

Gentamicin Sponge for Anti-infection, a Controversial Old Topic and Preliminary Exploration of the Solution, in Vitro and in Vivo Experiments

Yongduo Li

Beijing Chao-Yang Hospital: Beijing Chaoyang Hospital

Dong Wang

Beijing Chao-Yang Hospital: Beijing Chaoyang Hospital

Junlin Zhou (✉ junlinzhou_article@outlook.com)

Beijing Chao-Yang Hospital: Beijing Chaoyang Hospital <https://orcid.org/0000-0001-8646-8779>

Research article

Keywords: Gentamicin sponge, quantitative equation, prevention concentration, treatment scheme, in vitro and in vivo experiments

Posted Date: January 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-143756/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Purpose: Our study was to construct drug-loading and drug-release quantitative equation of gentamicin sponge, in addition, obtain the wound infection prevented and treated scheme of gentamicin sponge.

Methods: Sterile sponge was cut into 1×1×0.5cm size and immersed into 40mg/ml, 16mg/ml, 8mg/ml, 4mg/ml, 1.6mg/ml, 0.8mg/ml or 0mg/ml gentamicin solution for 12h, 24h, 48h, 96h or 120h to evaluate gentamicin-loading of sponge. Sponge was immersed in gentamicin solution of different concentrations for 48h and then air-dried. Air-dried sponge was immersed into 10ml 0.9% physiological saline to evaluate gentamicin-release. *Staphylococcus aureus* (MSSA) and *Pseudomonas aeruginosa* (*P. aeruginosa*) were used to explore infection prevented scheme of gentamicin sponge. Besides, femur fractured with wound infection rat model was used to discuss the infection treated scheme.

Results: The equation of gentamicin-loading of sponge was: $z=(0.03718\pm 0.01672)x+(-4.578e-4\pm 0.06253)y+(-2.50935e-4\pm 1.47521e-4)x^2+(0.00303\pm 0.00149)y^2+(0.00408\pm 3.52827e-4)xy$ (R^2 was 0.97) and drug-release equation was $z=(4.37205\pm 1.18048)x+(-7.05921\pm 3.09628)y+(-0.04596\pm 0.01287)x^2+(0.3309\pm 0.07912)y^2+(0.31559\pm 0.02754)xy$ (R^2 was 0.95). The antibacterial zone sizes of sponges immersed in 40mg/ml, 16mg/ml, 8mg/ml, 4mg/ml, 1.6mg/ml, 0.8mg/ml gentamicin solution were larger than 0mg/ml air-dried sponge, and the difference had statistically significant ($p<0.01$). Rats of groups of 40mg/ml air-dried sponge, 16mg/ml air-dried sponge, 8mg/ml air-dried sponge had no wound suppuration in both MSSA and *P. aeruginosa* infection rat model.

Conclusions: The quantified equation of gentamicin-loading and release of sponge was with high accuracy. 1.6 mg/ml air-dried sponge and 0.8mg/ml air-dried sponge were enough to prevent wound infection. Besides, if wound were confirmed to be sensitive bacteria infected, we recommended to use 40mg/ml air-dried sponge, 16mg/ml air-dried sponge or 8mg/ml air-dried sponge to treat.

Introduction

Gentamicin sponge, an implantable topical antibiotic agent, is approved for surgical implantation in 54 countries. Since 1985, more than 1 million patients have been treated with the sponges [1–3]. However, although gentamicin sponge has been studied for more than 30 years, the effectiveness of it still has been controversial [1, 4–8].

The finding of lots of articles are inspiring. Han, J.-S. et al. analyzed the results of applying gentamicin-impregnated sponge during the spinal operation and they found that gentamicin-impregnated sponge can significantly decrease the SSI [4]. Chang, W. K. et al. analyzed whether gentamicin sponges decrease the incidence of SSI. They included 15 RCTs encompassing a total of 6979 patients and concluded that gentamicin sponge decreased the rate of SSI [5]. Schimmer, C. et al. firstly used a controlled, prospective, randomized, double-blind study to investigate the efficacy of gentamicin sponge in sternal wound complications after heart surgery. They enrolled 720 patients and found that gentamicin sponge can effectively reduce the infection complications [6].

However, some studies have demonstrated that gentamicin impregnated sponge cannot reduce the SSI and even some researches proposed that gentamicin sponge can increase the risk of infections. Wouthuyzen-Bakker, M. et al. discussed that the efficacy of applying local gentamicin-impregnated sponges during debridement in early acute periprosthetic joint infections. They found that local gentamicin impregnated sponges cannot reduce the incidence rate of infection, so they discouraged use gentamicin impregnated sponge [7]. Uçkay, I. et al. evaluated the benefit of the treatment of gentamicin sponge with mild diabetic foot ulcer infection. Regrettably, although gentamicin sponge had a very well tissue tolerated, it cannot improve the outcomes of infection [8]. Bennett-Guerrero, E. et al. randomly assigned 602 patients undergoing open or laparoscopically assisted colorectal surgery at 39 U.S. sites to undergo either gentamicin sponges or no intervention. They found that gentamicin sponges was not an effective method at preventing SSI, besides, it increased the rate of SSI [1]. Many other article have the similar results.

Over all, there are still debates about the effectiveness of gentamicin sponges for anti-infection, in additions, there are some leaks in present studies. First, the amount of gentamicin carried on sponges was inconsistent in each study and in each surgical implantation approved by different countries, thus may result in conflicting conclusions. Second, the size of sponge is also different. We lack the uniform standards or guidelines. Third, whether there is local infection or not before using of gentamicin sponge is different. Gentamicin sponge in some studies are used in aseptic surgery to explore the preventive effect infection but in some studies are used in infected patients or animals to analyze the therapeutic effect of gentamicin sponge. Forth, the observation timepoint was not consistent.

Our study was to investigate some effects of gentamicin sponge in vitro and in vivo. First, we analyzed the gentamicin-loading of sponge to construct loading equation. Second, the release of gentamicin from air-dried gentamicin-saturated sponge was evaluated to get the release equation. Third, the antibacterial effect of gentamicin sponge was observed in vitro. Forth, the anti-infection effects and mortality-reduced effects of gentamicin sponge were explored in femur fractured with wound infection rat model. We give some ideas for clinical application of gentamycin sponge, especially in open fracture infection patients.

Methods

Reagents

Luria Broth (LB) powder (containing 10 g peptone, 5 g yeast powder and 10 g NaCl) was purchased from Beijing Solarbio Science & Technology Co., Ltd and phosphate buffer saline (PBS) was purchased from Merck KGaA. LB medium was made by LB powder dissolved in 1L sterile PBS and then filtered by 0.22 um, frozen at 4°C to prepare to use. 0.9% sterile physiological saline was purchased from Baxter Medical Products Co. Ltd and sodium pentobarbital was provided by the Medical Research Center of Beijing Chaoyang Hospital affiliated to Capital Medical University. Gentamicin ELISA kit was purchased from CUSABIO Bioengineering Co. Ltd (website: <https://www.cusabio.com/food/Gentamicin-GEN-ELISA-kit-154817.html>).

Bacterial strain.

Methicillin sensitive *Staphylococcus aureus* (MSSA) standard strain and *Pseudomonas aeruginosa* (*P. aeruginosa*) were provided by the Guangdong Microbial Species Preservation Center (China, website: <http://www.gimcc.net/database1.asp>).

Animals

Female adult Sprague-Dawley (SD) rats (Charles River Laboratories, weighing 200±20 g) were kept in separate cages under a 12 h light/dark cycle at 23.6 °C and 35 % humidity. Animals were fed with sterilized chow diet and water. All procedures were complied with the ARRIVE guidelines and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The present study was approved by the Capital Medical University Ethics Committee on the use of animals in research and education. At the end of the experiments, rats were euthanized by using an excessive dose of sodium pentobarbital (100 mg/kg, intraperitoneal injection).

femur fractured with wound infection rat model

The specific process was described in our other accepted article (The acceptance letter of the article is in the supplementary materials). Briefly, we built MSSA and *P. aeruginosa* wound infection rat model with femur fractured. LB medium was used to dissolved bacterial strain powders. Bacteria suspension was cultured at 37°C, 200 rpm/min overnight. The precipitate was acquired at 10,000 rpm/min, 3 min, then resuspended in LB medium. After 3 times bacterial passage performed, bacterial suspension was diluted 10-fold by sequential transfer of 100 ml into 900 ml PBS. 100 µl of different diluted bacterial suspensions were inoculated in different LB agar media at 37°C, overnight. The number of colonies yielded between 20 to 200 were counted and multiplied by the corresponding dilution times to obtain the bacterial quantity of the bacterial suspension. Finally, bacterial quantity of the bacterial suspension was adjusted to 10⁶, 10⁷, 10⁸ and 10⁹ CFU/ml by LB medium.

The rat femur fracture model was established on a small animal operating table in a super clean bench. We refer to Husodo, K. et al [9] for the rat femur fracture model. Briefly, the rats were fasted for 12 h and banned from drinking water for 3 h before anesthesia. Isoflurane (concentration of 3%, oxygen flow 3L/min, small animal anesthesia machine) was used to induce anesthesia and sodium pentobarbital (0.4 ml/100 g, 40 mg/kg, intraperitoneal injection) was used to maintain anesthesia. The right hind limb of rats was shaved and disinfected by 75% alcohol 3 times. Right lateral position was adopted, the right hind limb was draped using a sterile surgical towel. The femoral lateral approach was used. The incision was 1-1.5 cm. Skin, subcutaneous tissue, superficial and deep fascia were opened, and middle part of the femur was revealed from the tensor fascia lata and femoral lateral muscle gap. A bone saw was used to make a femur shaft transverse fracture. Kirschner wire (1.0 mm) was used to fix the fracture as an intramedullary nail. The movement of the hip and knee, the contraposition and alignment of the fracture, as well as the fixation of the fracture, were examined. The method of causing bacterial infection was described as the previously study [10]. Briefly, the bacterial suspension was administered to the rats by gradual dripping onto the surface of the muscle and embrocation with a sterile bacterial inoculation needle. The volume of the bacterial suspension was 0.5 ml at a concentration of 10⁶, 10⁷, 10⁸ and 10⁹ CFU/ml. The muscle the skin incision was sutured using a 3-0 silk suture. Rats were kept in separate cage with sterilized chow diet and water.

The mortality rate and wound tissue sections of rats were obtained following the establishment of the model to evaluate whether the model had succeeded and was stable. Finally, we chose 108 CFU/ml MSSA and 106 CFU/ml *P. aeruginosa* to establish the rat model. (related results are in the supplementary materials: DOI:10.17632/vpj7vjkz86.1).

Evaluation of the gentamicin-loading of sponge

Gentamicin powder was dissolved into 0.9% physiological saline and we adjusted the concentration of gentamicin were 40mg/ml, 16mg/ml, 8mg/ml, 4mg/ml, 1.6mg/ml, 0.8mg/ml or 0mg/ml. Gelatin sponge was cut into 1×1×0.5cm size and sterilized with ethylene oxide (Fig.1). According to the concentration of gentamicin solution, gentamicin sponges were divided into 7 groups. The sponge was immersed in gentamicin solution of different concentrations for 12h, 24h, 48h, 96h or 120h. Then, sponge was air dried until the quality no longer changed (about 48h). The weight of sponge was measured by electronic balance to evaluate the gentamicin-loading of sponge.

Preparation of air-dried sponge

We used the similar methods to prepare the air-dried sponge. Briefly, gentamicin powder was dissolved into 0.9% physiological saline and the concentration of gentamicin were adjust to 40mg/ml, 16mg/ml, 8mg/ml, 4mg/ml, 1.6mg/ml, 0.8mg/ml or 0mg/ml. Gelatin sponge was cut into 1×1×0.5cm size and sterilized with ethylene oxide. According to the concentration of gentamicin solution, gentamicin sponges were divided into 7 groups. The sponge was immersed in gentamicin solution of different concentrations for 48h. Then, sponge was air dried until the quality no longer changed (about 48h). Finally, sponges were sterilized with ethylene oxide and kept in -20°C until used.

Evaluation of the release of air-dried gentamicin-saturated sponge

The air-dried sponge was immersed into 10ml 0.9% physiological saline 30min,2h,6h, 12h,24h,48h and 96h. Gentamicin ELISA kit was used to test the concentration of gentamicin in 10ml 0.9% physiological saline. The specific operation steps are in accordance with the manual.

Evaluation of the antibacterial effect of gentamicin sponge in vitro

After 3 times bacterial passage performed as described before, 100ul bacterial suspension was inoculated in LB agar media at 37°C, overnight to make the surface of medium be full of colonies. The air-dried sponge was put on the center of the surface of the LB agar media at 37°C, 48h. Then, the sponge was removed and the antibacterial area (area that bacteria do not grow) was measured to evaluate the antibacterial effect of gentamicin sponge.

Evaluation of the effects of gentamicin sponge on femur fractured with wound infection rat model

70 rats were divided into 2 groups as MSSA infection (35 rats) and *P. aeruginosa* infection (35 rats) group. Then, each group was divided into 7 subgroups as: 40mg/ml air-dried sponge (5 rats), 16mg/ml air-dried sponge (5 rats), 8mg/ml air-dried sponge (5 rats), 4mg/ml air-dried sponge (5 rats), 1.6mg/ml air-dried sponge (5 rats), 0.8mg/ml air-dried sponge (5 rats) and 0mg/ml air-dried sponge (5 rats). As described before, we chose 10^7 CFU/ml MRSA and 10^6 CFU/ml *P. aeruginosa* to establish the femur fractured with wound infection rat model. After the bacterial suspension was administered to the rats, air-dried sponge was put in the bacteria inoculated muscle gap. Then, the muscle the skin incision was sutured using a 3-0 silk suture. Rats were kept in separate cage with sterilized chow diet and water. We recorded the mortality and wound suppuration rate of the rats at day 7.

Statistical analysis.

SPSS 23.0 was used to analyze the data. Measurement data were reported as median (interquartile), Kruskal-Wallis H test was used to compare multiple groups and Mann-Whitney U test was used to compare two groups. Enumeration data were as a percentage, in addition, χ^2 test and Fisher definite probability methods was used to compare groups. $P < 0.05$ was considered as statistically significant.

The equations of gentamicin-loading of sponge and release of air-dried gentamicin-saturated sponge was constructed by curve estimation of SPSS software. R2 was used to evaluate the matching degree of the constructed equation. We used the median of each group at each timepoint to construct the surface estimation in Origin2019b software. The maximum number of iterations was set at 400, tolerance was $1e-9$ and iterative algorithm was levenberg-Marquardt algorithm. Surface function was set to Poly2D and the coefficients of the surface equation are expressed by mean \pm standard deviation. R2 was also used to evaluate the matching degree of the constructed surface equation. Microsoft Excel 2016 was used to make the data visualization of wound suppuration rate of rats.

Results

Evaluation of the gentamicin-loading of sponge

The gentamicin-loading of sponge was shown in table.1. The original weight of air-dried sponges was no statistical difference in multiple groups ($p=0.92$). However, when the time of sponge immersed in gentamicin solution was prolonged, the difference was statistically significant in multiple groups. Sponge immersed in 40mg/ml gentamicin solution had the largest weight. The difference of weight of 1.6mg/ml air-dried sponge and 0.8mg/ml air-dried sponge had no statistically significant in all observation time point ($p > 0.05$). As the sponge was began to decompose when was immersed in the solution for 96 hours, the weight of 0mg/ml air-dried sponge at 96h, 120h was less than the original weight ($p=0.01$, $p=0.01$ respectively).

The equation of gentamicin-loading of sponge was shown in table.2. R2 was used to evaluate the matching degree and R2 of all constructed equations was above 0.9. Origin2019b software was used to construct the surface equation of gentamicin-loading of sponge. The equation of gentamicin-loading of sponge was:

$$z=(0.03718\pm 0.01672)x+(-4.578e-4\pm 0.06253)y+(-2.50935e-4\pm 1.47521e-4)x^2+(0.00303\pm 0.00149)y^2+(0.00408\pm 3.52827e-4)xy, R2 \text{ was } 0.97.$$

z was gentamicin-loading of sponge, x was the immersed duration of sponge in gentamicin solution, y was the concentration of gentamicin solution that sponge was immersed in. R2 of the surface equation was 0.97 (Fig.2).

Evaluation of the release of air-dried gentamicin-saturated sponge

The results of release of air-dried gentamicin-saturated sponge was shown in table.3. Since the sponge began to decompose after immersed in water for more than 96 hours, the longest observation time was set as 96 hours. The results showed that the amount of gentamicin released from sponge increased gradually with the extension of time, and the difference was statistically significant ($p < 0.01$). Moreover, the amount of gentamicin released from sponges immersed in gentamicin solution of different concentrations was also different ($p < 0.01$). The amount of gentamicin released from sponge immersed in 40 mg/ml gentamicin solution was the largest at each time point, and the difference was statistically significant ($p < 0.01$).

The equation of gentamicin release of air-dried gentamicin-saturated sponge was shown in table.4. R2 of all constructed equations was above 0.9. The surface equation of gentamicin release of air-dried sponge was:

$$Z=(4.37205\pm 1.18048)x+(-7.05921\pm 3.09628)y+(-0.04596\pm 0.01287)x^2+(0.3309\pm 0.07912)y^2+(0.31559\pm 0.02754)xy$$

z was gentamicin release of air-dried sponge, x was the immersed duration of sponge in gentamicin solution, y was the concentration of gentamicin solution that sponge was immersed in. R2 of the surface equation was 0.95 (Fig.3).

Evaluation of the antibacterial effect of gentamicin sponge in vitro

The antibacterial zone size of gentamicin sponge was shown in table.4. Air-dried sponge previously immersed in 40mg/ml gentamicin solution had the largest antibacterial zone size than others both in *P. aeruginosa* and MSSA ($p < 0.01$). The antibacterial zone sizes of 1.6mg/ml air-dried sponge and 0.8mg/ml air-dried sponge were no statistical differences both in *P. aeruginosa* and MSSA (both were $p > 0.99$). Besides, antibacterial zone sizes of sponges previously immersed in 40mg/ml, 16mg/ml, 8mg/ml, 4mg/ml, 1.6mg/ml, 0.8mg/ml gentamicin solution were larger than 0mg/ml air-dried sponge, and the difference had statistically significant ($p < 0.01$).

Evaluation of the effects of gentamicin sponge on femur fractured with wound infection rat model

Rats of all groups were no died at day 7 after modeled in femur fractured with wound infection, so the mortality of each group was 0.

The results of wound suppuration of rats with *P. aeruginosa* infected were shown in figure.4. Rats of groups of 40mg/ml air-dried sponge (5 rats), 16mg/ml air-dried sponge (5 rats), 8mg/ml air-dried sponge (5 rats) had no wound suppuration, the wound suppuration rates were 0. Although 4mg/ml air-dried sponge group(5 rats, 1 rat with wound suppuration), 1.6mg/ml air-dried sponge group(5 rats, 1 rat with wound suppuration), 0.8mg/ml air-dried sponge group(5 rats, 1 rat with wound suppuration) had rats with wound suppuration, the differences between the above groups and 40mg/ml air-dried sponge group were no statistically significant ($p>0.99$, $p>0.99$, $p>0.99$, respectively). However, rats of 0mg/ml air-dried sponge group (5 rats) were all with wound suppuration and the rate of wound suppuration in 0mg/ml air-dried sponge group was significantly higher than others ($p>0.99$, $p>0.99$, $p>0.99$, $p=0.048$, $p=0.048$, $p=0.048$, comparisons with 40mg/ml, 16mg/ml, 8mg/ml, 4mg/ml, 1.6mg/ml, 0.8mg/m air-dried sponge groups, respectively).

Wound suppuration rates of rats with MSSA infected were shown in figure.5. Rats of groups of 40mg/ml air-dried sponge (5 rats), 16mg/ml air-dried sponge (5 rats), 8mg/ml air-dried sponge (5 rats), 4mg/ml air-dried sponge group (5 rats,) had no wound suppuration, the wound suppuration rates were 0. However, 1.6mg/ml air-dried sponge group (5 rats, 1 rat with wound suppuration), 0.8mg/ml air-dried sponge group (5 rats, 1 rat with wound suppuration) had rats with wound suppuration. The differences between 1.6mg/ml air-dried sponge group and 40mg/ml air-dried sponge group were no statistically significant ($p>0.99$). Wound suppuration rates of 0mg/ml air-dried sponge group (5 rats) was 100%, which was much higher than others (all comparisons of two-groups were $p>0.99$).

Discussion

Gentamicin-sponge has been studied for more than 30 years, but its efficacy is still controversial [1, 11–15]. Many researches have demonstrated that the application of gentamicin-sponge can significantly reduce the incidence of wound infections and kill pathogenic bacteria, whether in animal experiments or clinical studies [5, 16–17]. However, also many scholars believe that gentamicin-sponge has no effect on preventing or treating wound infection, and it maybe play an opposite role as increase the incidence of infection [1, 7–8, 18–19]. Some randomized controlled studies with large sample size are used to confirm their opinion [1]. Therefore, it is unknown whether gentamicin-sponge is useful at present.

Gentamicin, as one of aminoglycoside antibiotics, has a clear spectrum of sensitive or resistant bacteria [20–21]. If wound is infected with bacteria, the bacterial resistance spectrum screening should be carried out first. Gentamicin-sponge is considered to use only the bacteria are sensitive to gentamicin. In this study, we chose two kinds of bacteria which are sensitive bacteria to gentamicin. At present, the controversy about the efficacy of gentamicin sponge is related to the drug-loading and release of gentamicin-sponge. As there are few studies on the drug-loading and release of gentamicin-sponge, we first quantified the drug-loading and release of gentamicin-sponge in vitro.

We cut the sponge into $1\times 1\times 0.5$ cm to uniform the sponge size. Seven concentrations of gentamicin solution were used and we chose 5 timepoint to measure the air-dried sponge. We found that the amount of gentamicin absorbed by sponge was increased gradually within 96 hours. As sponge began to obviously decompose in water about 96 h, drug-loading of sponge was significantly decreased in 4 mg/ml air-dried sponge group, 1.6 mg/ml air-dried sponge group, 0.8 mg/ml air-dried sponge group after 96 h. For this reason, we advised that the immersed time of sponge in water should not be exceed 96 hours when studying the drug loading experiment of sponge. We constructed the sponge drug-loading equation of gentamicin through in vitro experiments, and tested the fitting degree of the equation. The fitting degree of the equation is more than 0.9, which means that the accuracy of the equation is very high. It can provide a reference for the future study of sponge drug loading.

In vitro release experiment of gentamicin-sponge, we mainly discussed whether the amount of gentamicin released from gentamicin-sponge can reach its therapeutic concentration. The effective treatment concentration of gentamicin is 4–10 $\mu\text{g/ml}$ [20–21]. In group of 4 mg/ml air-dried sponge, 1.6 mg/ml air-dried sponge, 0.8 mg/ml air-dried sponge, the amount of gentamicin released did not reach the effective therapeutic concentration of gentamicin at 2 hours, but at six hours. The results mean that the air-dried gentamicin-saturated sponge acquired from being immersed in 4 mg/ml, 1.6 mg/ml and 0.8 mg/ml gentamicin solution could not exert its bactericidal ability within 2 hours. Besides, the amount of gentamicin released from 40 mg/ml air-dried sponge and 16 mg/ml air-dried sponge were 16.3 $\mu\text{g/ml}$ and 4.7 $\mu\text{g/ml}$ so these two kinds sponges could exert the bactericidal ability at 30 min. We constructed the release equation of gentamicin-sponge in vitro to quantify the release of gentamicin-sponge. The fitting degree of the equation was analyzed. R^2 is more than 0.9, which means that the accuracy of the equation is high. We can use the equation to analyze the bactericidal ability of gentamicin sponge.

In drug release test of gentamicin sponges in vitro, the amount of gentamicin released by some sponges was lower than the therapeutic concentration within 2 hours, so we used bacteria and animal experiments to analyze whether gentamicin-sponges of different groups can play a similar bactericidal effect. Although the bactericidal areas of 40 mg/ml air-dried sponge group and 16 mg/ml air-dried sponge group were significantly larger than other groups, 1.6 mg/ml air-dried sponge group and 0.8 mg/ml air-dried sponge group also had obvious bactericidal effects (compared with 0 mg / ml air-dried sponge group). Based on these, we think that 1.6 mg/ml air-dried sponge and 0.8 mg/ml air-dried sponge are enough to prevent wound infection.

In animal experiments, we first established a rat model of femoral fracture combined with wound MSSA or *P. aeruginosa* infection. The stability of the model was analyzed by animal mortality and wound suppuration rate. Finally, we chose 10^8 CFU/ml MSSA and 10^6 CFU/ml *P. aeruginosa* to establish the rat model. By using different gentamicin-sponges, we found that 40 mg/ml air-dried sponge, 16 mg/ml air-dried sponge and 8 mg/ml air-dried sponge could reduce the wound infection rate to 0. Therefore, if the wound has been confirmed to be infected and the bacteria are identified as sensitive bacteria, it is recommended to use 40 mg/ml air-dried sponge, 16 mg/ml air-dried sponge and 8 mg/ml air-dried sponge to treat wound infection.

The innovation of this study is that drug-loading and drug-release of gentamicin-sponge were quantified for the first time, and an equation with a fitting degree of more than 0.9 was constructed. Secondly, we proposed which gentamicin-sponge should be used to prevent and treat wound infection. Thirdly, we provided the basis for the future study of wound infection model or gentamicin-sponge.

There are also many limitations in this study. Firstly, the sponges that we used were produced by the same factory, so the results may be different from sponges made by other manufacturers in drug-loading and drug-release experiments in vitro. Secondly, we chose MSSA and *P. aeruginosa* to do bacteriostatic test in vitro and infect rats. The results cannot represent the other bacteria, such as *Escherichia coli*, *Staphylococcus epidermidis*, and so on. Thirdly, the incidence of wound infection in rodents is significantly different from humans, so we need further study to analyze which gentamicin-sponge is enough for prevention or treatment of wound infection.

Conclusion

In this study, we constructed the drug-loading and drug-release equations of gentamicin sponge for the first time, and the fitting degree of the equations were more than 0.9. The equations can be used to analyze the gentamicin sponge for further. Meanwhile, we proposed that 1.6 mg/ml air-dried sponge and 0.8 mg/ml air-dried sponge are enough to prevent wound infection, in addition, 40 mg/ml air-dried sponge, 16 mg/ml air-dried sponge and 8 mg/ml air-dried sponge to treat wound infection.

Abbreviations

RCTs: randomized controlled trials

SSI: Surgical site infection

LB: Luria Broth

PBS: phosphate buffer saline

MSSA: Methicillin sensitive *Staphylococcus aureus*

P. aeruginosa: *Pseudomonas aeruginosa*

SD: Sprague-Dawley

Declarations

Ethical Approval and Consent to participate

This article does not contain any studies with human participants performed by any of the authors. The present study was approved by the Capital Medical University Ethics Committee on the use of animals in research and education.

Consent for publication

All authors agree for publication.

Availability of supporting data

The supplemental data used to support the findings of this study have been deposited in the Mendeley Data repository (DOI:10.17632/vpj7vjkz86.1).

Competing interests

The authors have no conflict of interest or financial disclosures.

Funding

This work is Supported by Beijing Natural Science Foundation (7202049) which was obtained by Junlin Zhou. The role of the funding was in experiments and collection.

Authors' contributions

YDL and JLZ designed this study; YDL did the in vitro experiments and DW did the in vivo experiments; DW collected and analyzed the data; YDL wrote the article and JLZ revised the article. All the authors read and approved the final manuscript.

Acknowledgements

We thank that medical research center of Beijing Chaoyang Hospital affiliated to Capital Medical University provides the experimental place.

References

1. Bennett-Guerrero, E., Pappas, T. N., Koltun, W. A., Fleshman, J. W., Lin, M., Garg, J., Mark, D. B., Marcet, J. E., Remzi, F. H., George, V. V., Newland, K., & Corey, G. R. (2010). Gentamicin–Collagen Sponge for Infection Prophylaxis in Colorectal Surgery. *New England Journal of Medicine*. <https://doi.org/10.1056/nejmoa1000837>

2. Marson, B. A., Grindlay, D. J. C., Ollivere, B. J., Deshmukh, S. R., & Scammell, B. E. (2018). A systematic review of local antibiotic devices used to improve wound healing following the surgical management of foot infections in diabetics. In *Bone and Joint Journal*. <https://doi.org/10.1302/0301-620X.100B11.BJJ-2018-0720>
3. Bennett-Guerrero, E., Ferguson, T. B., Lin, M., Garg, J., Mark, D. B., Scavo, V. A., Kouchoukos, N., Richardson, J. B., Pridgen, R. L., & Corey, G. R. (2010). Effect of an implantable gentamicin-collagen sponge on sternal wound infections following cardiac surgery: A randomized trial. *JAMA - Journal of the American Medical Association*. <https://doi.org/10.1001/jama.2010.1152>
4. Han, J.-S., Kim, S.-H., Jin, S.-W., Lee, S.-H., Kim, B.-J., Kim, S.-D., & Lim, D.-J. (2016). The Use of Gentamicin-Impregnated Collagen Sponge for Reducing Surgical Site Infection after Spine Surgery. *Korean Journal of Spine*. <https://doi.org/10.14245/kjs.2016.13.3.129>
5. Chang, W. K., Srinivasa, S., MacCormick, A. D., & Hill, A. G. (2013). Gentamicin-collagen implants to reduce surgical site infection: Systematic review and meta-analysis of randomized trials. *Annals of Surgery*. <https://doi.org/10.1097/SLA.0b013e3182895b8c>
6. Schimmer, C., Özkur, M., Sinha, B., Hain, J., Gorski, A., Hager, B., & Leyh, R. (2012). Gentamicin-collagen sponge reduces sternal wound complications after heart surgery: A controlled, prospectively randomized, double-blind study. *Journal of Thoracic and Cardiovascular Surgery*. <https://doi.org/10.1016/j.jtcvs.2011.05.035>
7. Wouthuyzen-Bakker, M., Löwik, C. A. M., Knobben, B. A. S., Zijlstra, W. P., Ploegmakers, J. J. W., Mithoe, G., Al Moujahid, A., Kampinga, G. A., & Jutte, P. C. (2018). Use of gentamicin-impregnated beads or sponges in the treatment of early acute periprosthetic joint infection: A propensity score analysis. *Journal of Antimicrobial Chemotherapy*. <https://doi.org/10.1093/jac/dky354>
8. Uçkay, I., Kressmann, B., Di Tommaso, S., Portela, M., Alwan, H., Vuagnat, H., Maître, S., Paoli, C., & Lipsky, B. A. (2018). A randomized controlled trial of the safety and efficacy of a topical gentamicin–collagen sponge in diabetic patients with a mild foot ulcer infection. *SAGE Open Medicine*. <https://doi.org/10.1177/2050312118773950>
9. Husodo, K., Kamal, A. F., & Yusuf, A. A. (2016). Effect of povidone iodine and hydrogen peroxide on fracture healing: A histomorphometric study on rats. *Journal of Orthopaedic Surgery*. <https://doi.org/10.1177/1602400224>
10. Wang, D., Liu, Y., Zhao, Y., & Zhou, J. (2016). Low dose of protein A pretreatment can alleviate the inflammatory reaction and the bio-safety was evaluated in vivo. *Journal of the Chinese Medical Association*. <https://doi.org/10.1016/j.jcma.2016.01.010>
11. Kowalewski, M., Pawliszak, W., Zaborowska, K., Navarese, E. P., Szwed, K. A., Kowalkowska, M. E., Kowalewski, J., Borkowska, A., & Anisimowicz, L. (2015). Gentamicin-collagen sponge reduces the risk of sternal wound infections after heart surgery: Meta-analysis. *Journal of Thoracic and Cardiovascular Surgery*. <https://doi.org/10.1016/j.jtcvs.2015.01.034>
12. Rapetto, F., Bruno, V. D., Guida, G., Marsico, R., Chivasso, P., & Zebele, C. (2016). Gentamicin-impregnated collagen sponge: Effectiveness in preventing sternal wound infection in high-risk cardiac surgery. *Drug Target Insights*. <https://doi.org/10.4137/DTI.S39077>
13. Birgand, G., Radu, C., Alkholder, S., Al Attar, N., Raffoul, R., Dilly, M. P., Nataf, P., & Lucet, J. C. (2013). Does a gentamicin-impregnated collagen sponge reduce sternal wound infections in high-risk cardiac surgery patients? *Interactive Cardiovascular and Thoracic Surgery*. <https://doi.org/10.1093/icvts/ivs449>
14. Schimmer, C., Gross, J., Ramm, E., Morfeld, B. C., Hoffmann, G., Panholzer, B., Hedderich, J., Leyh, R., Cremer, J., & Petzina, R. (2017). Prevention of surgical site sternal infections in cardiac surgery: a two-centre prospective randomized controlled study. *European Journal of Cardio-Thoracic Surgery : Official Journal of the European Association for Cardio-Thoracic Surgery*. <https://doi.org/10.1093/ejcts/ezw225>
15. Hayes, G., Moens, N., & Gibson, T. (2013). A review of local antibiotic implants and applications to veterinary orthopaedic surgery. In *Veterinary and Comparative Orthopaedics and Traumatology*. <https://doi.org/10.3415/VCOT-12-05-0065>
16. Pochhammer, J., Zacheja, S., & Schäffer, M. (2015). Subcutaneous application of gentamicin collagen implants as prophylaxis of surgical site infections in laparoscopic colorectal surgery: a randomized, double-blinded, three-arm trial. *Langenbeck's Archives of Surgery*. <https://doi.org/10.1007/s00423-014-1232-4>
17. Formanek, M. B., Herwaldt, L. A., Perencevich, E. N., & Schweizer, M. L. (2014). Gentamicin/collagen sponge use may reduce the risk of surgical site infections for patients undergoing cardiac operations: A meta-analysis. *Surgical Infections*. <https://doi.org/10.1089/sur.2012.209>
18. Godbole, G., Pai, V., Kolvekar, S., & Wilson, A. P. R. (2012). Use of gentamicin-collagen sponges in closure of sternal wounds in cardiothoracic surgery to reduce wound infections. *Interactive Cardiovascular and Thoracic Surgery*. <https://doi.org/10.1093/icvts/ivr129>
19. Migaczewski, M., Zub-Pokrowiecka, A., Budzyński, P., Matłok, M., & Budzyński, A. (2012). Prevention of early infective complications after laparoscopic splenectomy with the Garamycin sponge. *Wideochirurgia I Inne Techniki Maloinwazyjne*. <https://doi.org/10.5114/wiitm.2011.27151>
20. Andersson, D. I., & Hughes, D. (2017). Selection and Transmission of Antibiotic-Resistant Bacteria. In *Microbial Transmission*. <https://doi.org/10.1128/microbiolspec.mtbp-0013-2016>
21. Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. V. (2015). Molecular mechanisms of antibiotic resistance. In *Nature Reviews Microbiology*. <https://doi.org/10.1038/nrmicro3380>

Tables

Table 1
the gentamicin-loading of sponge.

	Original weight(mg)	12 h(mg)	24 h(mg)	48 h(mg)	96 h(mg)	120 h(mg)	p-value
40 mg/ml air-dried sponge	8.10(7.65–8.45)	13.30(12.65–13.55)	18.20(18.00–18.65)	24.90(23.35–25.75)	28.40(28.10–28.55)	29.10(26.85–30.10)	< 0.01
16 mg/ml air-dried sponge	7.80(7.25–8.20)	8.70(8.45–9.20)	10.50(10.20–11.00)	12.90(12.60–13.30)	16.90(16.60–17.20)	16.10(15.85–17.15)	< 0.01
8 mg/ml air-dried sponge	8.20(7.40–8.50)	8.60(7.95–8.95)	9.30(9.05–9.50)	10.10(9.85–10.35)	11.20(10.85–11.55)	11.20(10.35–12.55)	< 0.01
4 mg/ml air-dried sponge	7.80(7.70–8.20)	8.10(7.80–8.30)	8.30(7.75–8.40)	9.40(9.20–9.70)	8.90(8.45–9.20)	8.20(7.75–8.95)	< 0.01
1.6 mg/ml air-dried sponge	8.30(7.65–8.55)	8.40(7.95–8.75)	8.60(7.85–8.95)	8.80(8.65–9.05)	8.10(7.75–8.45)	7.10(6.75–7.50)	0.01
0.8 mg/ml air-dried sponge	7.90(7.55–8.60)	8.10(7.85–8.70)	7.90(7.90–8.35)	8.80(8.45–9.15)	8.10(7.40–8.45)	6.70(6.20–7.10)	< 0.01
0 mg/ml air-dried sponge	8.20(7.45–8.50)	8.30(7.70–8.55)	7.70(7.60–8.50)	7.90(7.60–8.15)	6.30(6.05–6.75)	5.90(5.40–6.50)	< 0.01
p-value	0.92	0.01	< 0.01	< 0.01	< 0.01	< 0.01	

* The data were expressed in the median (quartile). Each group was repeated 5 times at each time point. The p-value was calculated by Kruskal-Wallis H test to compare multiple groups or multiple time point. Mg/ml was the concentration of gentamicin solution that sponge immersed in. The weight was sponge that was air-dried until the quality no longer changed (about 48 h).

Table 2
the equation of gentamicin-loading of sponge.

	drug loading equation of sponge(mg)	R2
40 mg/ml air-dried sponge	$0.5056x - 0.0038x^2 + 9.4828e-006(x^3)$	0.998
16 mg/ml air-dried sponge	$0.0582x + 0.0016x^2 - 1.1666e-005(x^3)$	0.996
8 mg/ml air-dried sponge	$0.0364x + 0.0004x^2 - 2.5544e-006(x^3)$	0.984
4 mg/ml air-dried sponge	$-0.0020x + 0.0008x^2 - 5.4735e-006(x^3)$	0.915
1.6 mg/ml air-dried sponge	$0.0097x + 0.0004x^2 - 3.3236e-006(x^3)$	0.916
0.8 mg/ml air-dried sponge	$-0.0079x + 0.0008x^2 - 5.8723e-006(x^3)$	0.821

* x was the immersed duration of sponge in gentamicin solution, R2 was the index of testing fitting degree of equation, range was 0 to 1 as fitting degree was bad to excellent.

Table 3
the drug-release of air-dried gentamicin-saturated sponge

	30 min(ug/ml)	2 h(ug/ml)	6 h(ug/ml)	12 h(ug/ml)	24 h(ug/ml)	48 h(ug/ml)	96 h(ug/ml)	p-value
40 mg/ml air-dried sponge	16.30(16.00-16.80)	56.90(51.80-59.40)	166.50(160.65-174.25)	338.60(327.30-343.95)	847.20(840.55-851.10)	1103.40(1100.85-1107.50)	1371.30(1367.85-1372.900)	< 0.01
16 mg/ml air-dried sponge	4.70(4.15-5.65)	16.90(15.00-17.85)	43.00(40.85-46.55)	49.80(47.45-51.65)	247.10(240.70-250.35)	324.40(177.80-328.25)	402.20(400.30-403.95)	< 0.01
8 mg/ml air-dried sponge	1.90(1.45-2.20)	5.70(5.05-6.50)	17.60(17.10-18.25)	40.90(37.60-42.00)	97.30(95.50-100.35)	138.10(132.50-140.65)	174.90(166.30-180.40)	< 0.01
4 mg/ml air-dried sponge	0.90(0.35-1.15)	3.80(3.40-4.00)	8.70(8.25-9.00)	24.10(22.85-27.20)	74.20(72.25-76.55)	99.60(97.75-102.35)	144.90(142.15-150.20)	< 0.01
1.6 mg/ml air-dried sponge	0.70(0.45-1.05)	3.10(2.60-3.70)	7.80(7.35-8.50)	19.30(18.35-20.00)	48.20(46.10-50.30)	64.40(63.30-67.25)	80.90(79.00-83.60)	< 0.01
0.8 mg/ml air-dried sponge	0.80(0.45-1.00)	3.10(2.80-3.40)	8.10(7.50-8.25)	18.30(16.15-19.70)	47.40(45.45-50.05)	65.80(64.75-66.90)	80.60(79.85-82.35)	< 0.01
0 mg/ml air-dried sponge	0	0	0	0	0	0	0	
p-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	

* The data were expressed in the median (quartile). Each group was repeated 5 times at each time point. ug/ml was the concentration of gentamicin in 10 ml 0.9% physiological saline that air-dried gentamicin-saturated sponge immersed in.

Table 4
antibacterial zone size of gentamicin sponge

	<i>P. aeruginosa</i> (cm2)	MSSA(cm2)
40 mg/ml air-dried sponge	6.90(6.75-7.45)	7.50(6.00-7.85)
16 mg/ml air-dried sponge	5.40(5.25-6.30)	5.40(5.20-6.60)
8 mg/ml air-dried sponge	4.90(4.35-5.40)	4.90(4.60-5.50)
4 mg/ml air-dried sponge	3.70(3.25-4.30)	4.20(3.65-4.50)
1.6 mg/ml air-dried sponge	2.90(2.65-3.20)	3.20(2.85-3.65)
0.8 mg/ml air-dried sponge	2.70(2.50-3.00)	3.30(2.45-3.85)
0 mg/ml air-dried sponge	2	2
p-value	< 0.01	< 0.01

*The data were expressed in the median (quartile). Each group was repeated 5 times at each time point.

Figures

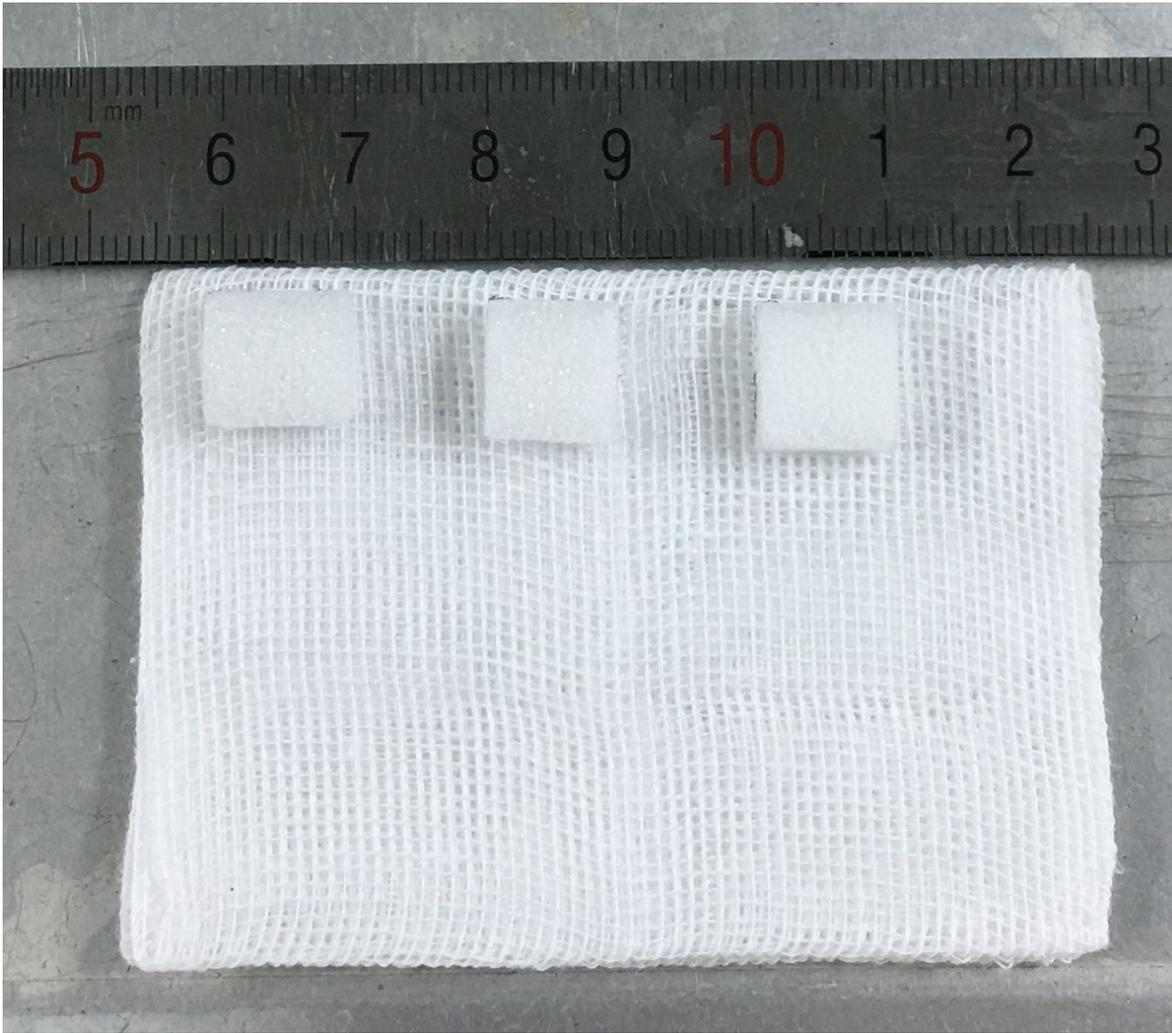


Figure 1

Gelatin sponge was cut into 1×1×0.5cm size.

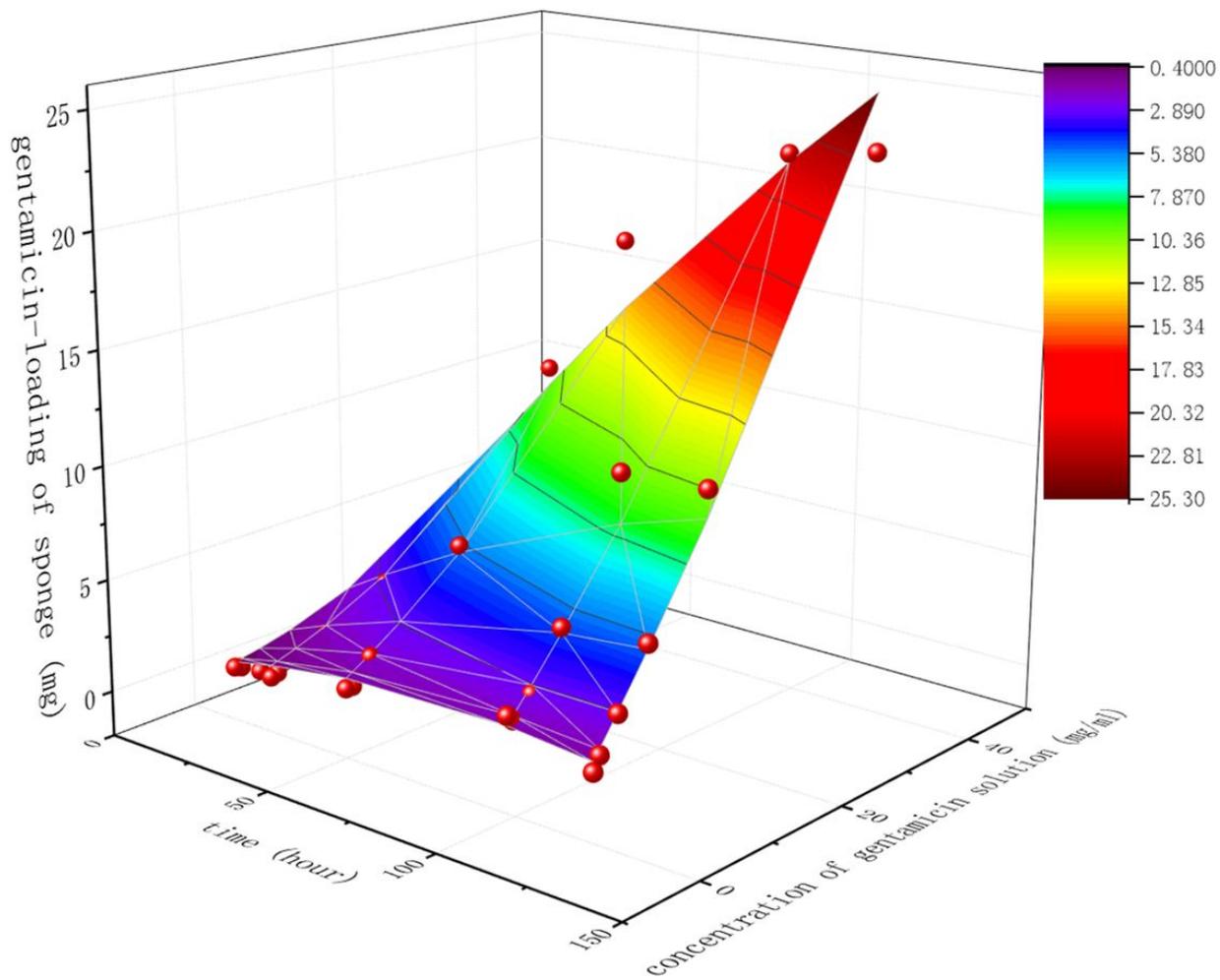


Figure 2

Equation surface of drug-loading of gentamicin sponge. x-axis was the immersed duration of sponge in gentamicin solution, y-axis was the concentration of gentamicin solution that sponge was immersed in, z-axis was gentamicin-loading of sponge. Point was the median of drug-loading of each group at each timepoint. The color ruler was presented the gentamicin-loading of sponge and unit was mg.

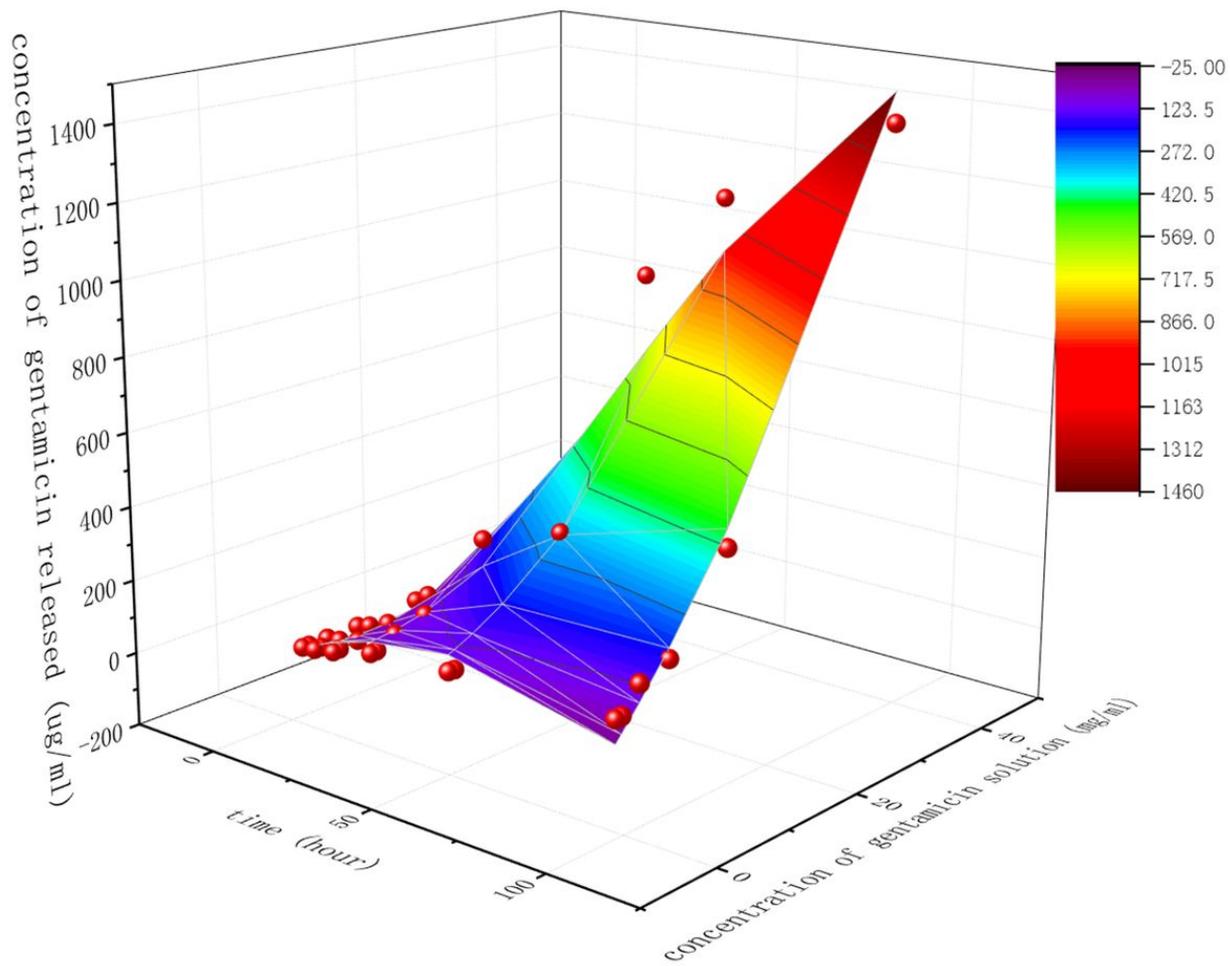


Figure 3
 Equation surface of drug-release of gentamicin-sponge. x-axis was the immersed duration of sponge in gentamicin solution, y-axis was the concentration of gentamicin solution that sponge was immersed in, z-axis was gentamicin release of air-dried sponge. Point was the median of drug-release of each group at each timepoint. The color ruler was presented the gentamicin-release of sponge and unit was ug/ml.

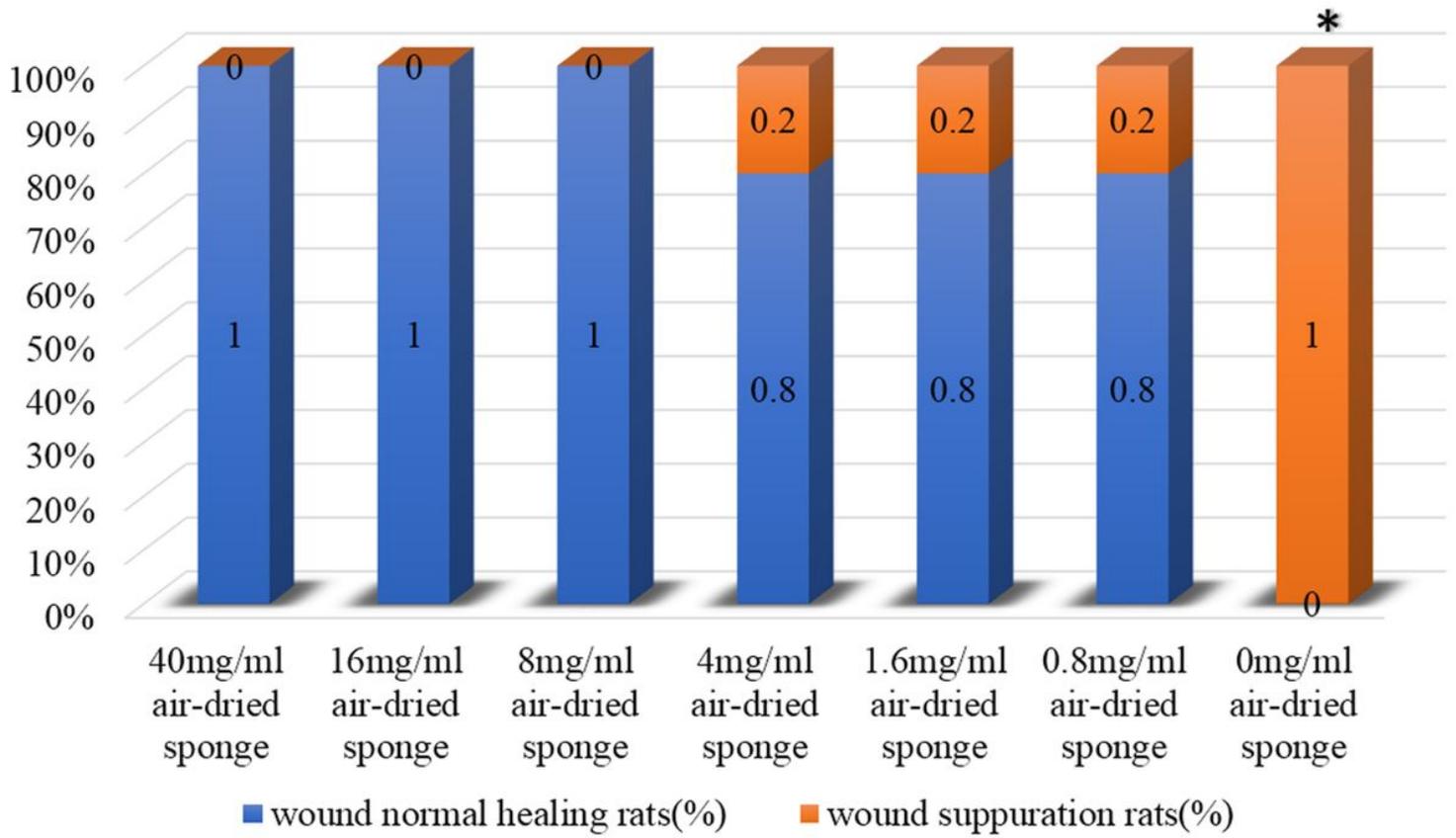


Figure 4

wound suppuration rates of rats with wound *P. aeruginosa* infected. *: $p < 0.05$, compared with 40mg/ml air-dried sponge group.

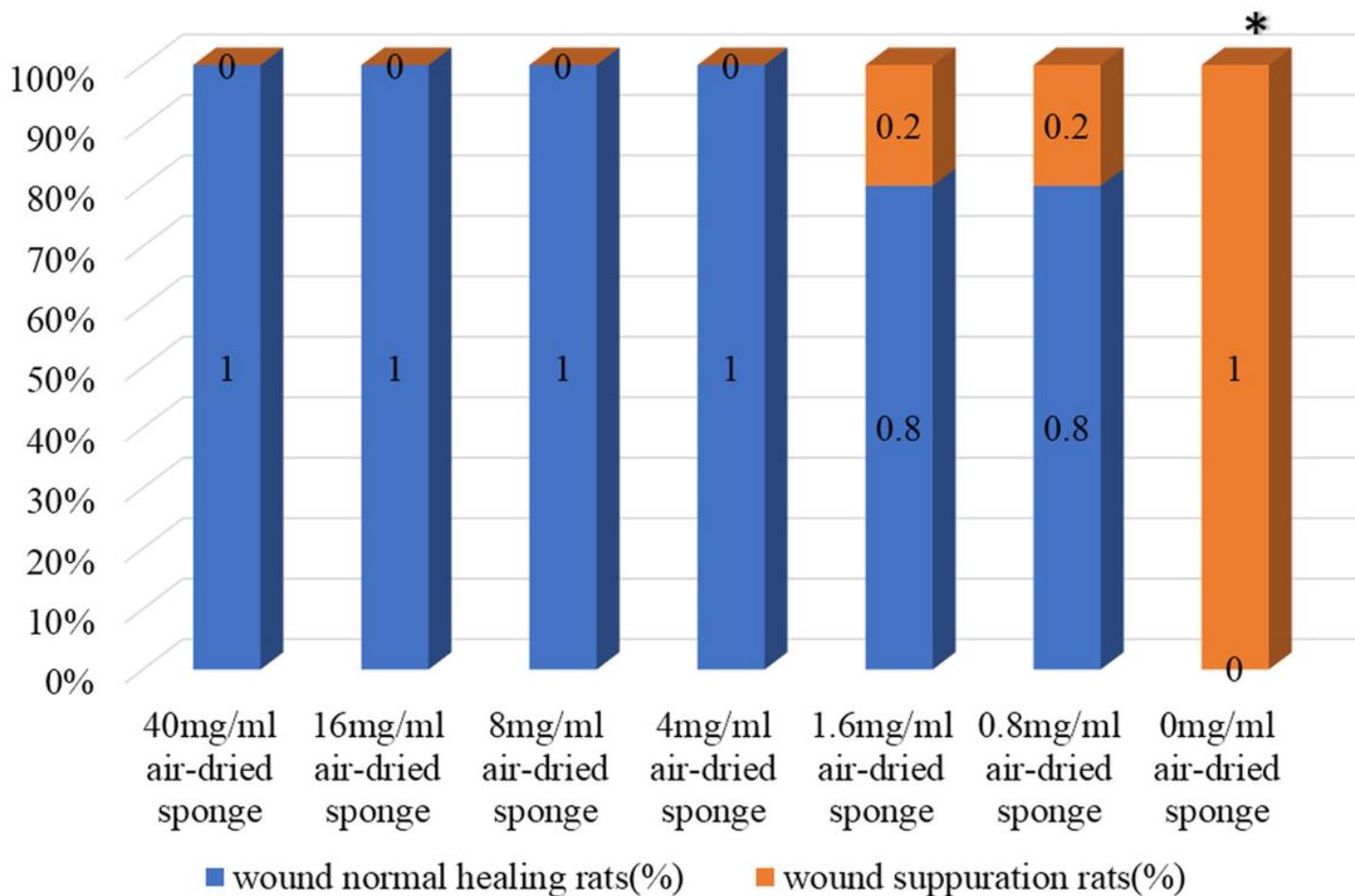


Figure 5

wound suppuration rates of rats with wound MSSA infected. *: p<0.05, compared with 40mg/ml air-dried sponge group.