



HAL
open science

Biocompatible analysis of marine magnetotactic ovoid strain MO-1 for in vivo application *

Chen Changyou, Wang Pingping, Long-Fei Wu, Song Tao

► To cite this version:

Chen Changyou, Wang Pingping, Long-Fei Wu, Song Tao. Biocompatible analysis of marine magnetotactic ovoid strain MO-1 for in vivo application *. Chinese Journal of Oceanology and Limnology, 2021, 10.1007/s00343-021-0420-7 . hal-03338462

HAL Id: hal-03338462

<https://amu.hal.science/hal-03338462>

Submitted on 8 Sep 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Biocompatible analysis of marine magnetotactic ovoid strain MO-1 for *in vivo* application *

CHEN Changyou^{1,2}, WANG Pingping^{1,2**}, Long-Fei WU^{2,3}, SONG Tao^{1,2,4}

¹ Beijing Key Laboratory of Bioelectromagnetism, Institute of Electrical Engineering, Chinese Academy of Sciences, Beijing 100190, China.

² France-China International Laboratory of Evolution and Development of Magnetotactic Multicellular Organisms, Beijing, China.

³ Aix Marseille University, CNRS, LCB, Marseille F-13402, France.

⁴ University of the Chinese Academy of Sciences, Beijing 100049, China.

* Supported by the National Natural Science Foundation of China (No. 51937011, No. 51907192) and the National Key Research and Development Program of China (Grant NO. 2017YFC0108501).

** **Corresponding author:** wangpp@mail.iee.ac.cn.

Abstract Magnetotactic bacteria are capable of biosynthesizing magnetic nanoparticles, also called magnetosomes, and swimming along magnetic field lines. The abilities endow the whole cells of magnetotactic bacteria with such applications as targeted therapy and manipulation of microrobots. We have shown that the intact marine magnetotactic bacteria MO-1 kill efficiently antibiotic-resistant pathogen *Staphylococcus aureus in vivo*, but the biocompatibility of this marine bacteria is unknown. In this study, the strain MO-1 was chosen to analyze its biocompatibility and potential for biomedicine applications. Results showed that MO-1 cells could be guided at 37 °C under an external magnetic field and swim in the blood plasma and urine. They could keep active locomotivity within 40 minutes in the plasma and urine, although their velocity slowed down. When incubated with human cells, magnetotactic bacteria MO-1 had no obvious effects on cellular viability at low dose, while the cell toxicity increased with the augmentation of the quantity of the MO-1 cells added. In the *in vivo* experiments, the median lethal dose of magnetotactic bacteria MO-1 in rats was determined to be 7.9×10^{10} cells/kg. These results provided the foundation for the biocompatibility and safety evaluations of magnetotactic bacteria MO-1 and suggested that they could be basically used in clinical targeted therapy.

Keywords: magnetotactic bacteria, motility, cell interaction, median lethal dose

1 INTRODUCTION

Magnetotactic bacteria are a group of phylogenetically diverse and Gram-negative microorganisms with the ability of synthesizing magnetite (Fe_3O_4) or greigite (Fe_3S_4) nanoparticles in a genetically controlled manner (R.P. Blakemore, 1982; Bazylinski and Frankel, 2004; Simmons et al., 2006; Faivre and Schüller, 2008). These nanoparticles, also called magnetosomes, are arranged in a chain-like manner, which helps magnetotactic bacteria to swim along magnetic lines (Lefèvre and Wu, 2013). Magnetotactic bacteria also have the flagella that enable them to swim autonomously and efficiently (Zhang and Wu, 2020). On the basis of different types of swimming behavior, magnetotactic bacteria can be divided into two main categories: one is polar strain that swims persistently in one direction along the magnetic field, and the other one is axial strain that swims in either direction along magnetic field lines (Bazylinski and Frankel, 2004). The features of magnetosomes and autonomous movements along the magnetic field endow them with rapid responsibility to external magnetic field.

As a biosynthetic magnetic nanoparticle, magnetosome is coated by a biomembrane, which could be easily decorated for *in vitro* uses, such as DNA extraction and immunoassays

(Matsunaga et al., 2004). Magnetosomes could also be used *in vivo* as a contrast agent (Hu et al., 2010; David Heinke, 2017), drug carriers (Sun et al., 2007; Alphandéry et al., 2017), and a tumor photo-/magneto-thermal transmitter (Alphandéry et al., 2011; Alphandéry et al., 2012; Chen et al., 2016). However, they are not capable of autonomous swimming and do not target the deep area in the body even when an external magnetic field is applied, thereby greatly limiting their applications.

Applications of the whole cell of magnetotactic bacteria have recently attracted considerable attention. The whole magnetotactic bacteria could not only be guided to target the deep area but also perform as energy converters, which are conducive to microrobot construction and targeted therapy (Fdez-Gubieda et al., 2020). Magnetotactic bacteria are suitable actuators of bacterial microrobot due to their autonomous swimming ability. *Magnetospirillum gryphiswalense* (MSR-1), an axial strain, was constructed as a guidable delivery vehicle for cargo transport and release (Alsaiani et al., 2016). It was also integrated with ciprofloxacin-loaded microtubes to build controllable microrobots, and the robots could target an infectious biofilm under the guidance of an external magnetic field and then release the antibiotics (Stanton et al., 2017). A polar magnetotactic bacterium may be more suitable as an actuator than the above axial strain because it swims persistently in one direction along the magnetic field. A polar marine *magnetotactic coccus*, designated strain MC-1 is one of the best actuators for building microrobots. MC-1 bacteria pushed a microsphere and swam along the magnetic field, thus showing a great potential in manipulated movement under large weight loading (Martel S, 2006). Further, MC-1 cells were used to transport drug-loaded nanoliposomes to target the tumor in the blood under the control of magnetic field (Taherkhani et al., 2014; Felfoul et al., 2016). Another polar magnetotactic bacterium, magnetotactic ovoid strain MO-1, is also a highly suitable candidate for constructing microrobots. Magnetotactic ovoid strain MO-1 was isolated from the Mediterranean Sea, and it is a kind of chemoautotrophic bacterium (Lefèvre et al., 2009). MO-1-derived microrobots were constructed by attaching microbeads to the cell surface, and the microrobots could swim in the microfluidic channel under the control of magnetic field (Ma et al., 2012). MO-1 cells have also been fabricated as a separating microrobot to target separate pathogens and then kill them using different external magnetic fields (Chen et al., 2014; Chen et al., 2017). By using magnetosomes

in the cell body, intact magnetotactic bacteria could produce positive magnetic resonance imaging contrast for visualizing mouse tumor xenografts, thereby providing a potential tool for improved cancer imaging visualization in preclinical and translational studies (Benoit et al., 2009). The whole cell of magnetotactic bacteria could also transmit the magnetic field energy into the thermal energy through the intracellular nanoparticles. When applied in the alternating magnetic field, they produced heat and then strongly affected cancer cell proliferation on clinical conditions (Gandia et al., 2019). Magnetotactic bacterium-based magnetic hyperthermia was also used to target and kill pathogenic cells, thus could potentially treat infectious diseases (Chen et al., 2016). Aside from thermal energy, magnetotactic bacteria could transmit the magnetic field energy to the mechanical force, which could inhibit the viability of pathogen (Chen et al., 2017).

Although the whole magnetotactic bacteria show many potentials in the construction of microrobots and targeted therapy, their biocompatibility and biosecurity should be considered seriously. On the one hand, when magnetotactic bacteria enter into the body fluid, such as blood and urine, for drug delivery and targeted treatment, their status and swimming ability require sufficient evaluation. When incubated in the blood, the terminal velocity of *magnetococcus marinus* strain MC-1 decayed for a duration of 40 minutes (Martel et al., 2009). On the other hand, magnetotactic bacteria, as foreign substances, are certainly attacked by the immune system in the human body, and the toxicity should be assessed. As for magneto-ovoid strain MO-1, which has been used for the construction of bacterial microrobots and targeted therapy, the biocompatible studies in the blood and urine are still unknown until now, and they need to be carried out.

In the present study, magneto-ovoid strain MO-1 was used to investigate its biocompatibility for *in vivo* applications. The swimming ability of MO-1 bacteria in the blood and urine was first assessed. The interaction of bacteria and human cells was examined to show the biocompatibility and the cellular safe dose. Finally, the median lethal dose (LD₅₀) of magnetotactic bacteria in rats was determined for *in vivo* biomedical utilization.

2 MATERIAL AND METHOD

2.1 Material

Chemical reagents containing NaCl, MgCl₂, Na₂SO₄, CaCl₂, KCl, NH₄Cl, NaHCO₃, K₂HPO₄,

and NaNO₃ were purchased from BBI Co., Ltd. Succinic acid, L-cysteine, tryptone, and yeast extract were bought from AMRESCO Commercial Finance, LLC. MTT was purchased from Sigma–Aldrich. The mineral solution and vitamin solution for magnetotactic ovoid strain MO-1 were prepared in accordance with previous reports (Frankel et al., 1997). Ferric quinate was also prepared. Artificial urine was purchased from Qingdao Jisskang Biotechnology Co., Ltd. (China), and its pH was adjusted to 7.5 by using NaOH solution.

2.2 Animal

Adult male Sprague–Dawley (SD) rats weighing 180–200 g were used in the LD₅₀ experiments and purchased from Beijing HFK Bio-Technology Co., Ltd (China). Blood was acquired after the rats were anesthetized, and then the plasma was obtained by centrifuging the rat blood.

2.3 Bacterial culture and transmission electron microscopy (TEM)

The magnetotactic ovoid strain MO-1 was grown at room temperature in EMS2 medium as previously reported (Lefevre, et al., 2009). The EMS2 medium was first added with (per L of deionized water; pH 6.8) 19.45 g NaCl, 5.9 g MgCl₂, 3.24 g Na₂SO₄, 1.8 g CaCl₂, 0.55 g KCl, 0.2 g NH₄Cl, 1.26 g NaHCO₃, 0.6 g L-cysteine, 3 g HEPES, and 0.35 g agarose. The medium was then autoclaved at 120°C for 20 min. After the medium was cooled to approximately 45 °C, the following solutions were added (per L) to previously sterile-filtered stock solutions in the following order: 0.5 mL vitamin solution, 0.5 mL ferric quinate (0.4 mM), 3 mL sodium thiosulfate (40% w/v), 4 mL dipotassium hydrogen phosphate (1.86% w/v), and 5 mL modified Wolfe’s mineral solution. Ultrathin copper grids were immersed in the bacterial suspensions to observe the MO-1 cell morphology, and then the grids were dried in a clean bench after retrieval for observation using a transmission electron microscope (Hitachi H-7650B; Hitachi, Japan).

2.4 Speed analysis of magnetotactic bacteria in plasma and urine

Speed analysis of magnetotactic bacteria MO-1 in the plasma and urine was carried out in a microfluidic chip. The microfluidic channel contains four corners, and its plane geometry was shown in the previous report (Ma et al., 2012). The width and depth of the channel were 200 µm and 20 µm, respectively. The rat plasma or artificial urine was pushed into a microfluidic channel. After one of the micropools in the chip was blocked, the MO-1 solution was injected into the other

micropool. A magnetic field was applied to guide the MO-1 cells in the microfluidic channel. The movement of MO-1 swarm in the plasma or artificial urine was observed under 40× magnification in the dark-field mode. The swimming behavior was recorded using a Canon 500D camera that recorded 1920 × 1080 pixel images at 20 frames per second. The experiments were conducted at 37 °C to mimic the *in vivo* biomedical applications of human physiological temperature and repeated thrice. The temperature controlling instrument of Live Cell Imaging System was used to keep the microfluidic chip with MO-1 cell samples at 37 °C. All the blood and urine solutions were at human physiological pH. The average swimming velocities were calculated using the MTrackJ plugin for ImageJ (Murat et al., 2015; Zhang et al., 2017).

2.5 Cell culture and MTT assay

Human immortalized epidermal cell HaCaT (No. 3111C0001CCC000373) and human cervical carcinoma cell Hela (No. 3111C0001CCC000011; China Infrastructure of Cell Line Resources, Beijing, China) were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco/Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Australian origin, Hyclone Laboratories, Logan, UT, USA), 100 IU/mL penicillin (Sigma–Aldrich, St. Louis, MO, USA), and 100 µg/mL streptomycin (Sigma-Aldrich) at 37 °C in 5% CO₂. The HaCaT and Hela cells were seeded at 10,000 cells/well in 100 µL DMEM in a 96-well plate and incubated for 24 h. The medium was then replaced with the magnetotactic bacteria MO-1 working solution for the succeeding experiments. The magnetotactic bacteria MO-1 working solution (per well in 100 µL DMEM) contains 0 (control sample), 2×10^5 , 4×10^5 , 8×10^5 , 1.6×10^6 , 3.3×10^6 , 6.5×10^6 , 1.3×10^7 , 2.5×10^7 , and 5×10^7 bacteria.

After incubation was performed for 24 h, the viability of the HaCaT and Hela cells was analyzed by MTT method. In brief, the cell medium was replaced with 100 µL DMEM without phenol red (Gibco) containing MTT agent and incubated at 37°C in the dark for 4 h. The medium was discarded, and 100 µL of dimethyl sulfoxide (Sigma–Aldrich) was added to the well. After shaking for 15 min, the cell plates were centrifuged at 3000 rpm for 5 min, and the supernatant was transferred to a new 96-well plate. The absorbance was measured at 570 nm with a reference at 630 nm by using a microplate absorbance reader (168-1130 model, Bio-Rad Laboratories, Hercules, CA, USA). The relative cell viability (%) relative to control was calculated using the

following equation: $[A(\text{test sample})/A(\text{control sample})] \times 100\%$ (where A is absorbance). The experiments were repeated thrice.

2.6 LD₅₀ of magnetotactic bacteria in rats

The magnetotactic ovoid strain MO-1 in exponential growth was collected using a magnetic block and suspended in phosphate buffer solution (PBS, pH7.0). Then, 16 SD rats were randomly assigned to four groups (n= 4), and 5×10^8 , 5×10^9 , 5×10^{10} and 5×10^{11} MO-1 cells in 3 mL PBS were injected into four groups of rats intravenously. The animals in each group were observed for 1 week. Afterwards, Behrens–Kärber method was used to calculate the LD₅₀ and 95% confidence interval (CI) (Zhang et al., 2015):

$$LD_{50} = \lg^{-1} \left[X_m - i \left(\sum p - 0.5 \right) \right], \quad S_{\lg LD_{50}} = i \sqrt{\sum \frac{pq}{n}},$$

$$95\% \text{ CI: } \left[\lg^{-1} \left(\lg LD_{50} - 1.96 S_{\lg LD_{50}} \right), \lg^{-1} \left(\lg LD_{50} + 1.96 S_{\lg LD_{50}} \right) \right]$$

where X_m is the logarithm of the maximum dose, i is the difference in the logarithm of adjacent concentrations, p and q are the lethal success and failure rates in each group, and n is the number of rats in each group.

3 RESULT AND DISCUSSION

3.1 Characterization of magnetotactic bacteria MO-1

Magneto-ovoid strain MO-1 is a marine species affiliated with zeta proteobacterial strains, and it grew at aerobic–anaerobic interfaces in the culture flasks. The TEM results showed that magnetotactic bacteria MO-1 owned two flagellar bundles on the long hemisphere of the cell (Fig. 1a, black arrows). They have an autonomous swimming ability toward north pole in a magnetic field. TEM images showed that they also synthesized bio-membrane-coated magnetosomes with an average number of 11.1 ± 2.94 (Fig. 1b), which assisted them to feel and move along the magnetic fields. The morphologies of most MO-1 magnetosome particles have an elongated cubo-octahedral shape, with the length of 77.06 ± 11.1 nm, width of 71.54 ± 10.59 nm respectively (n=149). The features of fast autonomous movement and magnetotaxis may lead them to reach the deep site inside the organism body for drug delivery and targeted therapy.

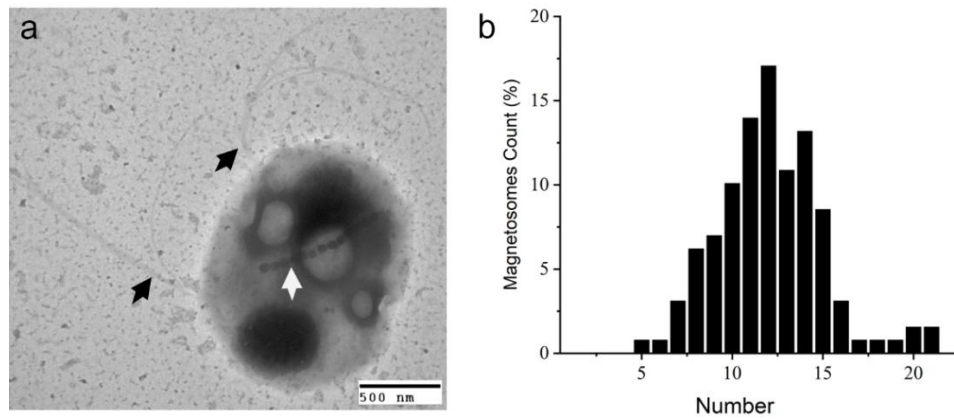


Fig. 1 Magnetotactic ovoid strain MO-1. a Magnetotactic ovoid strain MO-1. Black arrows indicated the two flagellar bundles and white arrow indicated the magnetosome chain. b Number of magnetosome (1432) in 129 MO-1 cells.

3.2 Speed of magnetotactic bacteria MO-1 in rat plasma and artificial urine

Movement ability is an important characteristic for MO-1 cells to perform a task. Generally, *in vivo* application scenarios in biomedical areas are always accompanied with the body liquid environment, such as blood and urine. Here, the magnetotactic velocity of magnetotactic bacteria MO-1 in the blood plasma and artificial urine was assessed at the human physiological temperature of 37 °C to mimic the *in vivo* biological application scenarios. The rat plasma was acquired, and the MO-1 cells and the plasma were injected into the microfluidic chip. At 37 °C, when a magnetic field with a strength of 2.2 mT was applied, MO-1 cells could swim along the magnetic field in the blood plasma liquid, the fresh MO-1 cells swam at the beginning speed of about 300 $\mu\text{m/s}$, while the swimming speed decreased as the time pasted (Fig. 2a and 2b). In the first 4 minutes, the MO-1 cells swam at the velocity of above 200 $\mu\text{m/s}$ and then travelled at more than 100 $\mu\text{m/s}$ in the following 20 minutes. In the next time, the MO-1 cells continued to swim at a lower speed below 100 $\mu\text{m/s}$. During the entire process, the MO-1 cells were capable of responding to the external magnetic field guidance at 37 °C (Fig. 2b). The experimental results showed that magnetotactic bacteria MO-1 could survive and keep their swimming motility in the plasma at the human physiological temperature for at least 45 minutes.

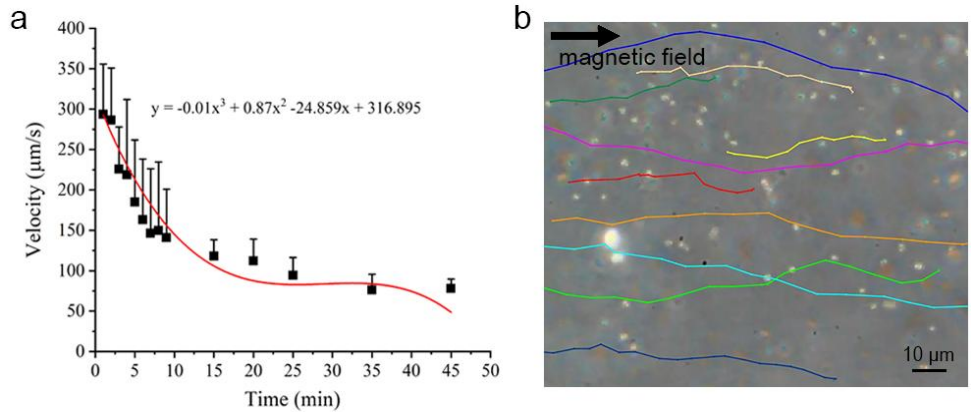


Fig. 2 Speed of magnetotactic bacteria MO-1 in rat plasma under external magnetic field. The swimming speed of magnetotactic bacteria MO-1 in the plasma at 37 °C was analyzed from 450 bacterial cells (a), and the movement trajectories of MO-1 cells along magnetic field in plasma at 37 °C were shown in (b).

When artificial urine was used as another model of body fluid, the state of magnetotactic bacteria MO-1 at the human physiological temperature (37 °C) was similar to that in the blood plasma (Fig. 3a). The MO-1 cells could be navigated by an external magnetic field and swim in the urine at a speed of above 200 µm/s during the first 6 minutes. The bacteria over time continued swimming at the speed of more than 130 µm/s for 20 minutes. Finally, their speed further decreased in the next time but still moved in the urine at a lower speed below 100 µm/s (Fig. 3a and 3b). The swimming experimental results confirmed that the MO-1 cells could survive and swim for at least 40 minutes in the urine at physiological temperature.

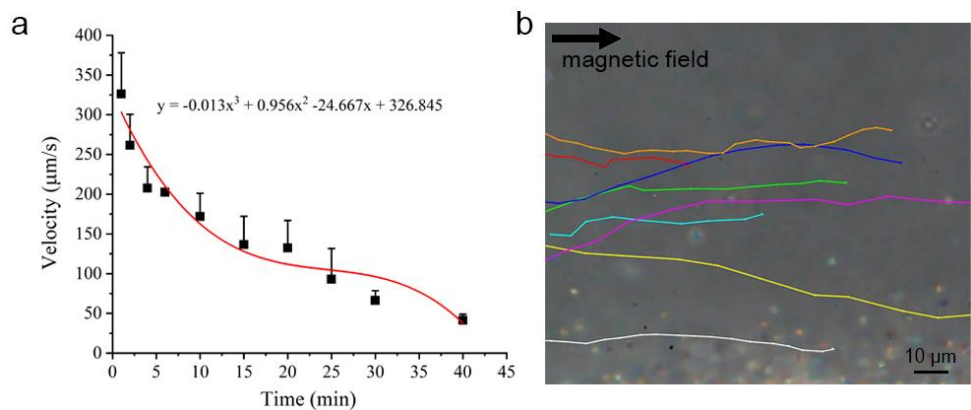


Fig. 3 Speed of magnetotactic bacteria MO-1 in artificial urine under external magnetic field. The swimming speed of magnetotactic bacteria MO-1 in the urine at 37 °C was analyzed from 296 bacterial cells (a) and the movement trajectories of MO-1 cells along magnetic fields in the urine at 37 °C were shown in (b).

Targeted therapy or drug delivery in the human body mediated by magnetotactic bacteria probably need the ability of these bacteria to swim autonomously in the body fluid. The results of this study demonstrated that magnetotactic bacteria MO-1 are capable of surviving and swimming in the blood and urine. According to the average speed and surviving time, the distance that magnetotactic bacteria MO-1 swam in the blood for 45 minutes or in the urine for 40 minutes at physiological temperature could even reach up to approximately 40 cm. Considering the size of human organ and the principle of proximal intervention with external carriers, the magnetotactic bacteria MO-1 swimming distance is enough to satisfy the requirements of drug delivery and targeted therapy. The blood results are also similar to those when another magnetotactic bacteria of MC-1 were used (Martel, et al., 2009). Other factors, such as blood flow velocity and vascular branch, were not considered in the present study. However, it's been reported that magnetotaxis enabled MTB to overcome counter-directional flow and migrate effectively through porous micromodels (Rismani Yazdi, et al., 2017; Rismani Yazdi, et al., 2018). As to the scene that the velocity of MO-1 cells actually decreased as the incubation time increased, the main reason is possibly the osmotic pressure induced by different salt concentrations. For example, the NaCl concentrations in the blood and urine are 8.5 and 4.2 g/L respectively, much lower than those (19.45 g/L) needed by MO-1 to grow. Consistently, it has also been implied that the velocity of the bacteria could also be controlled by varying the concentration of Na⁺ in the medium (Lefèvre et al., 2009). Besides, a magnetic field with a higher strength could slow down the speed of magnetotactic bacteria (Yang et al., 2012). It's also suggested from the survival and movement abilities that magnetotactic bacteria do not proliferate spontaneously inside an animal nor cause severe infection and pathogenicity. Thus, their safety for the *in vivo* application is much better than that of other pathogenic bacteria.

3.3 Interaction of magnetotactic bacteria MO-1 with human HaCaT and Hela cells

Although the infection and pathogenicity of magnetotactic bacteria as a therapeutic tool may be negligible according to the analysis, the dose effect at the cellular level and the *in vivo* toxicity of magnetotactic bacteria MO-1 remained to be studied. In the present study, the biocompatibility and biological safety dose of magnetotactic bacteria MO-1 at the cellular level were examined via MTT assay. HaCaT and Hela cells (1×10^4 cells) were treated with magnetotactic bacteria MO-1

at different bacterial cell concentrations ranging from 0 to 5×10^7 bacteria/well in 100 μ L DMEM in a 96-well plate for 24 h. As shown in Fig. 4a, the incubation of MO-1 cells at the concentration of less than 3.3×10^6 bacteria/well had no effect on the viability of the HaCaT cells. The cellular viability decreased by approximately 40% at the concentration of 6.5×10^6 bacteria/well and continued to decrease as the number of MO-1 cells was increased to 5×10^7 bacteria/well (Fig. 4a). As for HeLa cells, co-culture of MO-1 cells did not induce cell toxicity at the bacterial concentration of less than 1.6×10^6 bacteria/well (Fig. 4b). However, when the MO-1 concentration was at 3.3×10^6 bacteria/well, the viability of HeLa cells decreased significantly to 70% and continued to decrease with the increased concentration of magnetotactic bacteria MO-1. A potential reason for the increased cytotoxicity may be that increased magnetotactic bacteria touched with cells and then were phagocytosed, leading to increases in oxidative stress and reactive oxygen species inside the cell (Wang et al., 2014; Wang et al., 2017).

Previous reports showed that 6.5×10^6 bacteria of *magnetococcus marinus* strain MC-1 incubated with 1×10^5 murine J774A.1 and NIH/3T3 cells in a 96-well plate induced a huge decrease in cellular viability to approximately 30% (Taherkhani, et al., 2014). The decrease in cellular viability caused by magnetotactic bacteria MO-1 in the present study was much smaller than the results of strain MC-1. The difference may be attributed to the different cell lines and added bacterial species. The method of coating nanoliposome on the surface of MC-1 bacteria was proposed to reduce the toxicity of magnetotactic bacteria (Taherkhani, et al., 2014).

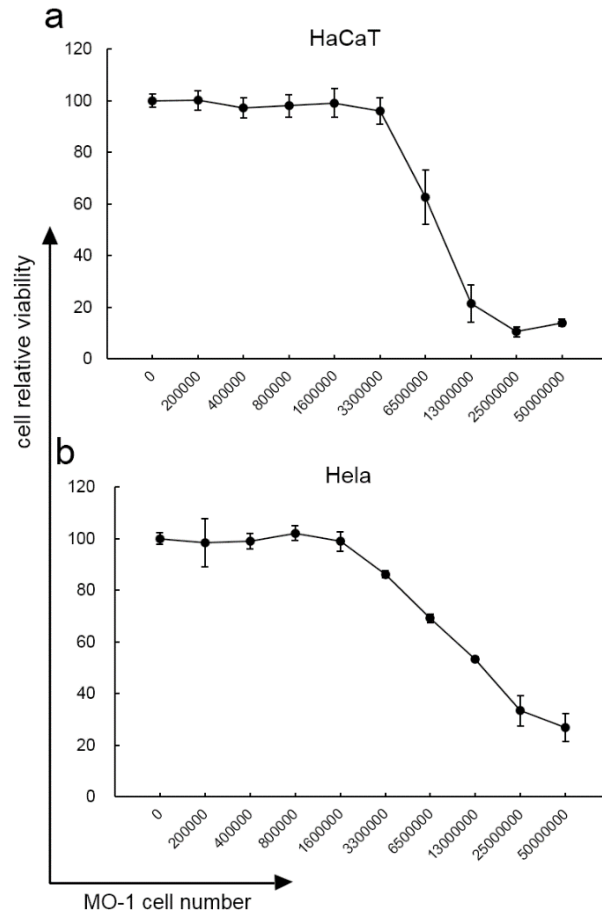


Fig. 4 Cell viability of HaCaT (a) and HeLa (b) cells after interaction with magnetotactic bacteria MO-1.

3.4 Determination of LD₅₀ of magnetotactic bacteria in rats

From an application perspective, the effect of magnetotactic bacteria on the whole human body must be considered. Different doses of magnetotactic bacteria MO-1 were injected into rats in each test group to acquire the LD₅₀, and the rats were observed persistently for 1 week. As shown in Fig. 5, the survival rate decreased as the doses of magnetotactic bacteria MO-1 were increased. The experimental animals did not die in the groups of 5×10^8 bacteria/3 mL, one died in the 5×10^9 bacteria/3 mL group, three died in the 5×10^{10} bacteria/3 mL group, and all animals died in the 5×10^{11} bacteria/3 mL group. Behrens–Kärber method was used to calculate the LD₅₀ of the rats (0.2 kg) at 7.9×10^{10} bacteria/kg, with a 95% CI of 1.99×10^{10} – 3.155×10^{11} bacteria/kg. According to a guidance (U.S. Food and Drug Administration, 2005), the dose per unit body weight of an adult (60 kg) is equivalent to 0.16 of that of rats (0.2 kg). Therefore, the LD₅₀ of an adult human (60 kg) was calculated to be 1.264×10^{10} MO-1 cells/kg.

With the abilities of magnetic nanoparticle synthesis and autonomous swimming,

magnetotactic bacteria have been used as contrast agents and *in vivo* drug delivery. As a contrast agent, *magnetospirillum magneticum* AMB-1 was intravenously injected into mice with 1×10^9 cells (Benoit, et al., 2009). The number of magnetotactic bacteria MC-1 intravenously injected into rats for drug delivery was reported to be 1×10^8 cells (Felfoul, et al., 2016). Thus, the actual quantity of magnetotactic bacteria for *in vivo* uses was much less than its LD₅₀ (7.9×10^{10} MO-1 cells/kg in rats) obtained in the present study. Of course, different species of magnetotactic bacteria may have different LD₅₀ values, thereby needing further investigations. Regardless of the species of magnetotactic bacteria, after entering the body from the blood vessels or urethra, these bacteria may interact with the initially encountered organ or tissue and then interact with different kinds of cells, especially immune cells. Whether magnetotactic bacteria induced an intense immune response or not depends mainly on the immunogenicity and quantity of the bacteria. In any case, as a kind of Gram-negative bacteria, the endotoxins of magnetotactic bacteria MO-1 should be paid with sufficient attention when used in biomedical applications (Alphandéry, et al., 2020). On one hand, agents such as poly-L-lysine could be modified to reduce or eradicate the endotoxins of magnetotactic bacteria (Hamdous, et al., 2017; Mandawala, et al., 2017). On the other hand, rats could actually overcome some endotoxins of MO-1 cells according to the study. Nevertheless, the endotoxins of magnetotactic bacteria / magnetosomes released from bacterial cells into the animal body should be considered seriously. Taken together, on the basis of the LD₅₀ obtained in the present study, the dose of MO-1 cells may be safe and it could satisfy the requirement of clinical tumor therapy.

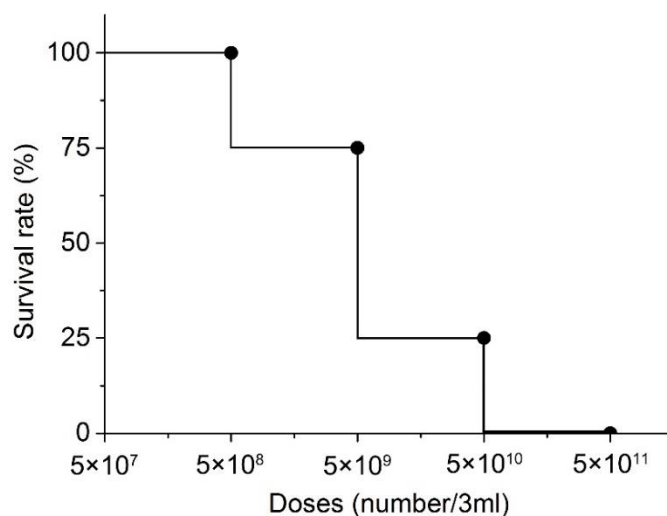


Fig. 5 Determination of LD₅₀ of magnetotactic bacteria in rats.

4 CONCLUSIONS

In summary, the biocompatibility of magnetotactic bacteria was determined in this study. The MO-1 cells were able to swim along the magnetic field lines in the blood and urine and survive at 37 °C for approximately 40 minutes. When the HaCaT and Hela cells were incubated with magnetotactic bacteria MO-1, their cellular effect was dependent on the dose and within the range of below 3.3×10^7 MO-1 cells/mL, which was well tolerated by the human cells. In the animal experiments, an LD₅₀ of 7.9×10^{10} bacterial cells/kg in rats was determined. These data provided the key to understand the biocompatibility and safety of magnetotactic bacteria to a certain extent and suggested that magnetotactic ovoid strain MO-1 could basically be used in targeted therapy. However, the distribution of whole bacteria in the body should be further investigated.

5 ACKNOWLEDGEMENT

We would like to thank Dr. Haitao Chen for the assistance in analyzing the swimming speed of magnetotactic bacteria MO-1 in the plasma and urine by using the MTrackJ plugin for ImageJ.

References

- Alphandéry E. 2020. Applications of magnetotactic bacteria and magnetosome for cancer treatment: A review emphasizing on practical and mechanistic aspects, *Drug Discov. Today*, 25(8): 1444-1452.
- Alphandéry E, Faure S, Seksek O, Guyot F, Chebbi I. 2011. Chains of Magnetosomes Extracted from AMB-1 Magnetotactic Bacteria for Application in Alternative Magnetic Field Cancer Therapy, *ACS Nano*, 5(8): 6279-6296.
- Alphandéry E, Guyot F, Chebbi I. 2012. Preparation of chains of magnetosomes, isolated from *Magnetospirillum magneticum* strain AMB-1 magnetotactic bacteria, yielding efficient treatment of tumors using magnetic hyperthermia, *Int. J. Pharm.*, 434(1-2): 444-452.
- Alphandéry E, Idbaih A, Adam C, Delattre J Y, Schmitt C, Guyot F, Chebbi I. 2017. Chains of magnetosomes with controlled endotoxin release and partial tumor occupation induce full destruction of intracranial U87-Luc glioma in mice under the application of an alternating magnetic field, *J. Control Release*, 262: 259-272.
- Alsaïari S K, Ezzedine A H, Abdallah A M, Sougrat R, Khashab N M. 2016. Magnetotactic

- bacterial cages as safe and smart gene delivery vehicles, *OpenNano*, 1: 36-45.
- Bazylnski D A, Frankel R B. 2004. Magnetosome formation in prokaryotes, *Nat. Rev. Microbiol.*, 2(3): 217-230.
- Benoit M R, Mayer D, Barak Y, Chen I Y, Hu W, Cheng Z, Wang S X, Spielman D M, Gambhir S S, Matin A. 2009. Visualizing implanted tumors in mice with magnetic resonance imaging using magnetotactic bacteria, *Clin. Cancer Res.*, 15(16): 5170-5177.
- Chen C, Chen L, Wang P, Wu L F, Song T. 2017. Magnetically-induced elimination of *Staphylococcus aureus* by magnetotactic bacteria under a swing magnetic field, *Nanomedicine*, 13(2): 363-370.
- Chen C, Chen L, Yi Y, Chen C, Wu L F, Song T. 2016. Killing of *Staphylococcus aureus* via Magnetic Hyperthermia Mediated by Magnetotactic Bacteria, *Appl. Environ. Microbiol.*, 82(7): 2219-2226.
- Chen C, Wang S, Li L, Wang P, Chen C, Sun Z, Song T. 2016. Bacterial magnetic nanoparticles for photothermal therapy of cancer under the guidance of MRI, *Biomaterials*, 104: 352-360.
- Chen C Y, Chen C F, Yi Y, Chen L J, Wu L F, Song T. 2014. Construction of a microrobot system using magnetotactic bacteria for the separation of *Staphylococcus aureus*, *Biomed. Microdevices*, 16(5): 761-770.
- Chen L, Chen C, Wang P, Chen C, Wu L-F, Song T. 2017. A compound magnetic field generating system for targeted killing of *Staphylococcus aureus* by magnetotactic bacteria in a microfluidic chip, *J. Magn. Magn. Mater.*, 427: 90-94.
- David Heinke A K, Dietmar Eberbeck, Daniel Schmidt, Patricia Radon, René Uebe, Dirk Schüler, Andreas Briel. 2017. MPS and MRI efficacy of magnetosomes from wild-type and mutant bacterial strains, *Int. J. Mag. Part. Imag.*, 13(2): 1-6.
- Faivre D, Schuler D. 2008. Magnetotactic Bacteria and Magnetosomes, *Chem. Rev.*, 108(11): 4875-4898.
- Fdez-Gubieda M L, Alonso J, García-Prieto A, García-Arribas A, Fernández Barquín L, Muela A. 2020. Magnetotactic bacteria for cancer therapy, *J. Appl. Phys.*, 128: 070902.
- Felfoul O, Mohammadi M, Taherkhani S, de Lanaude D, Zhong Xu Y, Loghin D, Essa S, Jancik S, Houle D, Lafleur M, Gaboury L, Tabrizian M, Kaou N, Atkin M, Vuong T, Batist G,

- Beauchemin N, Radzioch D, Martel S. 2016. Magneto-aerotactic bacteria deliver drug-containing nanoliposomes to tumour hypoxic regions, *Nat. Nanotechnol.*, 11(11): 941-947.
- Frankel R B, Bazylinski D A, Johnson M S, Taylor B L. 1997. Magneto-aerotaxis in marine coccoid bacteria, *Biophys. J.*, 73(2): 994-1000.
- Gandia D, Gandarias L, Rodrigo I, Robles-Garcia J, Das R, Garaio E, Garcia J A, Phan M H, Srikanth H, Orue I, Alonso J, Muela A, Fdez-Gubieda M L. 2019. Unlocking the Potential of Magnetotactic Bacteria as Magnetic Hyperthermia Agents, *Small*, 15(41): 1-12.
- Hu L L, Zhang F, Wang Z, You X F, Nie L, Wang H X, Song T, Yang W H. 2010. Comparison of the ¹H NMR Relaxation Enhancement Produced by Bacterial Magnetosomes and Synthetic Iron Oxide Nanoparticles for Potential Use as MR Molecular Probes, *IEEE T. Appl. Supercon.*, 20(3): 822-825.
- Hamdous Y, Chebbi I, Mandawala C, LeFèvre R, Guyot F, Seksek O, Alphandéry E. 2017. Biocompatible coated magnetosome minerals with various organization and cellular interaction properties induce cytotoxicity towards RG-2 and GL-261 glioma cells in the presence of an alternating magnetic field, *J. Nanobiotechnol.*, 15: 1-18.
- Lefèvre C T, Bernadac A, Yu-Zhang K, Pradel N, Wu L F. 2009. Isolation and characterization of a magnetotactic bacterial culture from the Mediterranean Sea, *Environ. Microbiol.*, 11(7): 1646-1657.
- Lefèvre C T, Wu L F. 2013. Evolution of the bacterial organelle responsible for magnetotaxis, *Trends Microbiol.*, 21(10): 534-547.
- Ma Q, Chen C, Wei S, Chen C, Wu L F, Song T. 2012. Construction and operation of a microrobot based on magnetotactic bacteria in a microfluidic chip, *Biomicrofluidics*, 6(2): 24107.
- Mandawala C, Chebbi I, Durand-Dubief M, Le Fèvre R, Hamdous Y, Guyot F, Alphandéry E. 2017. Biocompatible and stable magnetosome minerals coated with poly-L-lysine, citric acid, oleic acid, and carboxy-methyl-dextran for application in the magnetic hyperthermia treatment of tumors, *J. Mater. Chem. B*, 5: 7644-7660.
- Martel S, Mohammadi M, Felfoul O, Lu Z, Pouponneau P. 2009. Flagellated Magnetotactic Bacteria as Controlled MRI-trackable Propulsion and Steering Systems for Medical

- Nanorobots Operating in the Human Microvasculature, *Int. J. Rob. Res.*, 28(4): 571-582.
- Martel S T C C, Ngakeng S, Langlois G. 2006. Controlled manipulation and actuation of micro-objects with magnetotactic bacteria, *Appl. Phys. Lett.*, 89(23): 1.
- Matsunaga T, Okamura Y, Tanaka T. 2004. Biotechnological application of nano-scale engineered bacterial magnetic particles, *J. Mater. Chem.*, 14(14): 2099-2015.
- Murat D, Herisse M, Espinosa L, Bossa A, Alberto F, Wu L F. 2015. Opposite and Coordinated Rotation of Amphitrichous Flagella Governs Oriented Swimming and Reversals in a Magnetotactic Spirillum, *J. Bacteriol.*, 197(20): 3275-3282.
- R.P. Blakemore. 1982. magnetotactic bacteria, *Ann. Rev. Microbiol*, 36: 217-238.
- Rismani Yazdi S, Nosrati R, Stevens CA, Vogel D, Davies PL, Escobedo C. 2017. Magnetotaxis Enables Magnetotactic Bacteria to Navigate in Flow, *Small*, 14(5): 1702982.
- Rismani Yazdi S, Nosrati R, Stevens C A, Vogel D, Escobedo C. 2018. Migration of magnetotactic bacteria in porous media, *Biomicrofluidics*, 12(1): 011101.
- Simmons S L, Bazylynski D A, Edwards K J. 2006. South-seeking magnetotactic bacteria in the Northern Hemisphere, *Science*, 311(5759): 371-374.
- Stanton M M, Park B W, Vilela D, Bente K, Faivre D, Sitti M, Sanchez S. 2017. Magnetotactic Bacteria Powered Biohybrids Target E. coli Biofilms, *ACS Nano*, 11(10): 9968-9978.
- Sun J B, Duan J H, Dai S L, Ren J, Zhang Y D, Tian J S, Li Y. 2007. In vitro and in vivo antitumor effects of doxorubicin loaded with bacterial magnetosomes (DBMs) on H22 cells: the magnetic bio-nanoparticles as drug carriers, *Cancer Lett.*, 258(1): 109-117.
- Taherkhani S, Mohammadi M, Daoud J, Martel S, Tabrizian M. 2014. Covalent Binding of Nanoliposomes to the Surface of Magnetotactic Bacteria for the Synthesis of Self-Propelled Therapeutic Agents, *ACS Nano*, 8(5): 5049-5060.
- U.S. Food and Drug Administration, 2005. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, 7.
- Wang P, Chen C, Chen C, Li Y, Pan W, Song T. 2017. The interaction of bacterial magnetosomes and human liver cancer cells in vitro, *J. Magn. Mater.*, 427: 105-110.
- Wang P, Chen C, Zeng K, Pan W, Song T. 2014. Magnetic nanoparticles trigger cell proliferation arrest of neuro-2a cells and ROS-mediated endoplasmic reticulum stress response, *J. Nanopart.*

Res., 16(11): 2718.

Yang C, Chen C, Ma Q, Wu L, Song T. 2012. Dynamic Model and Motion Mechanism of Magnetotactic Bacteria with Two Lateral Flagellar Bundles, *J. Bionic Eng.*, 9(2): 200-210.

Zhang C H, Ma W Q, Yang Y L, Wang H M, Dong F T, Huang Z X. 2015. Median effective effect-site concentration of sufentanil for wake-up test in adolescents undergoing surgery: a randomized trial, *BMC Anesthesiol.*, 15: 1-27.

Zhang W J, SD Z, L-F W. 2017. Measurement of Free-Swimming Motility and Magnetotactic Behavior of *Magnetococcus massalia* Strain MO-1, *Meth. Mol. Biol.*, 1593: 305-320.

Zhang W J, Wu L F. 2020. Flagella and Swimming Behavior of Marine Magnetotactic Bacteria, *Biomolecules*, 10(3): 460.