Effects of lipid emulsion on the formation of *Escherichia coli – Candida albicans* mixed species biofilm on PVC

Shanshan Li
Department of Anesthesiology, the Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital, Yunnan Cancer Center)

Wanshi Duan
Department of Thoracic Surgery, Tangdu Hospital, the Second Affiliated Hospital of Air Force Medical University

Yujie Lei
Department of Thoracic Surgery I, Key Laboratory of Lung Cancer Research of Yunnan Province, the Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital, Yunnan Cancer Center)

Zhonghui Wang
Department of Anesthesiology, the Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital, Yunnan Cancer Center)

Chaojiang Fu
Department of Intensive Care Unit, the Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital, Yunnan Cancer Center)

Liang He
Department of Clinical Laboratory, the Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital, Yunnan Cancer Center)

Zhenghai Shen
Department of Thoracic Surgery I, Key Laboratory of Lung Cancer Research of Yunnan Province, the Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital, Yunnan Cancer Center)

Minjie Li
Department of Thoracic Surgery I, Key Laboratory of Lung Cancer Research of Yunnan Province, the Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital, Yunnan Cancer Center)

Ying Chen
Department of Thoracic Surgery I, Key Laboratory of Lung Cancer Research of Yunnan Province, the Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital, Yunnan Cancer Center)

Yunchao Huang (huangych2001@aliyun.com)
Department of Thoracic Surgery I, Key Laboratory of Lung Cancer Research of Yunnan Province, the Third Affiliated Hospital of Kunming Medical University (Yunnan Cancer Hospital, Yunnan Cancer Center)

Research Article

Keywords: Lipid emulsion, Central venous catheter (CVC), Candida albicans, Escherichia coli, Mixed species biofilm, PVC

Posted Date: March 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-251445/v1

Version of Record: A version of this preprint was published at Scientific Reports on August 19th, 2021. See the published version at https://doi.org/10.1038/s41598-021-96385-6.

Abstract

Patients receiving lipid emulsion have an increased risk of central venous catheter-related bloodstream infections (CRSBI) in the clinic. More than 15% of CRBSI are found to be polymicrobial. The objective of the study was to explore the mechanism and effects of lipid emulsion on the formation of *Escherichia coli-Candida albicans* mixed species biofilm (BF) on the surface of polyvinylchloride (PVC) materials. Mixed BFs were achieved by co-culturing *Escherichia coli* (*E.coli*) and *Candida albicans* (*Ca*) with PVC in various concentrations of lipid emulsion respectively. Crystal violet (CV) staining and XTT assay were performed to test the formation of BFs and the viability of bacteria in BFs. The microstructures of BFs were observed by a combined approach of fluorescence in situ hybridization (FISH), confocal laser scanning microscopy (CLSM), and scanning electron microscopy (SEM). The study found that lipid emulsion promoted the formation of *Escherichia coli-Candida albicans* mixed biofilm, especially 10% lipid emulsion. The mechnism of lipid emulsion prompting mixed biofilm formation may be significantly up-regulating the expression of *flhDC, iha, HTA1,* and *HWP1* genes associated with bacterial motility, adhesion and biofilm formation. For prevention of central venous catheter-associated infections, medical professionals should maintain strict aseptic precautions when handling lipid emulsion and avoid using high concentrations of lipid emulsion for a long time as much as possible. The study provides interesting information for future research in biomaterial related infection.

Introduction

Polyvinylchloride (PVC) is one of the most used medical materials due to its unique properties, availability and low cost. Medical devices for infusion and artificial nutrition are essentially made of plasticized PVC. Central venous catheters (CVCs) are no exception. CVCs are widely used in clinics and essential to patients that need central venous access for blood transfusion, chemotherapy, antibiotic infusion, fluid management, stem cell infusion, long-term hemodialysis, and total parenteral nutrition [1-3]. CVC-related bloodstream infections (CRBSI) are severe and joint complications in these patients requiring central venous catheterization. It is estimated that a total of 200,000~400,000 episodes of CRBSI occur annually in the USA[4,5]. A report published in 2013 estimated the cost associated with catheters related infections to be the highest among healthcare-associated infections [6]. Patients are at increased risk of CRBSI, resulting in a delay in primary disease treatment, increased morbidity and mortality, prolonged hospital stay, and higher hospital cost [7,8]. Prospective surveillance studies carried out during 2013~2020 reported the incidence of CRBSI to be 0.5~5.8% in adults [9,10]. The incidences of CRBSI were more than 10% in children, even with antimicrobial central venous catheters. According to recent reports, more patients were detected with CRBSI caused by Gram-negative bacteria than those caused by Gram-positive bacteria [11,12]. Gram-negative pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella* spp. were the most common pathogenic bacteria isolated in CRBSI, of which *E. coli* was the number one [13,14]. *Candida* spp. is also a leading pathogen in infections caused by central venous catheters [15]. A large number of clinical reports have indicated that the incidence of CRBSI increases in patients undergoing gastrointestinal surgery or those with advanced cancer, intestinal failure, or severe

pancreatitis receiving a long period of parenteral nutrition [16,17]. Lipid emulsion is an indispensable component of parenteral nutrition and can provide energy and essential fatty acids for patients receiving parenteral nutrient solution. The parenteral nutrient solution needs to be injected through the central venous catheter. It forms a high-glucose and high-lipid environment around the catheter, conducive to the adhesion, growth, and diffusion of pathogenic microorganism. After implantation of biomaterials in the human body, micropathogenic bacteria can more easily adhere and proliferate on the surface of biomaterials and form biofilms with complex structures. Once the biofilm forms, pathogenic microbes in mixed BFs can effectively resist immune destruction and antibiotic therapy, which can induce persistence of infection. Several studies have reported that patients receiving lipid emulsion have an increased risk of central venous catheter-related infections in the clinic. Still, these studies were limited to studying of infections caused by a single microorganism [18,19]. In fact, pathogenic bacteria are often coinfected by two or more strains or species that have been shown to influence disease virulence in nature. More than 15% of CRBSI caused during parenteral nutrition are found to be polymicrobial [20]. Studies have confirmed that 27~57% of *Ca* infections are associated with other pathogens or opportunistic pathogens. *Ca* and other pathogens can colonize on the surface of implants and accelerate the formation of mixed species BFs [21,22]. Clinical biomaterial infections that were polymicrobial had poorer clinical prognosis and double mortality rate than a single microbe infection.

Our research group has successfully constructed hybrid biofilm models of various biological implant materials, such as mixed biofilm of *Staphylococcus epidermidis-Candida albicans* on PVC [23], mixed biofilm of *Staphylococcus aureus-Escherichia coli* on titanium plate [24], and a tree shrew biomaterial-centered infection model [25]. In the present study, a model of the mixed biofilm of *Escherichia coli-Candida albicans* in vitro was constructed on the surface of PVC of a central venous catheter. In order to explore the pathogenic mechanism to reduce the infection associated with central venous catheters, we studied the effects of various concentrations of lipid emulsion on mixed biofilm formation and structure as well as the expression of *flhDC, iha, HTA1,* and *HWP1* genes that correlated with biofilm formation.

Results

Effects of lipid emulsion on bacterial adhesion and mixed BFs formation

After 4 h, 12 h, 24 h, 48 h, and 72 h co-culture, semi-quantitative detection of bacterial adhesion and biofilm formation on the surface of PVC pieces were conducted in each group. The results showed that the different concentration of lipid emulsion groups showed more bacterial adhesion and mixed BFs formation than did the control group, which was treated with TSB solution alone at each time point ($P<0.05$). The 10% lipid emulsion group had the most significant effect. The detection results of each group at 4~72 h showed that the ability of bacterial adhesion and BFs formation increased with the incubation time ($P<0.001$), and it was the highest at 48 h (*Figure 1a, 1b*).

Effects of lipid emulsion on bacterial viability in mixed BFs

After 4 h, 12 h, 24 h, 48 h, and 72 h co-culture, the viability of bacteria in mixed BFs on the surface of PVC pieces were examined by XTT in each group. There were some significant differences between the different concentration of lipid emulsion groups and the control group at 12~72 h ($P<0.05$). The 10% lipid emulsion group showed the most significant effect among all of groups. The results showed that the viability of each group increased with the incubation time ($P<0.001$), and it was the highest at 48 h (*Figure 2a, 2b*).

The thickness and live&dead bacteria of mixed BFs detected by CLSM

Mixed BFs formation on the surface of PVC pieces were found to be a dynamic process. The thickness of BFs in each group increased rapidly in 24 h of co-culture, and peaked at 48 h. There was a slight decline at 48~72 h in each group. The mixed BFs of the lipid emulsion groups were more complex and denser than that of the control group. The thickness of mixed BFs were thickest in the 10% lipid emulsion at 24 h, 48 h, and 72 h ($P<0.05$) (*Figure 3a*).

Both dead and live bacteria were observed in mixed BFs by CLSM. The dead bacteria stained red, whereas the live bacteria stained green. At 24 h, there were only small proportions of dead bacteria in mixed BF in each group. At 48~72 h, the proportions of live bacteria declined, and that of the dead bacteria increased gradually from the outer layers to the inner layers of biofilm (*Figure 3b*).

The structure of mixed BFs detected by FISH

The green channel is FAM, labeled by *E.coli* of 16s RNA. The red channel is Cy3, labeled by *Ca* of 18s RNA. The blue channel is the bacterial nucleus marked by DAPI. The 72 h biofilm is thicker and more complex than the 24 h biofilm. The structure of biofilm in the lipid emulsion groups was more complicated than that in the control group, especially in the 10% lipid emulsion group. In the lipid emulsion groups, *E.coli* were found to be growing around the mycelium of *Ca* overlapping and interlacing (*Figure 4*).

The ultrastructure of mixed BFs detected by SEM

It was found that the 72 h biofilm was more complex and denser than the 24 h biofilm-forming a three-dimensional network structure. Lipid emulsion remained a part of the mixed biofilm and attached to the surface of bacteria, mycelia and spores forming more complicated and mature biofilms, especially in the 10%, 15%, 20% lipid emulsion groups. The microstructures of mixed BFs of lipid emulsion groups showed that *E.coli* was the superior strains and adhered to the spores, pseudohyphae and mycelium of *Ca* forming a mixed growth. With the increase lipid emulsion concentration, more and more *E. coli* was found in the mixed BFs, but the growth of *Ca* was inhibited when the concentration of lipid emulsion exceeded 15% (*Figure 5*).

Biofilm-related gene expression

Certain differences were noted in the expression levels of various genes between the 10% lipid emulsion group and the control group at various time points. After 24 h of culture, the expression levels of *flhDC*, *iha*, *HTA1*, *and HWP1* genes were up-regulated in the 10% lipid emulsion group as compared to those in the control group (P<0.05) (*Figure 6a*). The expression of these four genes decreased gradually overtime after 48 h of culture (*Figure 6b*). At 72 h time point, the expression of these four genes was lower in the 10% lipid group than that in the control group (P<0.05) (*Figure 6c*).

Discussion

The opportunistic pathogens *Staphylococcus epidermidis* and *Candida albicans* that colonize the skin can easily enter the human body with the implantation of biological materials [26]. Conditioned pathogens in the intestinal tract such as *Escherichia coli* and *Coprococcus* can conduct bacterial translocation in the process of trauma, stress, shock, hypotension and parenteral nutrition, causing bacteria to adhere to the surface of biomaterials and even form biofilms [27,28]. Biofilms are film-like structures of microbial communities attached to surfaces of implanted biomaterials, where aggregates of microbes adhere to each other, undergo reproduction and self-cloning, and are embedded in a self-secreted extracellular slime substance [23]. When a bacterial biofilm is formed, the bacterial family within it resists attack by the body's immune system, multiplying resistance to antibiotics and leading to persistent infections [29]. Especially found in tumor patients with immune dysfunction and patients consuming glucocorticoids for a long time, the biofilm is often a mixture of bacteria and fungus [30,31]. In nature and man-made environments, micropathogenic bacteria reside in mixed-species biofilms, in which the growth and metabolism of an organism are different from these behaviors in single-species biofilms [32]. Compared to single-species bacterial biofilm, the quantity and diversity of present species raise the complexity in mixed biofilm. The treatment of mixed biofilm is more difficult than that of a single. The management of long-term central venous catheter-associated infections by multidrug-resistant bacteria in these patients is a major challenge.

Clinically, central venous catheter-related infections are the most common. Removal of CVC is the main therapeutic intervention, especially encouraged and recommended in the case of sepsis or septic shock [33]. A recent retrospective study reported that patients with candidemia had lower survival if the CVC was not removed or removed after more than 72 h [34]. Antimicrobials should be continued for at least seven days, depending on the causative pathogen antibiotic treatment. However, there were also certain serious complications such as endocarditis, thrombophlebitis, and septic metastasis after adequate antimicrobial therapy. The risk of infection depends on host factors, catheter type, and routine care procedures.

In this paper, PVC was used as the carrier of the central venous catheter. *Ca* and *E.coli* mixture was used as the source of opportunistic pathogens coinfections. The results of XTT and crystal violet in this study showed that the biofilm formation ability, bacterial adhesion, and viability of the lipid emulsion groups were higher than that of the control group. The effects of different concentrations of lipid emulsions on

the formation of mixed biofilm were observed and analyzed by SEM and CLSM. The activity of pathogenic bacteria and the thickness of biofilm formation were most superior when lipid emulsion concentration was 10%. With the increase lipid emulsion concentration, more and more *E. coli* was found in the mixed BFs, but the growth of *Ca* was inhibited and transformed from hyphae phase to yeast phase when the concentration of lipid emulsion exceeded 15%. The results indicated that there was interspecies competition between *E.coli and Ca*, and 10% of lipid emulsion was the optimum concentration for the growth and formation of *E.coli-Ca* mixed biofilm. Swindell K. pointed out that 10% of lipid emulsion was conducive to the germination of *Ca* spores and the growth of mycelia (18). The spores and mycelia of *Ca* could provide a lot of adhesion sites for *E. coli*, meanwhile, lipid emulsion provided rich nutrients, and promoted the growth of pathogenic bacteria and biofilm formation, which may partly explain why lipid emulsion has a catalytic effect on *E. coli-Ca* mixed biofilm formation [35].

flhDC mainly regulates the biosynthesis of *E.coli* flagellum, bacterial division, and the expression of bacterial virulence genes [36]. Overexpressing *flhDC* increased the motility and ability to colonize on biomaterials [37]. A number of K-12 strains exhibit limited motility due to low expression levels of *flhDC*. Motility is beneficial for cells to access and explore new environments and to escape detrimental ones.

iha gene is iron-regulated gene homolog adhesion. The gene encodes a bacterial outer membrane protein associated with the adhesion of bacterial [38]. It mediates bacterial adhesion to host cells, and after *iha* gene knockout, the ability of bacterial cell biofilm formation is significantly reduced.

HWP1 gene of *Ca* encodes for a fungal cell wall protein required for hyphal development and yeast adhesion to epithelial cells [39]. *HWP1* is an important gene in *Ca* biofilm formation. When *HWP1* gene was knocked out, its bacterial cells could not form a complete biofilm in vitro and in vivo. Doramectin S4 is a type of antimicrobial peptide, which can potentially be used in antifungal therapy. The mechanism of DS4 as an antifungal agent is via significantly decreasing the expression of *HWP1* gene as evidenced by the gene expression analyses [40].

HTA1 gene encodes the *Ca* histone H2A. Histones play an essential role in DNA replication. Therefore, the expression of *HTA1* gene reflects the growth and reproduction ability of *Ca* cells to a certain extent [41].

qRT-PCR results showed that *flhDC, iha, HWP1, and HTA1* genes were up-regulated in the 10% lipid emulsion group compared with that of the control group at 24 h. This indicated that lipid emulsion could promote the early formation of *E. coli-Ca* mixed biofilm, especially the significant promotion of movement and adhesion of *E. coli* at early stage. In the middle stage, lipid emulsion promotes the adhesion and aggregation of *E.coli* and *Ca*. This may be the molecular mechanism by which lipid emulsion promotes the formation of biofilm. Some studies demonstrated that pathogenic *Ca* could enhance virulence determinants of a bacterium in vivo with devastating consequences to the host [42,43]. After 72 h culture, *flhDC, iha, HWP1, and HTA1* genes were downregulated in the 10% lipid emulsion group compared with that of the control group, which may be related to nutrient consumption the medium.

Thus, lipid emulsion can potentially prompt the formation of *Escherichia coli-Candida albicans* mixed biofilm. To prevent central venous catheter-associated infections, medical professionals should maintain strict aseptic precautions when handling lipid emulsion and avoid using high concentrations of lipid emulsion for a long time as much as possible.

Methods

Bacterial strains, reagents, and equipment

Escherichia coli (MC1000) was gifted by The Yale Coli Genetic Stock Center. *Candida albicans* (ATCC10231) was purchased from the Institute of Microbiology, Chinese Academy of Sciences. Primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. In situ hybridization kit was purchased from Boxin Biology (Guangzhou) Co., Ltd. The cDNA Synthesis kit was procured from Bio-Rad Inc. Live&Dead bacterial staining kit was obtained from Life, USA. Polypropylene 24-well and 96-well cell culture plates were purchased from Corning, USA. MH agar plates and Shapaul agar plates were from Tu'an Biological Engineering (Zhengzhou) Co., Ltd. Tryptic Soy Broth (TSB) medium was from Huankai Microbiological Reagent (Guangdong) Co., Ltd. S-3000N scanning electron microscope was from HITACHI, Japan and FV1000 confocal laser scanning microscope from Olympus, Japan. Total RNA extraction kit was supplied by Tiangen Biotech (Beijing) Co., Ltd. The qRT-PCR assay was conducted using the SuperReal PreMix Plus (Tiangen, China),and was performed on the ABI 7500 PCR system (ABI, USA).

Bacterial culture and Experimental grouping

The standard strains of *Ca* and *E.coli* were inoculated on Sarpaul agar plates and MH agar plates respectively and incubated at 37°C for 24 h. Subsequently, 5 mL TSB culture medium was added into test tube, and the liquid level junction in the test tube was gently vibrated. An inoculation ring was used to select a single colony on the bacterial plate of each group and inoculate into the test tube containing 5 mL TSB medium. These tubes were incubated in a constant humidity oscillator at 37°C and 200 r/min for 16~18 h. After the growth of bacterial cells to the logarithmic growth phase, the concentration of the bacterial solution in each group was adjusted to 1.1×10^7 CFU/mL using TSB medium in an ultraviolet spectrophotometer for later use. The mixed bacterial solution in the study was prepared in 1:1 ratio, that is, 2 mL of bacterial solution was prepared by mixing 1 mL *Ca* bacterial solution and 1 mL *E. coli* bacterial solution. Bacterial suspensions treated with various concentrations of lipid emulsion were added to a 96-well cell culture plate and incubated in a 37 °C thermostatic incubator. The experiment included five groups: a control group with TSB medium alone and four lipid emulsion groups. The concentrations of lipid emulsion in the other four groups were 5%, 10%, 15%, and 20%. The TSB medium was mixed with lipid emulsion in various proportions to modulate the lipid emulsion concentration to 0%, 5%, 10%, 15%, and 20%.

Detection of bacterial adhesion and biofilm formation by CV

A total of 100 μL of the previously prepared lipid emulsion of different concentrations was inoculated into 96-well cell culture plates, and then 10 μL of the prepared bacterial solution and a PVC piece were added into each well. Six wells were inoculated in each group at each time point and incubated in a 37 °C incubator. The medium was removed from the 96-well plates at 4 h, 12 h, 24 h, 48 h, and 72 h after co-culturing. 100 μL PBS was added to wash and remove the floating bacteria on the PVC piece for three times. After gently washing and discarding PBS, 100 μL 2% CV dye solution was added to each well, and incubated at 37°C for 30 minutes. Then, CV dye solution was sucked and discarded, and 100 μL PBS was added to rinse for three times. The ability of bacterial adhesion and mixed biofilm formation was determined by crystal violet staining, and measured at an absorbance of 490 nm wavelength by using a multifunctional marker in various concentrations of lipid emulsion. Experiments were repeated three times.

Detection of bacterial viability by XTT

After 4 h, 12 h, 24 h, 48 h, and 72 h co-culture with different concentrations of lipid emulsion, the PVC pieces were gently washed three times with cold PBS solution to remove the floating bacteria on the PVC pieces. After gently washing and discarding PBS, 100 μL TSB culture medium and 20 μL XTT solution were added to each well and incubated at 37°C for 2 h in the absence of light. Following incubation, 120 μL medium was removed from each well to measure the viability of bacteria at an absorbance of 450 nm wavelength by using a multifunctional marker in various concentrations of lipid emulsion. Experiments were repeated three times.

Observation of mixed biofilm thickness and live/dead bacteria by CLSM

The fluorescent stains for bacterial viability in the BFs were identified using Live&Dead Bacterial viability kit. The PVC pieces were taken out for 24 h, 48 h, and 72 h after culture and gently washed with normal saline three times to remove the floating bacteria on the PVC pieces. They were then immersed in the fluorescent stains and stained for 20 min at room temperature in the absence of light. After absorbing excess fluorescent dye, the PVC pieces were put on a fluorescent image slide under the CLSM. CLSM observation was carried out using an argon laser. The green fluorescence excitation wavelength is 488 nm, and the emission wavelength is 519 nm. The red fluorescence excitation wavelength is 559 nm, and the emission wavelength is 567 nm. The live and dead bacteria on the PVC pieces at each time point were evaluated according to the area occupied by the green fluorescence of live bacteria and red fluorescence of dead bacteria. Each PVC piece was scanned from internal to external to measure the thickness of the biofilm.

Observation of mixed biofilm composition by FISH

The probe sequences of *E. coli* and *Ca* were designed according to GenBank (*Table 1*). The PVC pieces were taken out for FISH after 24 h and 72 h incubation according to the manufacturer's instructions. All the samples of PVC pieces were observed under the CLSM. The green fluorescence excitation wavelength is 488 nm, and the emission wavelength is 519 nm. The red fluorescence excitation wavelength is 559

nm, and the emission wavelength is 567 nm. The blue fluorescence excitation wavelength is 340 nm, and the emission wavelength is 488 nm. Each PVC piece was scanned from internal to external to observe the structure and composition of mixed BFs.

Observation of mixed biofilm ultrastructure by SEM

The PVC pieces were taken out after 24 h and 72 h co-culture, and gently washed with PBS solution three times and fixed with 2% glutaraldehyde phosphate buffer on slides for SEM analysis. Then, the PVC pieces were washed three times with PBS and dried in critical CO_2. The surface of PVC pieces turned to golden brown because of ions sputtering on the surface. The ultrastructure of the mixed biofilm was observed by SEM.

Quantitation of biofilm-related gene expression

A 24-well cell culture plate was removed each from the control group and 10% lipid emulsion group at 24 h, 48 h, and 72 h, and the BFs were scraped and transferred to 1.5 mL centrifuge tubes. Total RNA was extracted by the total RNA extraction kit for RNA quantitation. Reverse transcription was conducted with the extracted RNA samples by the BIO-RAD iscript cDNA Synthesis kit. Primers were designed for *flhDC, iha*, *HTA1*, *HWP1*, and 16 sRNA, *act1* which were used as the reference gene. Primer sequences are shown in (*Table 2*). The primers and cDNA template synthesized from the reverse transcription reaction were used for the quantitative RT- PCR reaction. The $2^{-\Delta\Delta Ct}$ method was used for the comparison of the relative levels of mRNAs.

Statistical Analysis

SPSS 24.0 statistical software was used for statistical analyses. The experimental data conforming to the normal distribution or meeting the normal distribution after conversion were expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used for intragroup and intergroup comparisons, and a *t*-test was used for pairwise comparisons. A value of $P<0.05$ was considered statistically significant difference. $P<0.01$ and $P<0.001$ suggested significant difference.

Declarations

Acknowledgments

Funding: This study was partly supported by the Grants from the National Natural Science Foundation of China (No.81960335).

Author contributions

(I) Conception and design: W Duan, S Li; (II) Administrative support: Y Huang, Y Chen, Y Lei; (III) Provision of study materials or patients: L He, C Fu; (IV) Collection and assembly of data: W Duan, Y Chen; (V) Data

analysis and interpretation: S Li, M Li, Z Shen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Competing interests

The authors have no conflicts of interest to declare.

Data availability

All dates in the paper are available.

References

1 Bellesi, S. *et al.* Peripherally inserted central catheters (PICCs) in the management of oncohematological patients submitted to autologous stem cell transplantation. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer* **21**, 531-535, doi:10.1007/s00520-012-1554-0 (2013).

2 Christensen, L. D. *et al.* Comparison of complications associated with peripherally inserted central catheters and Hickman™ catheters in patients with intestinal failure receiving home parenteral nutrition. Six-year follow up study. *Clinical nutrition (Edinburgh, Scotland)* **35**, 912-917, doi:10.1016/j.clnu.2015.06.009 (2016).

3 Kim, J. S., Holtom, P. & Vigen, C. Reduction of catheter-related bloodstream infections through the use of a central venous line bundle: epidemiologic and economic consequences. *American journal of infection control* **39**, 640-646, doi:10.1016/j.ajic.2010.11.005 (2011).

4 Mariggiò, E. *et al.* Peripherally inserted central catheters in allogeneic hematopoietic stem cell transplant recipients. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer* **28**, 4193-4199, doi:10.1007/s00520-019-05269-z (2020).

5 Nuckols, T. K. *et al.* Economic Evaluation of Quality Improvement Interventions for Bloodstream Infections Related to Central Catheters: A Systematic Review. *JAMA internal medicine* **176**, 1843-1854, doi:10.1001/jamainternmed.2016.6610 (2016).

6 Zimlichman, E. *et al.* Health care-associated infections: a meta-analysis of costs and financial impact on the US health care system. *JAMA internal medicine* **173**, 2039-2046, doi:10.1001/jamainternmed.2013.9763 (2013).

7 Buetti, N. *et al.* Short-term dialysis catheter versus central venous catheter infections in ICU patients: a post hoc analysis of individual data of 4 multi-centric randomized trials. *Intensive care medicine* **45**, 1774-1782, doi:10.1007/s00134-019-05812-w (2019).

8 Sheng, K. X. *et al.* Comparative efficacy and safety of lock solutions for the prevention of catheter-related complications including infectious and bleeding events in adult haemodialysis patients: a systematic review and network meta-analysis. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **26**, 545-552, doi:10.1016/j.cmi.2019.12.003 (2020).

9 Dube, W. C. *et al.* Comparison of Rates of Central Line-Associated Bloodstream Infections in Patients With 1 vs 2 Central Venous Catheters. *JAMA network open* **3**, e200396, doi:10.1001/jamanetworkopen.2020.0396 (2020).

10 Chopra, V., O'Horo, J. C., Rogers, M. A., Maki, D. G. & Safdar, N. The risk of bloodstream infection associated with peripherally inserted central catheters compared with central venous catheters in adults: a systematic review and meta-analysis. *Infect Control Hosp Epidemiol* **34**, 908-918, doi:10.1086/671737 (2013).

11 Lee, Y. M. *et al.* Clinical impact of delayed catheter removal for patients with central-venous-catheter-related Gram-negative bacteraemia. *The Journal of hospital infection* **99**, 106-113, doi:10.1016/j.jhin.2018.01.004 (2018).

12 Hajjej, Z. *et al.* Incidence, risk factors and microbiology of central vascular catheter-related bloodstream infection in an intensive care unit. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy* **20**, 163-168, doi:10.1016/j.jiac.2013.08.001 (2014).

13 Braun, E. *et al.* Predominance of Gram-negative bacilli among patients with catheter-related bloodstream infections. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **20**, O627-629, doi:10.1111/1469-0691.12565 (2014).

14 Marcos, M. *et al.* Changing epidemiology of central venous catheter-related bloodstream infections: increasing prevalence of Gram-negative pathogens. *The Journal of antimicrobial chemotherapy* **66**, 2119-2125, doi:10.1093/jac/dkr231 (2011).

15 Green, J. V. *et al.* Heparin-binding motifs and biofilm formation by Candida albicans. *The Journal of infectious diseases* **208**, 1695-1704, doi:10.1093/infdis/jit391 (2013).

16 Touré, A. *et al.* Propensity score analysis confirms the independent effect of parenteral nutrition on the risk of central venous catheter-related bloodstream infection in oncological patients. *Clinical nutrition (Edinburgh, Scotland)* **32**, 1050-1054, doi:10.1016/j.clnu.2012.12.006 (2013).

17 Ishizuka, M., Nagata, H., Takagi, K., Iwasaki, Y. & Kubota, K. Systemic inflammatory response predicts perioperative central venous catheter-related bloodstream infection in patients Undergoing colorectal cancer surgery with administration of parenteral nutrition. *Anticancer research* **32**, 4045-4050 (2012).

18 Swindell, K., Lattif, A. A., Chandra, J., Mukherjee, P. K. & Ghannoum, M. A. Parenteral lipid emulsion induces germination of Candida albicans and increases biofilm formation on medical catheter surfaces. *The Journal of infectious diseases* **200**, 473-480, doi:10.1086/600106 (2009).

19 Chheng, M. L., Heidbreder, C., Btaiche, I. F. & Blackmer, A. B. Infectious complications with nondaily versus daily infusion of intravenous fat emulsions in non-critically ill adults. *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition* **28**, 737-744, doi:10.1177/0884533613507085 (2013).

20 Santarpia, L. *et al.* Central venous catheter related bloodstream infections in adult patients on home parenteral nutrition: Prevalence, predictive factors, therapeutic outcome. *Clinical nutrition (Edinburgh, Scotland)* **35**, 1394-1398, doi:10.1016/j.clnu.2016.03.009 (2016).

21 Todd, O. A. *et al.* Candida albicans Augments Staphylococcus aureus Virulence by Engaging the Staphylococcal agr Quorum Sensing System. *mBio* **10**, doi:10.1128/mBio.00910-19 (2019).

22 Sheehan, G., Tully, L. & Kavanagh, K. A. Candida albicans increases the pathogenicity of Staphylococcus aureus during polymicrobial infection of Galleria mellonella larvae. *Microbiology (Reading, England)* **166**, 375-385, doi:10.1099/mic.0.000892 (2020).

23 Chen, Y. *et al.* Study on the Structure of Candida Albicans-Staphylococcus Epidermidis Mixed Species Biofilm on Polyvinyl Chloride Biomaterial. *Cell biochemistry and biophysics* **73**, 461-468, doi:10.1007/s12013-015-0672-y (2015).

24 Dong, J. *et al.* Research on the effect of TiO(2) nanotubes coated by gallium nitrate on Staphylococcus aureus-Escherichia coli biofilm formation. *Journal of clinical laboratory analysis* **34**, e23417, doi:10.1002/jcla.23417 (2020).

25 Lei, Y. *et al.* The effects of TGF-β1 on staphylococcus epidermidis biofilm formation in a tree shrew biomaterial-centered infection model. *Annals of translational medicine* **9**, 57, doi:10.21037/atm-20-4526 (2021).

26 Broekhuizen, C. A. *et al.* Staphylococcus epidermidis is cleared from biomaterial implants but persists in peri-implant tissue in mice despite rifampicin/vancomycin treatment. *Journal of biomedical materials research. Part A* **85**, 498-505, doi:10.1002/jbm.a.31528 (2008).

27 Liu, J., Huang, L., Luo, M. & Xia, X. Bacterial translocation in acute pancreatitis. *Critical reviews in microbiology* **45**, 539-547, doi:10.1080/1040841x.2019.1621795 (2019).

28 Zhou, Q. & Verne, G. N. Intestinal hyperpermeability: a gateway to multi-organ failure? *The Journal of clinical investigation* **128**, 4764-4766, doi:10.1172/jci124366 (2018).

29 Hathroubi, S., Mekni, M. A., Domenico, P., Nguyen, D. & Jacques, M. Biofilms: Microbial Shelters Against Antibiotics. *Microbial drug resistance (Larchmont, N.Y.)* **23**, 147-156, doi:10.1089/mdr.2016.0087

(2017).

30 Souza, J. G. S. *et al.* Role of glucosyltransferase R in biofilm interactions between Streptococcus oralis and Candida albicans. *The ISME journal* **14**, 1207-1222, doi:10.1038/s41396-020-0608-4 (2020).

31 Garcia-Perez, J. E. *et al.* A Framework for Understanding the Evasion of Host Immunity by Candida Biofilms. *Frontiers in immunology* **9**, 538, doi:10.3389/fimmu.2018.00538 (2018).

32 Oxaran, V. *et al.* Behavior of Foodborne Pathogens Listeria monocytogenes and Staphylococcus aureus in Mixed-Species Biofilms Exposed to Biocides. *Appl Environ Microbiol* **84**, e02038-02018, doi:10.1128/AEM.02038-18 (2018).

33 Mermel, L. A. *et al.* Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **49**, 1-45, doi:10.1086/599376 (2009).

34 Liu, C. Y. *et al.* Candidemia in cancer patients: impact of early removal of non-tunneled central venous catheters on outcome. *The Journal of infection* **58**, 154-160, doi:10.1016/j.jinf.2008.12.008 (2009).

35 Willems, H. M. E., Stultz, J. S., Coltrane, M. E., Fortwendel, J. P. & Peters, B. M. Disparate Candida albicans Biofilm Formation in Clinical Lipid Emulsions Due to Capric Acid-Mediated Inhibition. *Antimicrobial agents and chemotherapy* **63**, doi:10.1128/aac.01394-19 (2019).

36 Fahrner, K. A. & Berg, H. C. Mutations That Stimulate flhDC Expression in Escherichia coli K-12. *Journal of bacteriology* **197**, 3087-3096, doi:10.1128/jb.00455-15 (2015).

37 Huang, W. C. *et al.* The role of the bacterial protease Prc in the uropathogenesis of extraintestinal pathogenic Escherichia coli. *Journal of biomedical science* **27**, 14, doi:10.1186/s12929-019-0605-y (2020).

38 Paniagua-Contreras, G. L. *et al.* Comprehensive expression analysis of pathogenicity genes in uropathogenic Escherichia coli strains. *Microbial pathogenesis* **103**, 1-7, doi:10.1016/j.micpath.2016.12.008 (2017).

39 Orsi, C. F. *et al.* Impact of Candida albicans hyphal wall protein 1 (HWP1) genotype on biofilm production and fungal susceptibility to microglial cells. *Microbial pathogenesis* **69-70**, 20-27, doi:10.1016/j.micpath.2014.03.003 (2014).

40 Samot, J. & Rouabhia, M. Effect of Dermaseptin S4 on C. albicans Growth and EAP1 and HWP1 Gene Expression. *Probiotics and antimicrobial proteins*, doi:10.1007/s12602-020-09685-0 (2020).

41 Tenea, G. N. *et al.* Overexpression of several Arabidopsis histone genes increases agrobacterium-mediated transformation and transgene expression in plants. *The Plant cell* **21**, 3350-3367, doi:10.1105/tpc.109.070607 (2009).

42 Todd, O. A., Noverr, M. C. & Peters, B. M. Candida albicans Impacts Staphylococcus aureus Alpha-Toxin Production via Extracellular Alkalinization. *mSphere* **4**, doi:10.1128/mSphere.00780-19 (2019).

43 Nash, E. E., Peters, B. M., Fidel, P. L. & Noverr, M. C. Morphology-Independent Virulence of Candida Species during Polymicrobial Intra-abdominal Infections with Staphylococcus aureus. *Infection and immunity* **84**, 90-98, doi:10.1128/iai.01059-15 (2016).

Tables

Table 1 The prime probe sequence

strains	Probe sequence	fluorescent
Escherichia coli（MC1000）	AGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCA TAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACT	FAM(green)
Candida albicans （ATCC10231）	ACCAGACTTGCCCTCC	Cy3(red)

Table 2 *16 sRNA, flhDC, iha, act1, HTA1 and HWP1* primer sequences

Target gene	Forward primer(5’—3’)	Reverse primer(5’—3’)
flhDC	GCGGTTTGTTGAAAGTGGAT	GATGGCGGTTGACATAAGC
iha	ATGATAACCGGGATGGTCAA	CCCATTTGTCGCTCTTCAGT
16 sRNA	GAGAGCAAGCGGACCTCATA	GCAGACTCCATTCCGGACTAC
HTA1	ATGTCAGGTGGTAAAGGTAAAG	CTACAATTCTTGAGAAGCCTTAAC
HWP1	GGTTGTGAGCCATTAGGGTTA	GGTTGTGAGCCATTAGGGTTA
act1	ACCACCGGTATTGTTTTGGA	TGGACAAATGGTTGGTCAAG

16 sRNA, *act1* were used as the reference gene.

Figures

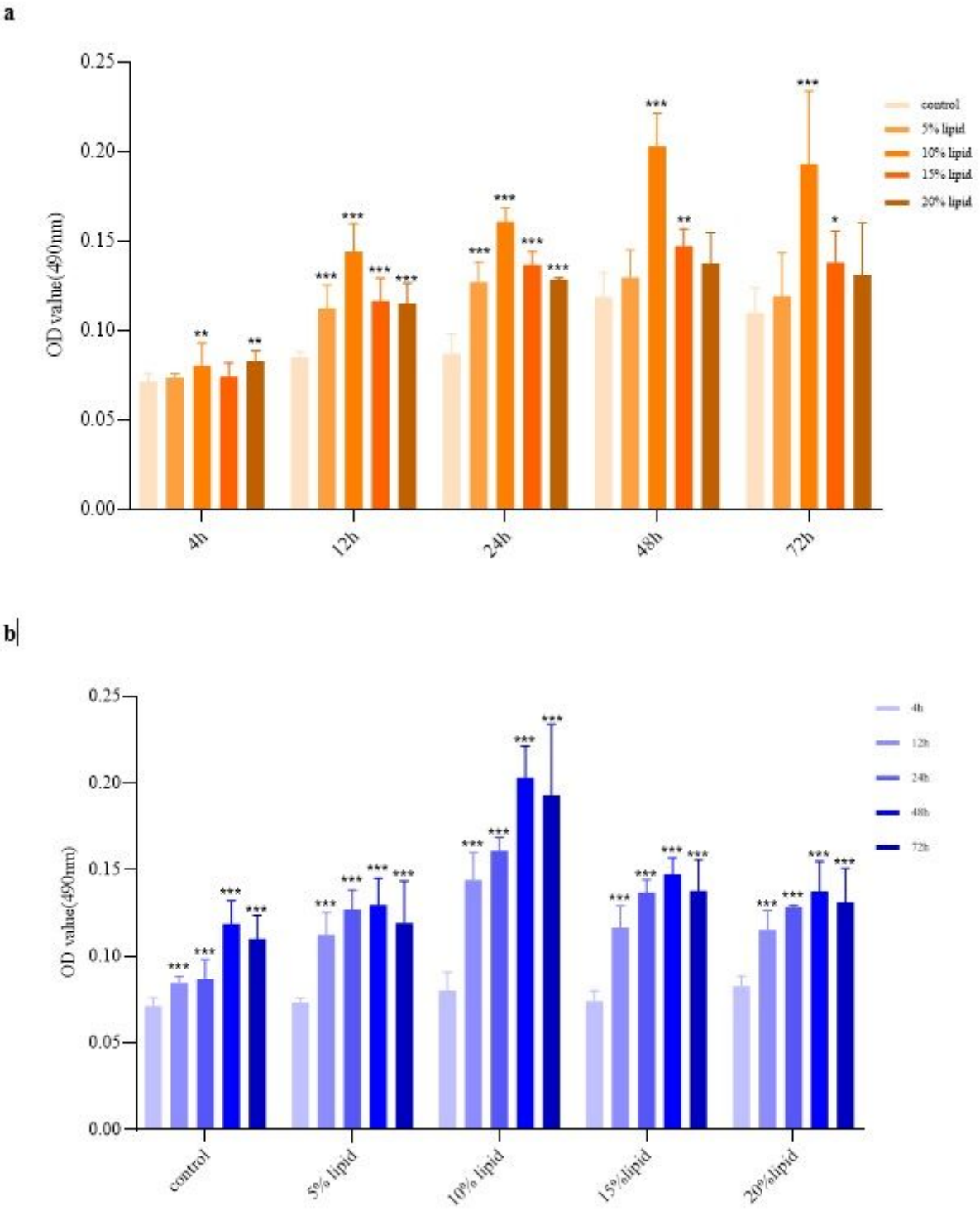

Figure 1

Effect of lipid emulsions on bacterial adhesion. Compared with the control group at the same time, * P<0.05, ** P<0.01, *** P<0.001 (1a). Compared with the 4 h at the same concentration, *P<0.05, ** P<0.01, *** P<0.001 (1b).

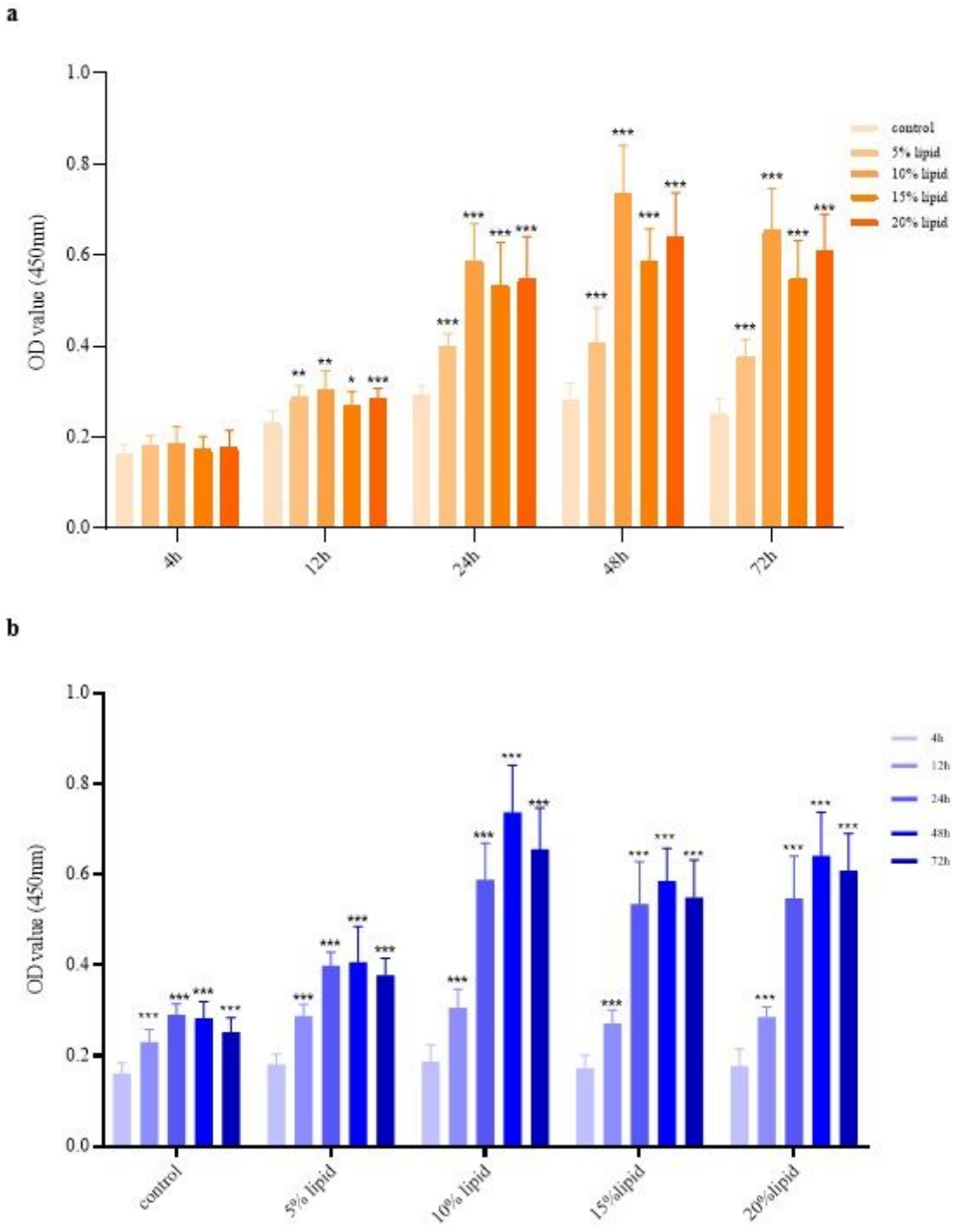

Figure 2

Effect of lipid emulsions on mixed biofilm formation ability. Compared with the control group at the same time, * P<0.05, ** P<0.01, ***P<0.001 (2a). Compared with the 4 h at the same concentration, * P<0.05, ** P<0.01, *** P<0.001 (2b).

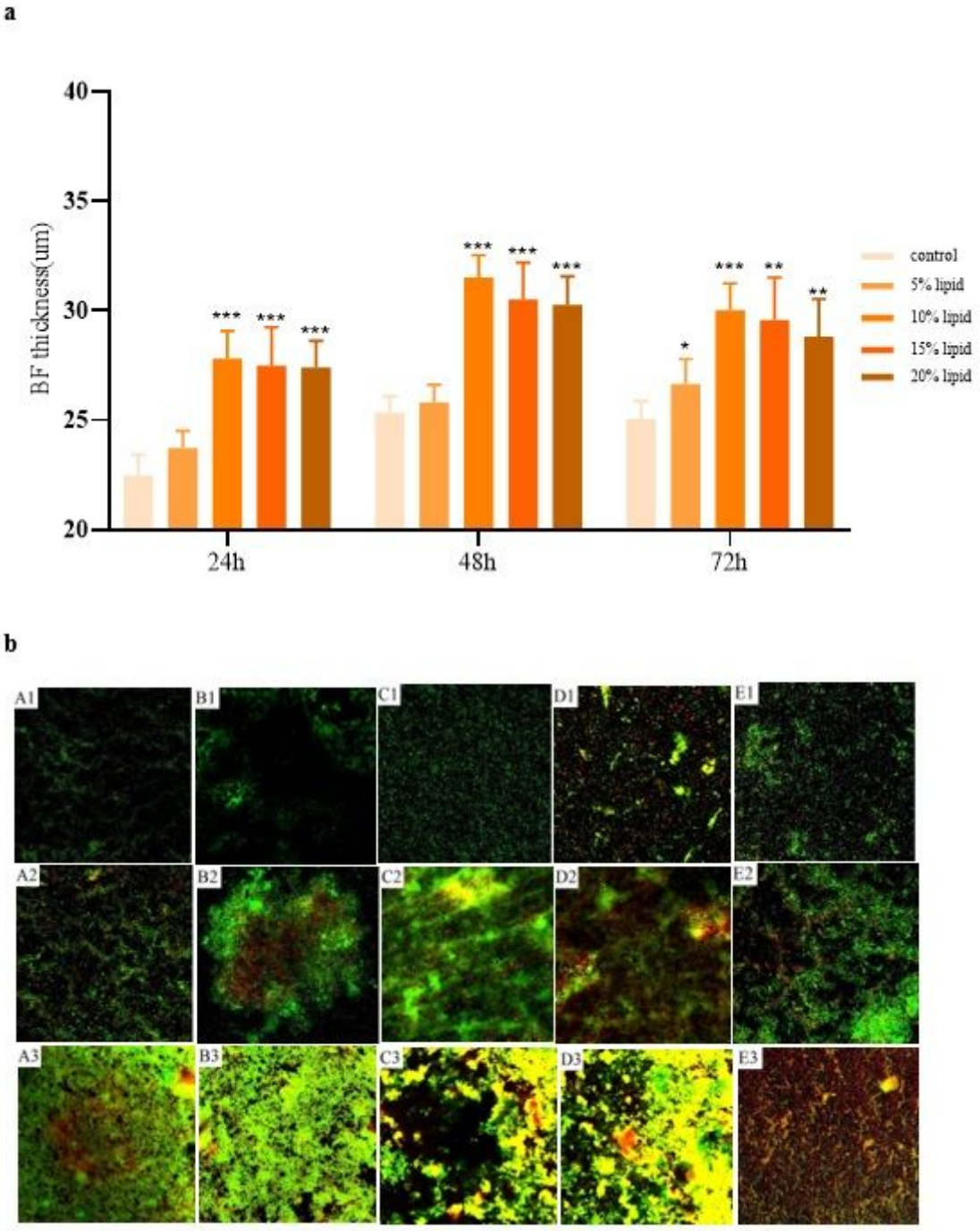

Figure 3

The thickness of biofilm in each group at 24, 48, 72 h. Compared with the control group at the same time, * P<0.05, ** P<0.01, *** P<0.001 (3a). Observation of mixed biofilm by CLSM at 24, 48, 72 h points. The images in each group were taken at 200× magnifications (3b). (A1) Control group (24 h); (B1) 5% lipid group (24 h); (C1) 10% lipid group (24 h); (D1) 15% lipid group (24 h); (E1) 20% lipid group (24 h); (A2) Control group (48 h); (B2) 5% lipid group (48 h); (C2) 10% lipid group (48 h); (D2) 15% lipid group (48 h);

(E2) 20% lipid group (48 h); (A3) Control group (72 h); (B3) 5% lipid group (72 h); (C3) 10% lipid group (72 h); (D3) 15% lipid group (72 h); (E3) 20% lipid group (72 h).

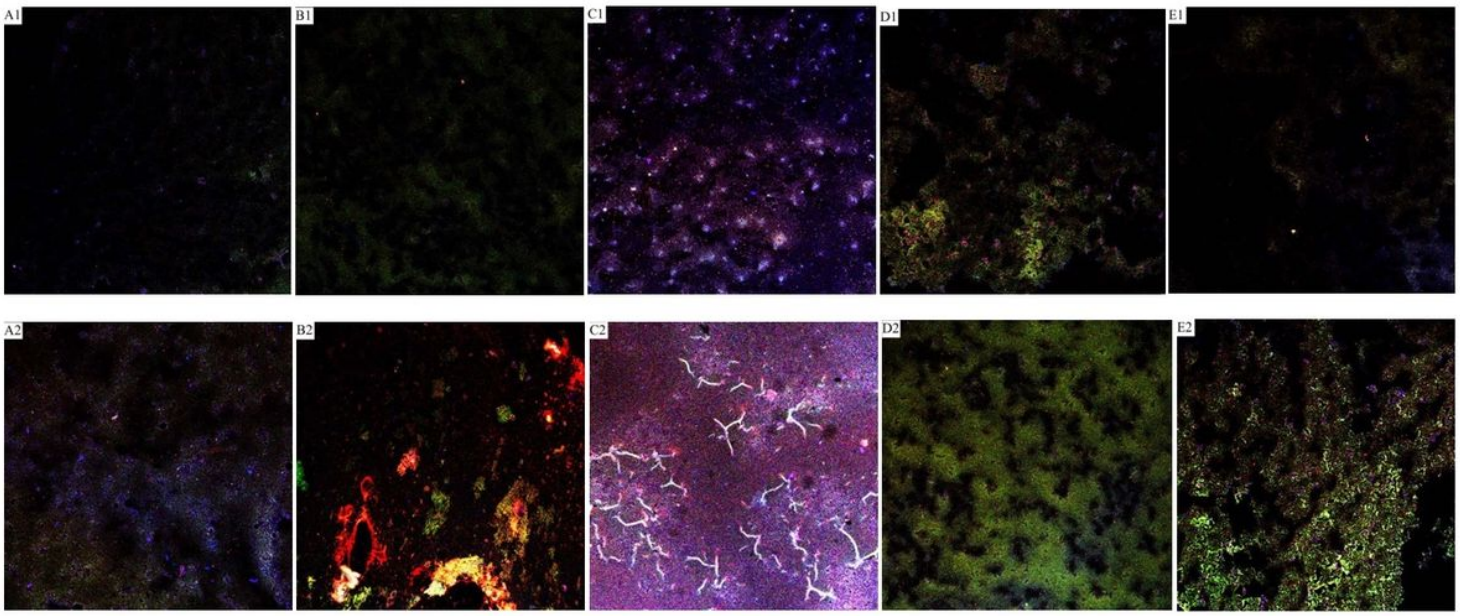

Figure 4

Observation of mixed biofilm by FISH at 24 h, 72 h points. The images in each group were taken at 200× magnifications. (A1) Control group (24 h); (B1) 5% lipid group (24 h); (C1) 10% lipid group (24 h); (D1) 15% lipid group (24 h); (E1) 20% lipid group (24 h); (A2) Control group (72 h); (B2) 5% lipid group (72 h); (C2) 10% lipid group (72 h); (D2) 15% lipid group (72 h); (E2) 20% lipid group (72 h).

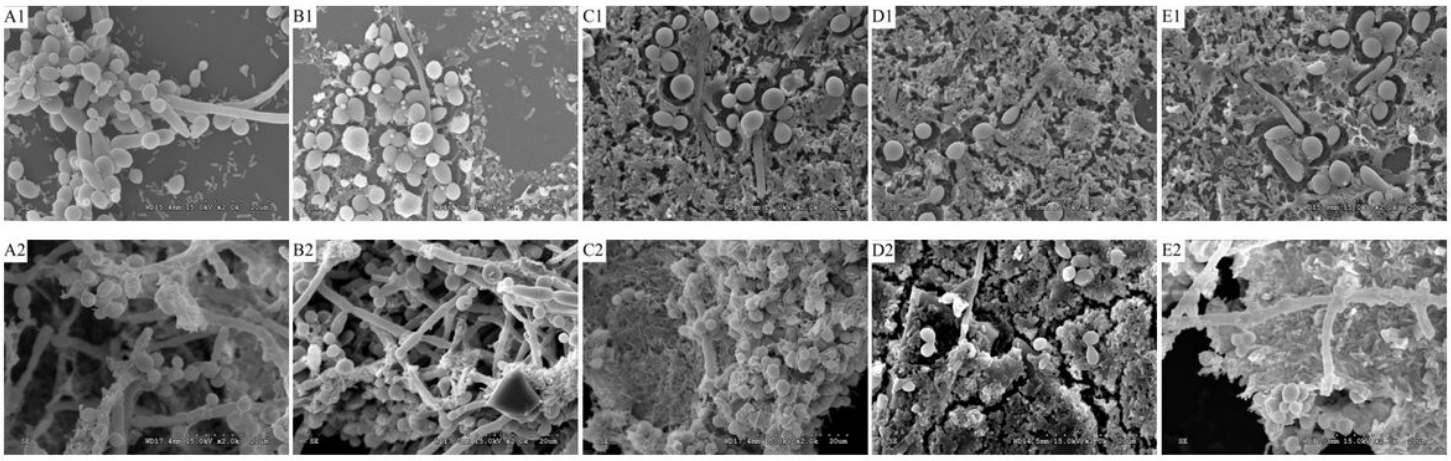

Figure 5

SEM observation of mixed biofilm at 24 h, 72 h points. The representative SEM images in different groups were taken at 2000× magnifications. (A1) Control group (24 h); (B1) 5% lipid group (24 h); (C1) 10% lipid group (24 h); (D1) 15% lipid group (24 h); (E1) 20% lipid group (24 h); (A2) Control group (72 h);

(B2) 5% lipid group (72 h); (C2) 10% lipid group (72 h); (D2) 15% lipid group (72 h); (E2) 20% lipid group (72 h).

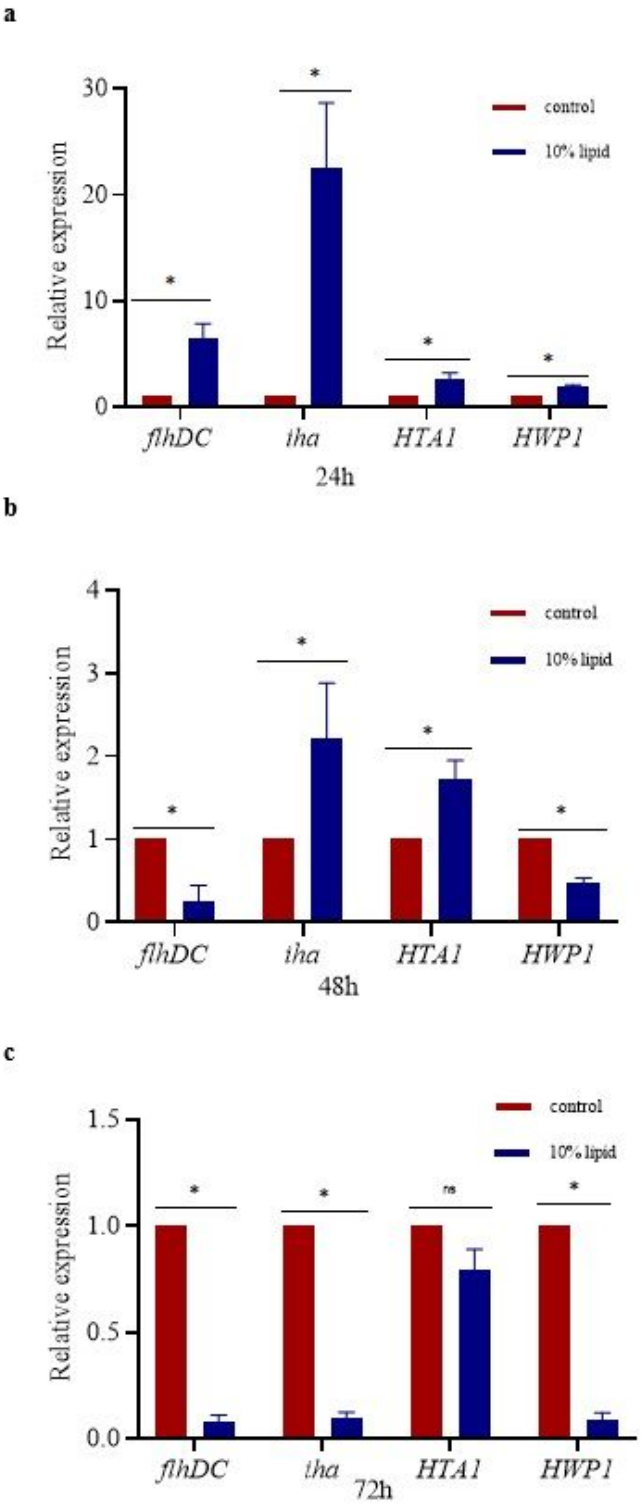

Figure 6

Comparison of qRT-PCR results of gene expression at 24 h, 48 h, 72 h between the control group and 10% lipid group, * P<0.05.