement of *S. aureus* attached to MO-1 from the below (the down white arrows in Fig. 5a, c) to the right (the right two arrows in Fig. 5b, d) was observed. It could be found that some *S. aureus* cells not attached to MO-1 (the top right arrows in Fig. 5) also had rapid but random motions. It was probably due to the local chaos of the water stirred by MO-1 nearby.

3.4. *The killing effect of* S. aureus

Before analyzing of the killing effect, the temperature increase during the sMF procedure was observed, which were from 25.4 to 34.6 °C in *S. aureus* alone group and from 26.2 to 36.1 °C in MO-1 attached *S. aureus* group. We also measured it in the sMF area without samples, which was from 24.6 to 34.1 °C. It could be basically sure that the temperature rise was caused by thermal radiation of the copper coils around the sample. We could reduce the temperature rise by improving the heating dissipation effect of the system in the future.

The killing effect was analyzed by the conventional plate counting method. As the pictures of incubated blood plates shown (Fig. 6), the viability of *S. aureus* alone was not affected under sMF ($P > 0.05$). By contrast, the *S. aureus* attached to MO-1 exposed to sMF had a large reduction of *S. aureus* colonies compared to that without the sMF exposure ($P < 0.05$). Since the temperature around 35 °C was not fatal, we have reasons to believe that sMF caused the death of *S. aureus* attached to MO-1. The pressure intensity induced by the sMF on *S. aureus* cells was calculated to approximately 8.3 kPa, which was proved to be able to affect the viability of *S. aureus* in the other work [23]. The statistical analysis (Fig. 6b) illustrated that the killing ratio caused by antibody-conjugated MO-1 cells under the sMF was approximately 47.1%. The result of significant killing effect suggested that this method may provide an alternative tool to the therapy of infections induced by *S. aureus*.

4. Conclusion

In this work, we have built a compound system which could generate a compound magnetic field including gMF, rMF and sMF in the same device. In the microfluidic chip, the gMF realized the targeting movement of MO-1 cells, then the rMF contributed to the attachment of *S. aureus* and antibody-conjugated MO-1 cells. Finally, the sMF induced the death of *S. aureus* under the help of MTB MO-1. The experimental results suggest that MTB under the compound magnetic field is potential to be used in bacterial infection. It also sheds lights on the targeted therapy of cancer, which needed be

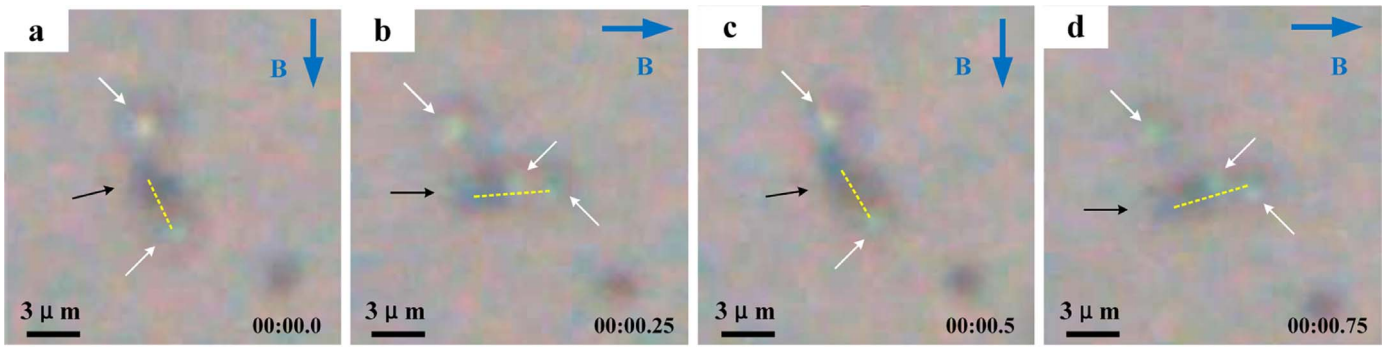

Fig. 5. *S. aureus* swung with MO-1 under sMF. (a)–(d) show the displacements of bacteria during one min when the magnetic field direction changed shown in upright with blue arrows. The white arrows point to *S. aureus* cells, and the black ones show the MO-1 cells. The swinging motion of *S. aureus* and MO-1 could be seen from the direction change of yellow dashed line, which represent the attachment of MO-1 and two *S. aureus* cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

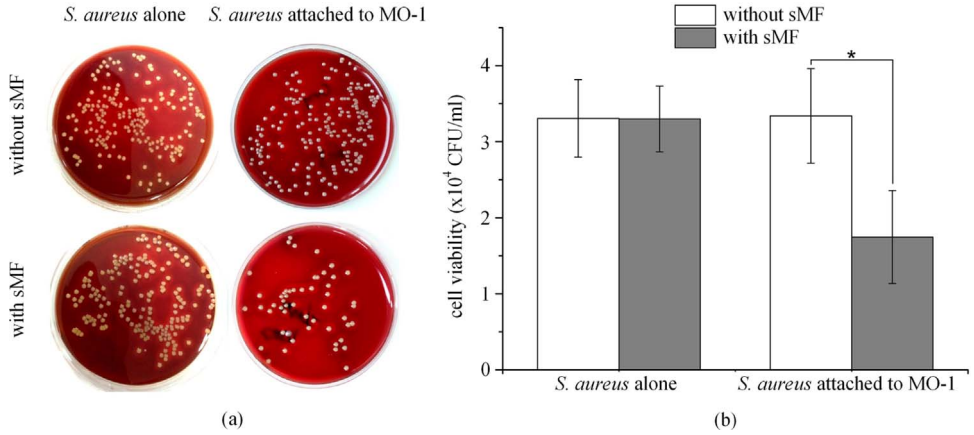

Fig. 6. The killing effect of antibody-conjugated MO-1 cells on *S. aureus* under the sMF. (a) *S. aureus* colonies in blood LB agar plates of *S. aureus* alone and MO-1 attached groups, with or without sMF respectively. (b) the killing effect on *S. aureus*. $^{*}P < 0.05$.

confirmed in our future work.

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