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An Experimental Study on Antimicrobial Activity of Silicone Oil in vitro

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Key Words
Silicone oil · Bacterium · Fungus

Abstract

Objective: To study the antimicrobial activity of silicone oil against *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Candida albicans* in vitro. ***Methods:*** *S. aureus, S. epidermidis, P. aeruginosa* and *C. albicans* were prepared to 1 McFarland turbidity, 10^5 and 10^3 CFU/ml. The bacteria and fungi were inoculated into silicone oil (Acri.Sil-ol 5000), physiologic saline, meat extract broth for bacteria and Sabouraud broth for fungi, respectively. From each sample, 10 μl were taken and plated in petri dishes with the addition of blood agar media for bacteria and Sabouraud dextrose agar for fungi. After overnight incubation, CFUs were enumerated. Cultured from the initially prepared specimens, overnight incubation and CFU counting were repeated until no growth of microorganisms was seen in the silicone oil-containing media. ***Results:*** (1) In the group 1 McFarland, *S. aureus, S. epidermidis, P. aeruginosa* and *C. albicans* survived for 13, 6, 20, and 16 days in silicone oil, respectively, which was shorter than in the other media. (2) In the group 10^5 CFU/ml, *S. aureus, S. epidermidis, P. aeruginosa* and *C. albicans* survived for 2, 6, 20, and 1 days in silicone oil, respectively, which was shorter than in the other media. (3) In the group 10^3 CFU/ml, *S. aureus, S. epidermidis, P. aeruginosa* and *C. albicans* survived for 1, 1, 20, and 1 days in silicone oil, respectively, which was shorter than in the other media. ***Conclusion:*** Silicone oil has a significant antimicrobial activity against *S. aureus, S. epidermidis, P. aeruginosa* and *C. albicans* in vitro.

Acute bacterial endophthalmitis is a suppurative inflammation of the uvea and retina. If it is not treated in time, vision loss and even phthisis bulbi will occur [1]. The major pathogenic bacteria are *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Candida albicans*. Clinical observations validate that vitrectomy combined with silicone oil filling is an effective and safe method for treatment of endophthalmitis [2]. The combined surgeries not only completely remove the inflammation focus, but also inhibit further damage of bacteria to the ocular tissue with the use of silicone oil filling. In this study, we will test and discuss the antimicrobial activity of silicone oil on bacteria and fungi in vitro.

Materials and Methods

S. aureus (ATCC 25923), *S. epidermidis* (ATCC 12228), *P. aeruginosa* (ATCC 27853) and *C. albicans* (ATCC 90028) were prepared to 1 McFarland turbidity, 10^5 and 10^3 CFU/ml, respectively. In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range. In the group 1 McFarland,

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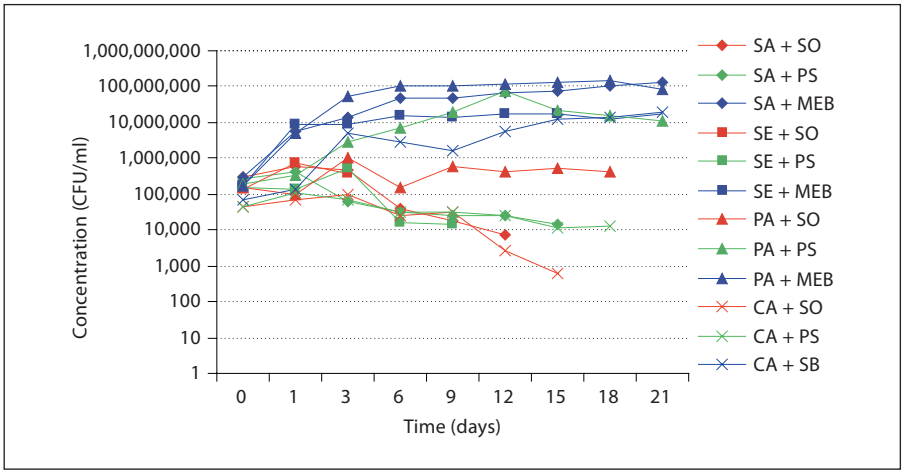

Fig. 1. Changes of bacteria and fungi in the group 1 McFarland turbidity in three media (CFU/ml). SO = Silicone oil; PS = physiological saline; MEB = meat extract broth; SB = Sabouraud broth; SA = *S. aureus;* SE = *S. epidermidis;* PA = *P. aeruginosa;* CA = *C. albicans.*

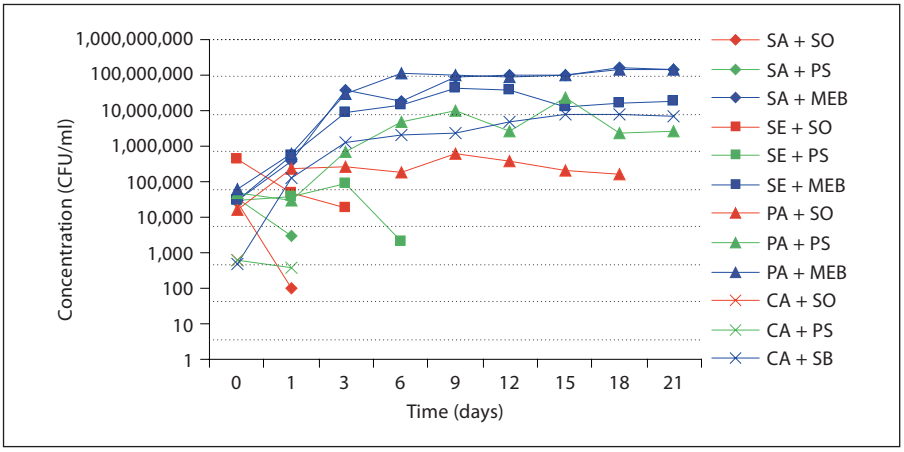

Fig. 2. Changes of bacteria and fungi in the group 10^5 CFU/ml in three media (CFU/ml). SO = Silicone oil; PS = physiological saline; MEB = meat extract broth; SB = Sabouraud broth; SA = *S. aureus;* SE = *S. epidermidis;* PA = *P. aeruginosa;* CA = *C. albicans.*

a sample of 0.2 ml and two samples of 0.5 ml were separately taken from the bacterium and fungus suspension. The sample of 0.2 ml was inoculated into 1.8 ml silicone oil (Acri.Sil-ol 5000), and the 0.5-ml samples were inoculated into 4.5 ml physiologic saline solution and 4.5 ml meat extract broth for bacteria or 4.5 ml Sabouraud broth for fungi, respectively. The final concentrations of bacteria and fungi in silicone oil, physiologic saline solution, meat extract broth and Sabouraud broth were 10%. All tubes containing the resultant suspensions were vortexed to equally distribute the inoculum. The resultant suspension (10 μl) samples were taken and plated in petri dishes with the addition of blood agar media for bacteria and Sabouraud dextrose agar for fungi, respectively. All tubes and petri dishes were incubated at 37°C overnight. After overnight incubation, CFUs were enumerated. Cultured from the initially prepared specimens, overnight incubation and CFU counting were repeated until no growth of microorganisms was seen in the silicone oil-containing media. The tubes containing growth medium such as meat extract broth for bacteria and Sabouraud broth for fungi were kept at 4°C after day 6 to prevent bacteria and fungi from turning into the decline phase of the growth period. The inoculation and incubation procedures in the groups 10^5 and 10^3 CFU/ml were the same as in the group 1 McFarland.

Results

Group 1 McFarland

S. aureus, S. epidermidis, P. aeruginosa and *C. albicans* survived for 13, 6, 20, and 16 days in silicone oil, respectively, which was shorter than in the other media (fig. 1).

Group 10^5 CFU/ml

S. aureus, S. epidermidis, P. aeruginosa and *C. albicans* survived for 2, 6, 20, and 1 days in silicone oil, respectively, which was shorter than in the other media (fig. 2).

Group 10^3 CFU/ml

S. aureus, S. epidermidis, P. aeruginosa and *C. albicans* survived for 1, 1, 20, and 1 days in silicone oil, respectively, which was shorter than in the other media (fig. 3).

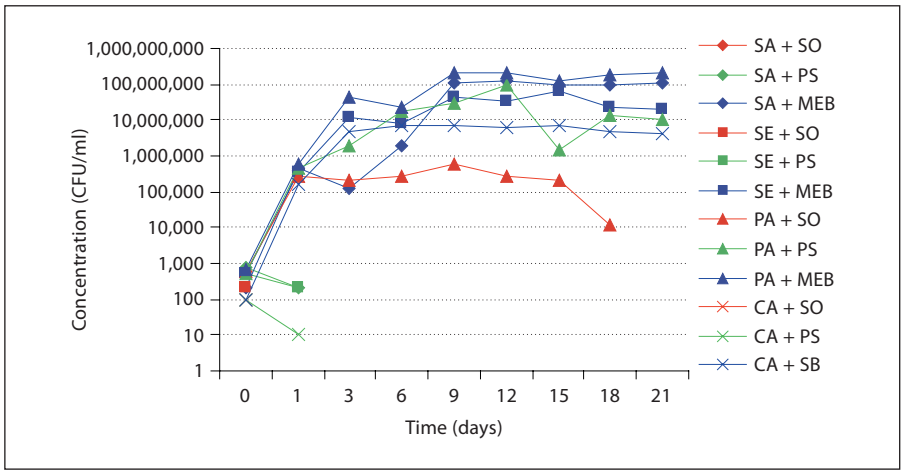

Fig. 3. Changes of bacteria and fungi in the group 10^3 CFU/ml in three media (CFU/ml). SO = Silicone oil; PS = physiological saline; MEB = meat extract broth; SB = Sabouraud broth; SA = *S. aureus;* SE = *S. epidermidis;* PA = *P. aeruginosa;* CA = *C. albicans.*

Discussion

The concentrations of bacteria and fungi which result in endophthalmitis cannot be easily tested in the clinic. Liang et al. [3] reported on an endophthalmitis model in rabbits with 10^3 CFU/ml *S. aureus;* Kim et al. [4] presented an endophthalmitis model in rabbits with 2×10^3 CFU/ml *P. aeruginosa,* and Serracarbassa et al. [5] developed an endophthalmitis model in rabbits with 3×10^3 CFU/ml *C. albicans.* Therefore, we chose 1 McFarland turbidity, 10^5 and 10^3 CFU/ml as the experimental concentrations of bacteria and fungi.

In the group 1 McFarland, 10^5 and 10^3 CFU/ml, *S. aureus, S. epidermidis, P. aeruginosa* and *C. albicans* survived for a shorter time in silicone oil than in the other media used in our study, which might be due to nutritional deprivation and toxicity of silicone oil against bacteria and fungi. On the one hand, silicone oil does not contain any nutrients, which may lead to the death of microorganisms and inhibition of the growth of microorganisms. On the other hand, the low-molecular-weight components and slightly active catalyst in silicone oil may be related with the toxicity of silicone oil, and may be the main reason of the antimicrobial activity of silicone oil.

In the group 1 McFarland, the amount of *P. aeruginosa* in silicone oil decreased during the first several days, and disappeared at last. However, *P. aeruginosa* in physiological saline proliferated quickly. As neither silicone oil nor physiology saline contains nutrients, these results indicate that the toxicity caused by the low-molecular-weight components and slightly active catalyst in silicone oil may be the major reason for bacterium and fungus death.

In the group 10^5 and 10^3 CFU/ml, *S. aureus, S. epidermidis* and *C. albicans* in silicone oil and physiological saline disappeared during the first several days. However, the bacteria and fungi in meat extract broth or Sabouraud broth proliferated quickly. Although bacteria combined with silicone oil or physiological saline were inoculated in petri dishes with the addition of blood agar media, and fungi combined with silicone oil or physiological saline were inoculated in petri dishes with the addition of Sabouraud dextrose agar, there were less nutrients in silicone oil and physiological saline than in meat extract broth or Sabouraud broth. Therefore, the microorganisms in silicone oil and physiological saline died soon. The purpose of using lower concentrations of bacteria, such as 10^5 and 10^3 CFU/ml in silicone oil, was to screen the lowest volume of bacteria and fungi inhibited by silicone oil during the first several days, which is in accordance with the clinical treatment of endophthalmitis with vitrectomy combined with silicone oil filling.

In the group 10^5 and 10^3 CFU/ml, *P. aeruginosa* in silicone oil and physiological saline presented an increase at first and then a decrease in CFUs. However, it increased in meat extract broth. This is because *P. aeruginosa* has a stronger ability of proliferation and resistance. It can survive longer in moist conditions, and also has a stronger resistance against dryness, ultraviolet and chemical antiseptics such as aldehyde, hydrargyrum and surface-acting agents. Although the results in our experiment indicate that silicone oil can inhibit *P. aeruginosa,* there may be some other reasons for inhibiting *P. aeruginosa* proliferation which include the lower concentrations of Pseudomonas and the lower content of nutrients in silicone oil. We think that bacteria cannot live

in silicone oil, thus they can only survive in the nonoil part of the emulsion.

In our experiment, the amount of CFUs of *C. albicans* was less than that of other bacteria on day 1. The possible reason is that *C. albicans* belongs to fungi, it proliferates by budding, and the growth velocity is slow. The fungi grow into colonies after cultivating at 37°C for 2–3 days. However, the bacteria proliferate by binary division, and their growth velocity is fast. *S. aureus, S. epidermidis* and *P. aeruginosa* grow into colonies after cultivating for 24–48 h. In our study, in order to guarantee the use of the same experimental method and condition, CFUs were enumerated 24 h after cultivating.

This experiment reveals that silicone oil can inhibit the growth of bacteria and fungi in vitro which might avoid the interference of autoimmunity in humans or animals. It provides the theoretical evidence of treating endophthalmitis with vitrectomy combined with silicone oil filling.

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