JOURNAL OF ORAL MICROBIOLOGY
2024, VOL. 16, 2299538
https://doi.org/10.1080/20002297.2023.2299538

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Effective disinfecting of negative pressure pipelines of DCUs reduces the risk of cross infection in dental care

Xuerong Lv[a*], Ke Yan[a*], Xiang Han[a], Feiyang Wang[a], Yuzhuo Ma[a], Shougang Zhang[b] and Xiaoqian Wang[a]

[a]Department of Periodontology, Jiangsu Province Key Laboratory of Oral Diseases, Jiangsu Province Engineering Research Center of Stomatological Translational Medicine, The Affiliated Stomatological Hospital of Nanjing Medical University, Nanjing, China; [b]Department of Disinfection and Vector Control, Nanjing Center for Disease Control and Prevention, China

ABSTRACT

Objectives: Microbial contamination of various accessory parts of the dental chair units (DCUs) is an essential source of cross infection, while the accessories of the crucial suction function are usually overlooked. In this study, we aim to find an effective disinfectant and a cost-effective method to remove bacterioplankton and bacterial biofilm deposited in the negative pressure suction pipelines to control cross infection during dental treatment.

Methods: Double-chain quaternary ammonium salt disinfectant (Orotol Plus®), 3% hydrogen peroxide solution plus multi-enzyme cleaning agent and chlorine disinfectant are used to clean and disinfect the negative pressure pipelines of DCUs. Microbiological examinations, air condition detection, corrosion tests and gene sequencing are performed.

Results: Little bacteria grow in the pipelines disinfected with double-chain quaternary ammonium salt disinfectants, destruction of biofilms in these pipelines appears, and multi-resistant bacteria cannot be detected. Minimal damage to metal sheets and fittings is caused by double-chain quaternary ammonium salt disinfectants.

Conclusion: Double-chain quaternary ammonium salt disinfectant has excellent bactericidal ability and anti-biofilm effect, and it is less corrosive to the fittings of the pipelines. Thus, the double-chain quaternary ammonium salt disinfectant is a potential novel disinfectant for negative pressure suction pipelines of DCUs to control cross infection during dental treatment.

Clinical significance: It is essential to add all these data to our dental practice to control cross infection with a broader landscape.

ARTICLE HISTORY
Received 19 July 2023
Revised 13 December 2023
Accepted 14 December 2023

KEYWORDS
Dental chair unit; negative pressure pipeline; cross infection; disinfectant; dental care; biofilm

Introduction

The Dental Chair Unit (DCU) is a complex piece of dental medical equipment used to perform various dental examinations and treatments. The DCUs are mainly used for oral therapy which consist of the dental chair, a pedestal unit furnishing provision, the control of equipment and auxiliary attachments, which involve the negative pressure suction system [1].

The negative pressure suction system is composed of two evacuators attached to a vacuum source supplied to the body of the DCUs, which are called high volume evacuator (HVE) and low volume evacuator (LVE). The role of this system is to effectively reduce the release of aerosols, sprays and droplets, and timely remove the liquids and debris in patients' mouths [2]. In the process of oral treatment, LVE is often used to suck the blood, saliva and other organic substances in the patient's mouth and the coolant of the tooth drill to ensure the clarity of the operation area [3]. When the patient has too much fluid in mouth, he may wrap the suction tip with the lips to suck away the liquid; meanwhile, the oral cavity temporarily forms a partial vacuum space, which is easy to result in the occurrence of backflow [4]. The bacteria and biofilms exist steadily in pipeline connected to evacuators, which provides interfaces for bacteria and biofilms to form, grow, proliferate and shedding. The potential liquid backflow in LVE exposes the patient's oral mucosa or incomplete soft or hard tissues to fluids suctioning from previously treated patients which can predispose them to potential cross-infection risk [3]. The biofilms shedding off from the inner wall of the suction system pipelines may be back into the patients' mouths which can lead to the high risk of infection during dental treatment, especially for immune deficiency patients, and may easily result in multi-drug-resistant bacteria and cross infection of viruses among different patients.

As for HVE, it is a suction device that draws in large quantities of air or even liquid droplets suspended in the air during ultrasonic scaling procedures. Choudhary [5] clarified that the greatest

CONTACT Xiaoqian Wang xiaoqian0103@hotmail.com The Affiliated Stomatological Hospital of Nanjing Medical University, No.1 Shanghai Road, Gulou District, Nanjing, Jiangsu Province 210029, China
*These authors contributed equally to this work.

concentrations of aerosols are produced by ultrasonic scaling and high-speed drilling of anterior teeth, which is evident in dental procedures on the upper incisors where large amounts of water are splashed back into the atmosphere around the patient and where HVE has enough space to reduce aerosols. Many previous researches [6] also proved that HVE significantly reduced the aerosol concentration during all types of dental procedures sampled.

There were researches [7,8] focusing on the Waterline Disinfectants, and the study [7] has shown that plumbing disinfectants are effective in reducing dental aerosols. The cleaning and disinfection of the suction pipeline system is a longtime problem in the infection control of the dental treatment process [9]. However, due to the heavy workload in the outpatient clinics of dental specialty hospitals, it is easy to neglect the cleaning and disinfection of the saliva suction pipeline [10]. Nowadays, there is no recommended domestic or international standard for the cleaning and disinfection of negative pressure pipelines on DCUs, and the cleaning and disinfection methods adopted by various medical institutions are mainly the use of tap water for suction between consultations, and the use of a certain concentration of chlorine disinfectant for soaking and flushing after consultation. Chlorine disinfectant can only reduce or inhibit planktonic microorganisms in the negative pressure pipelines but make no effort on removing and inhibiting the biofilm of plaque on the pipeline wall. Otherwise, the long-term use of chlorine disinfectant may have a corrosive effect on the pipeline fittings [11–13]. It has also been reported that 3% hydrogen peroxide solution with multi-enzyme cleaning agent is used to soak and rinse the negative pressure pipelines of the DCUs, while the hydrogen peroxide may produce abundant foam when it meets blood stain, which may lead to blockage of the negative pressure pipelines and spittoon downpipes, and may cause gas backflow in the pipelines [12–14]. Scholars have found that long-term used DCUs leaked due to corrosion of negative pressure pipeline fittings, and opportunistic pathogenic bacteria such as Pseudomonas aeruginosa were isolated from the rusted negative pressure line connections, with a positive rate of 61% [1]. It has become a possible source of cross-infection in dental practice.

Double-chain quaternary ammonium salt disinfectants belong to the fourth generation of new quaternary ammonium salt disinfectants with surface activity, whose surface activity and bactericidal function have many unique advantages compared with the traditional quaternary ammonium salt surfactants in the past [14]. They also have stronger bactericidal activity than benzalkonium bromide and better killing effect on bacterial propagules and lipophilic viruses. Quaternary ammonium compounds have shown significant antibacterial effects against dental caries and periodontitis and some of them also have exhibited antiviral activity against coronaviruses, indicating their potential as treatments for COVID-19 [15].

In this study, the growth of bacteria in biofilm in the pipelines, the effect of different cleaning and disinfection methods on planktonic bacteria, biofilm removal, biofilm prevention, air quality, and long-term corrosion of pipelines fittings are investigated, while the negative pressure pipelines of DCUs are taken as the object of the study. The materials used in this research to disinfect the negative pressure pipelines of the DCUs are Orotol Plus® negative pressure pipeline disinfectant, 3% hydrogen peroxide solution with multi-enzyme cleaner and 500 mg/L chlorine disinfectant to observe the effect of removing biofilm and preventing biofilm generation. The aim of this research is to find effective disinfectant and methods to reduce negative pressure pipeline contamination, to reduce the risk of cross-infection between patients, to reduce the adverse effects of negative pressure pipelines on the air in the dental clinic and reduce the possible occupational exposure of dental health-care providers.

Methods and materials

Dental chair units

From June 2020 to June 2022, the HVE and LVE negative pressure suction pipelines of the DCUs (Sinora intego, $n = 15$) of the Department of Periodontology, Affiliated Stomatological Hospital of Nanjing Medical University are studied as the research objects. Among them, the suction pipelines on the first to fifth days are in working state, and on the sixth to seventh days are in off-working state. The negative pressure pipelines under working state are cleaned and disinfected twice a day, while the negative pressure pipelines under non-working condition are kept dry.

Grouping

The experiments are divided into preliminary (5 days) and further (4 weeks) experiments according to the maintenance time, besides pipelines are divided into the following groups according to the protocol: A1 (control group, $n = 5$), pipelines of DCUs are immersed with 1:100 multi-enzyme detergent plus 3% H_2O_2. A2 (experimental group, $n = 5$), pipelines are soaked with the original solution of double-chain quaternary ammonium salt disinfectant. A3 (control group, $n = 5$), pipelines of DCUs are soaked in 250 mL chlorine disinfectant (500 mg/L available chlorine). The preliminary experiment above lasts for 5 days.

As for the duration of the experiment for group B1, B2, and B3, it is extended to as long as 4 weeks. B1 (control group, $n = 5$), pipelines of DCUs are immersed with 1:100 multi-enzyme detergent plus 3% H_2O_2. B2 (experimental group, $n = 5$), pipelines are soaked with the 1:50 diluent of double-chain quaternary ammonium

salt disinfectant. B3 (control group, $n = 5$), pipelines of DCUs are soaked in 250 mL chlorine disinfectant (500 mg/L available chlorine).

At the end of each half-day work, each DCU was disinfected. Before each sampling, the articulation buckle was disinfected and replaced with a brand-new suction pipeline which was connected to be used after being disinfected.

The suction pipelines are cleaned by immersion. The disinfectants solution volume, room temperature and duration of disinfecting time of immersion are identical for all groups. The volume of the soaking solution is 250 ml, the soaking time is 30 min, and the action temperature is room temperature (approximately 25°C). After every immersion, 1000 mL tap water is pumped into the immersed pipelines to remove the disinfectants.

Laboratorial processing

During this study, the daily workload of each DCU and the pressure of negative pressure suction are kept almost the same, and the time of use is around 6 hours. Before each sampling, the connecting buckles are disinfected with 75% alcohol, and the new suction pipelines are replaced and connected for use after disinfection. The operator and assistant are through systematic stomatology training, and all experimental samples are taken by the same operator. Sampling time points: (1) baseline: dismantle the original negative-pressure pipeline and replace the negative-pressure saliva suction pipeline; (2) first time: after 5 days of continuous work and disinfection treatment; (3) second time: after 4 weeks of continuous work and disinfection; sampling once a week, before the opening of the clinic on Monday morning, dismantling the original saliva suction pipeline and replacing it with a brand-new saliva suction pipeline. The first to fifth days of the week were in working condition, and the sixth to seventh days were in non-working condition. The time point for each sample was 8:00 a.m. the following morning, and the pipeline was disassembled at the sampling time point and placed in a clean container for transfer to the laboratory.

Methods

Detection of plankton removal effect in negative pressure pipelines

(1) Dismantlement and replacement the suction pipelines: After the whole day working, researchers remove the negative pressure pipelines from the DCUs with sterile gloves and empty the liquid in the cavity of the pipelines which are afterwards transferred to the laboratory in sterilized containers.
(2) Detection of planktonic bacteria removal effect: The external surface of the suction pipelines is wiped with an antiseptic wipe for cleaning and disinfection, then wiped with sterile wet gauze infiltrated in sterilized PBS solution twice after 3 min. Adding 100 mL sterilized PBS solution into the HVE pipelines and 40 mL PBS is added to LVE pipelines which are gently shaking for 5 times and poured into the sterilized centrifuge tubes.
(3) Inoculation and culture of bacteria: the PBS solution of the control groups and the experimental groups swabbed above is diluted with a series of 10 times diluent. Suspension of all groups with appropriate dilution is selected to form the mixed solution, 0.1 mL of which is taken with a sterile straw, inoculated on the plate containing nutrient agar and then coated uniformly on the plate. The collected nutrient agar Petri dish (BKMAM) is inverted into a 37°C constant temperature incubator for bacterial colony culture, and the culture time is 48 hours.
(4) Bacterial colony counting: The number of bacterial colonies on the surface of each square centimeter of suction lumen is calculated (CFU/ml). Actual bacterial colony count (CFU/ml) = Visible colony count × Dilution ratio/Sampling area.

Detection of biofilm removal effect in negative pressure pipelines

(1) Interception of the negative pressure pipelines: The length of every negative pressure pipeline is 120 cm and about 10 cm at both ends are discarded while 100 cm in the middle would be reserved. The pipelines are cut into three sections taking 30 cm as one unit, then saved for future observations. All cutting steps are implemented by sterile surgical scissors.
(2) Scanning Electron Microscope (SEM) observation: the new negative pressure pipelines, the used negative pressure pipelines and the section of the negative pressure pipelines cleaned and disinfected by different methods are taken separately and placed in a glutaraldehyde solution with a temperature of 4°C and a concentration of 2.5% for 2 hours, and then rinsed with PBS solution with pH 7.2 three times, for 10 min each time. After that, the pipelines are dehydrated with acetone gradient (30%, 50%, 70%, 90%, 100%) for 10 min each time, and then replaced with isoamyl acetate, dried at the critical point of carbon dioxide, sprayed gold coating with ion, and finally observed under SEM (TESCAN, TESCAN MAIA 3 GMU, 5.92 mm).
(3) Biofilm swab collection: About 12 cm length of the end of the negative pressure pipelines are cut off by sterilized scissors. The orifices of

pipelines are penetrated into about 5 cm with sterilized cotton swabs, which are closely stuck to the walls of pipelines to rotate and wipe back and forth for 5 times and then taken out carefully to be put in 50 mL of sterile PBS solution. There is eddy vibration for 1 min and centrifugation at 12,000 rpm for 3 min for collected cotton swabs. In the end, the supernatant is discarded and the sediment is precipitated at the temperature of −20°C.

(4) Detection of biofilm biodiversity: The bacterial plaque precipitates obtained from the above steps are extracted for genomic DNA extraction. Second-generation sequencing technology is used to sequence 16S rDNA for prokaryotes, 18S rDNA for eukaryotes and ITS sequencing and target region amplicon sequencing for in-depth analysis of the distribution and changes in microbial diversity after disinfection treatment of the DCUs.

(5) Detection of multi-drug resistant bacteria in biofilms: The pipeline eluates and biofilm swabs collected in the previous steps are isolated and cultured, respectively, and drug sensitivity tests are performed to detect the distribution of multi-drug resistant bacteria in both samples.

Impact of different disinfection methods on air quality

Air around the opening of the pipelines is collected as sample for the experiment. A Plexiglas cover is made to cover the assistant position of the DCUs, sterilized and placed over the gateway of the high and low volume evacuator. The 9 cm diameter Petri dish (R2A) is opened and placed in the Plexiglas cover, left for 30 min, removed and placed upside down in a 37°C incubator for a 48-hour incubation period, then bacterial colonies are counted.

Corrosion effect of different disinfectants on pipe fittings

The disinfectants are conducted immersion experiments, the brand new pipeline fittings attached to the negative pressure system called moisture separation covers (made of PP-TD30) and several metal sheets (copper, stainless steel, pure aluminum, aluminum alloy) are soaked in the above four disinfectants for 3 months, the corrosion of the surface of the pipeline fittings is observed under SEM, the corrosion of the metal sheets is observed under the metallographic microscope, and the corrosion rate of moisture separation covers is weighed and calculated, so as to detect the influence of different disinfection methods on the corrosion effect of the negative pressure system. Disinfectants in group C1 are 1:100 multi-enzyme cleaning agents plus 3% H_2O_2, in group C2 is double-chain quaternary ammonium salt disinfectant stock solution, in group C3 is double-chain quaternary ammonium salt disinfectant diluent (1:50), in group C4 are chlorine disinfectants, and the corrosion of accessories and metal sheets after soaking in each group of disinfectant is observed.

Statistical analysis

The data are analyzed using SPSS 20.0 software. After testing for normal distribution and chi-square in groups A1, A2, A3, B1, B2, B3 and groups C1, C2, C3, C4, one-way analysis of variance (ANOVA) is applied to those data that met the normal distribution; within-group comparisons are statistically analyzed using the LSD method ($\alpha = 0.05$). In the statistical analysis, differences are considered statistically significant when $p < 0.05$ and mean ± standard deviation are used to express the number of bacterial colonies in the suction duct.

Results

Double-chain quaternary ammonium salt disinfectant appears effective in disinfecting plankton in negative pressure pipelines. Sterilized PBS solution is added to the tubes as eluent, diluted and inoculated in nutrient agar Petri dishes, then bacterial colonies are incubated in a constant temperature incubator for 48 hours, and the formula mentioned above is applied to derive the number of bacterial colonies per square centimeter of the lumen surface of the suction pipelines. The treatment lasted for 5 days, results of which are shown in Table 1. The results reveal that no bacterial growth is found out in the HVE and LVE pipelines disinfected with double-chain quaternary ammonium salt disinfectant stock solution.

In the further experiment, the results after 4 weeks of treatment with double-chain quaternary ammonium salt disinfectant diluents are shown in Table 1.

Double-chain quaternary ammonium salt disinfectant appears effective in removing biofilm in negative pressure pipelines as well. The new negative pressure pipelines, used negative pressure pipelines, the cleaned and disinfected negative pressure pipelines with different treatment methods are separately taken and disposed, thus the biofilms in the pipelines are observed by SEM, which illustrates a visual growth of bacteria in biofilms in negative pressure pipelines. Figure 1 reveals the comparison between the three experimental groups with brand new and the used pipelines without disinfection treatment. It is found that the bacteria in the HVE negative pressure pipelines grow well and are not significantly damaged in group A1 and A3 during the 5 days experiment, while the biofilms in the HVE pipelines treated with double-chain quaternary ammonium

Table 1. Counting of bacterial cultures in eluates of pipelines disinfected by group A1, A2, A3 for 5 days and by group B1, B2, B3 for 4 weeks.

Groups	HVE (CFU/ml)	LVE (CFU/ml)
A1	0	11.53±10.61
A2	0	0
A3	0	0.99±0.30
B1	15.23±37.30	1216.02±1861.75
B2	0	43.51±19.97
B3	0.053±0.14	1401.84±637.85

A1 (control group, $n = 5$), 1:100 multi-enzyme detergent plus 3% H_2O_2, 5 days. A2 (experimental group, $n = 5$), original solution of double-chain quaternary ammonium salt disinfectant, 5 days. A3 (experimental control group, $n = 5$), chlorine disinfectant (500 mg/L available chlorine), 5 days. B1 (control group, $n = 5$), 1:100 multi-enzyme detergent plus 3% H_2O_2, 4 weeks. B2 (experimental group, $n = 5$), double-chain quaternary ammonium salt disinfectant diluent (1:50), 4 weeks. B3 (control group, $n = 5$), chlorine disinfectant (500 mg/L available chlorine), 4 weeks. Groups A1, A3, B1, and B3 are control groups, and groups A2 and B2 are experimental groups. ** p(A1-LVE, A2-LVE) <0.05, p(A1-LVE, A3-LVE) <0.05, p(B2-LVE, B3-LVE) <0.05.

salt disinfectant stock solution show a reduction in bacterial species, number, structure with a severe biofilm structure damage. In the LVE pipelines, bacteria in groups A1 and A3 still grow better than in group A2, and biofilms in groups A1 and A3 are relatively well structured compared to the LVE pipelines treated with double-chain quaternary ammonium salt disinfectant stock solution for 5 days (Figure 1).

The SEM images (Figure 1) of the effect of the different disinfection methods on the removal of biofilm from the negative pressure pipelines over a 4-week trial. The results show a reduction in bacterial species, numbers and structure in the biofilms of both HVE and LVE pipelines disinfected with double-chain quaternary ammonium salt disinfectant diluents.

Differences in biofilm diversity in the pipeline wall under different disinfection methods are illustrated in Figure 2. α-diversity is an indicator of species in terms of richness, diversity and evenness in locally homogeneous habitats, also known as intra-habitat diversity, expressed using the Chao1 index (richness) and the Shannon index (diversity). Results show that after 5 days of disinfectant treatment, the α-diversity of the double-chain quaternary ammonium salt disinfectant stock solution group (O5q, O5r) is higher than that of the 500 mg/L chlorinated disinfectant group (C5q, C5r) in the biofilm flora in HVE and LVE pipelines, which have statistically significant differences, but there is no statistically difference from that of the 1:100 multi-enzyme detergent + 3% H_2O_2 (Meh5q, Meh5r) in Figure 2a. After 28 days of disinfectant treatment, the Shannon index is higher and statistically different in the HVE pipeline biofilm flora in the 1:100 multi-enzyme cleaner + 3% H_2O_2 group (Meh28q) than in the 500 mg/L chlorine-containing disinfectant group (C28q), but not in the rest of the groups (Figure 2a). Figure 3b illustrates the β-diversity and shows that after 5 days of disinfectant treatment, the microbial community composition differs more in the HVE pipeline biofilm flora in the double-chain quaternary ammonium salt disinfectant group (O5q) versus the 500 mg/L chlorinated disinfectant group (C5q) samples, and less in the 1:100 multi-enzyme cleaner + 3% H_2O_2 (Meh5q) versus O5q and C5q. After 5 days of disinfectant treatment, the microbial community composition differs more between the double-chain quaternary ammonium salt disinfectant group (O5r) and the 500 mg/L chlorinated disinfectant group (C5r) samples and less from the 1:100 multi-enzyme cleaner + 3% H_2O_2 group (Meh5r) in the LVE pipeline biofilm community. However, no statistical difference between the groups exists in the 28-day disinfection experiment. LEfSe (LDA Effect Size) analysis emphasizes the search for robustly differential species between subgroups. In the 5-day disinfection experiment, the large abundance of bacteria exists in the double-chain quaternary ammonium salt disinfectant stock solution group (O5q, O5r), while in the 28-day disinfection experiment, the large abundance of bacteria exists in the 1:100 multi-enzyme cleaner + 3% H_2O_2 group (Meh5r). These results have no statistical significance (Figure 2c).

For the detection of multi-drug resistant bacteria in the biofilm, the pipeline eluates and biofilm swabs collected are isolated and cultured in several, and drug sensitivity tests are performed to detect the distribution of multi-drug resistant bacteria in both samples. The results of each group of HVE and LVE pipelines are illustrated in Table 2. After the disinfection of HVE and LVE pipelines with double-chain quaternary ammonium salt disinfectant original solution for 5 days, the bacterial species in the biofilms are Bacillus spp. and most of which are environmentally benign microorganisms. While in the eluates of these pipelines, there are no bacteria cultured. After 4 weeks of disinfection of the HVE and LVE pipelines with double-chain quaternary ammonium salt disinfectant dilution, no multi-drug resistant bacteria are detected in biofilms and eluates while multiple Staphylococcus aureus is detected in biofilms of LVE pipelines treated with chlorinated disinfection solution (Table 2).

Air samples around the suction pipelines show a significant reduction or even absence of airborne colonies. As shown in Figure 4a after 5 days of treatment, no significant colonies are found in group A2 (both HVE and LVE) treated with double-chain quaternary ammonium salt disinfectants at stock concentration compared to groups A1 and A3. The results of the experiment after 4 weeks of treatment are shown in Figure 3b. It shows that no significant colonies are found in the HVE pipelines treated with double-chain quaternary ammonium salt disinfectant diluents, but colonies are still present in the LVE pipelines.

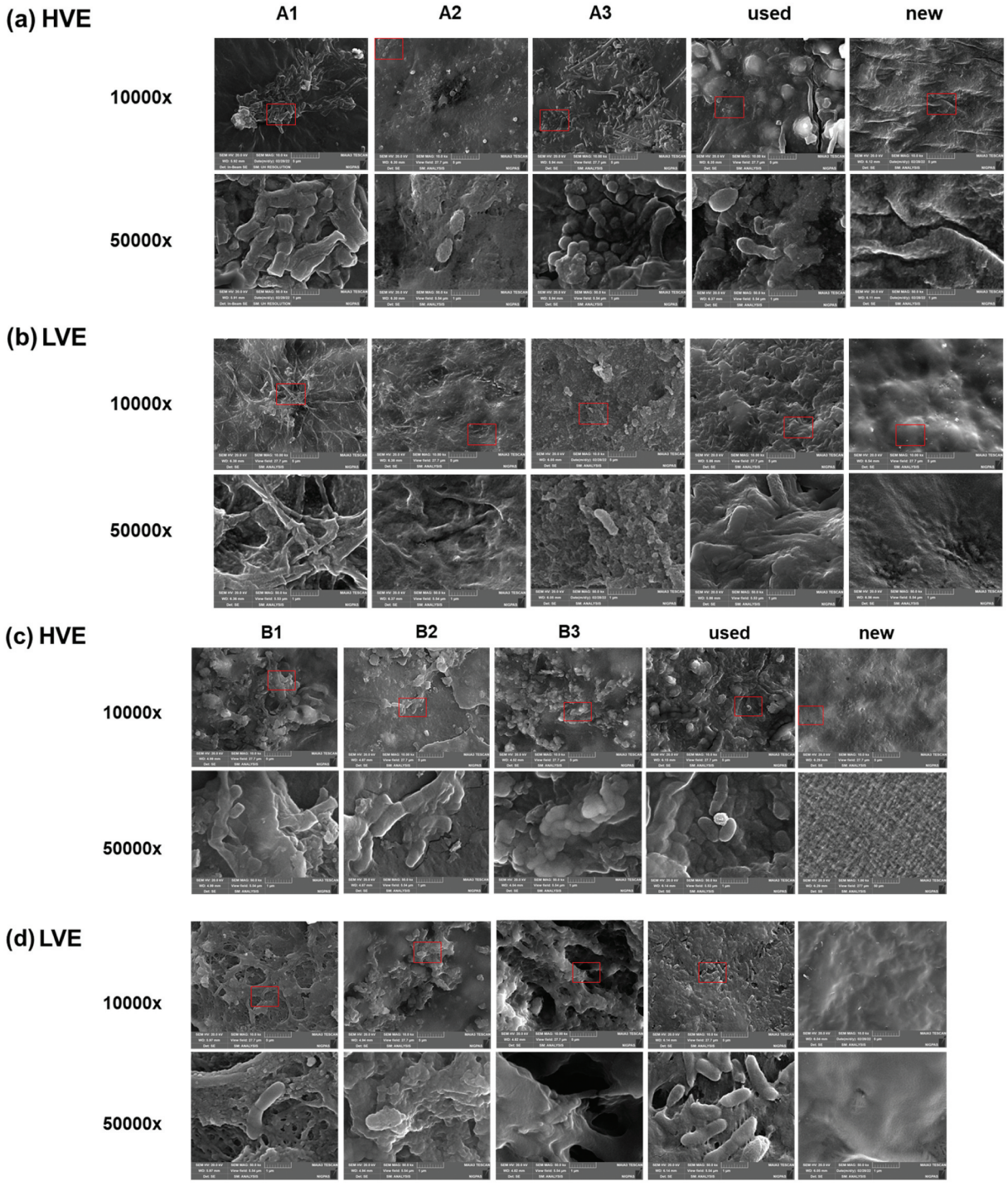

Figure 1. Bacteria species, number, and structure in biofilms of pipelines which are new, used or disinfected by different methods are observed under SEM. (a) Biofilms in the HVE pipelines. (b) Biofilms in the LVE pipelines. (c) Biofilms in the HVE pipelines. (d) Biofilms in the LVE pipelines. A1 (control group, *n*=5), 1:100 multi-enzyme detergent plus 3% H_2O_2, 5 days. A2 (experimental group, *n*=5), original solution of double-chain quaternary ammonium salt disinfectant, 5 days. A3 (experimental control group, *n*=5), chlorine disinfectant (500mg/L available chlorine), 5 days. B1 (control group, *n*=5), 1:100 multi-enzyme detergent plus 3% H_2O_2, 4 weeks. B2 (experimental group, *n*=5), double-chain quaternary ammonium salt disinfectant diluent (1:50), 4 weeks. B3 (control group, *n*=5), chlorine disinfectant (500mg/L available chlorine), 4 weeks. The area in the red box of the image at 10000x is magnified to be viewed at 50000x.

The results below show that the double-chain quaternary ammonium disinfectant has the least corrosive effect on various pipeline fittings. Moisture separation covers are taken as samples, soaked in four disinfectants to detect the influence of different disinfection methods on the corrosion effect of the negative pressure system in this study. Findings including the weights and images of corrosion effects in Table 3 and Figure 4 suggest that the double-chain quaternary ammonium salt disinfectant diluent has the weakest corrosion effect on the negative pressure system, while the 500 mg/L chlorine-containing disinfectant is opposite.

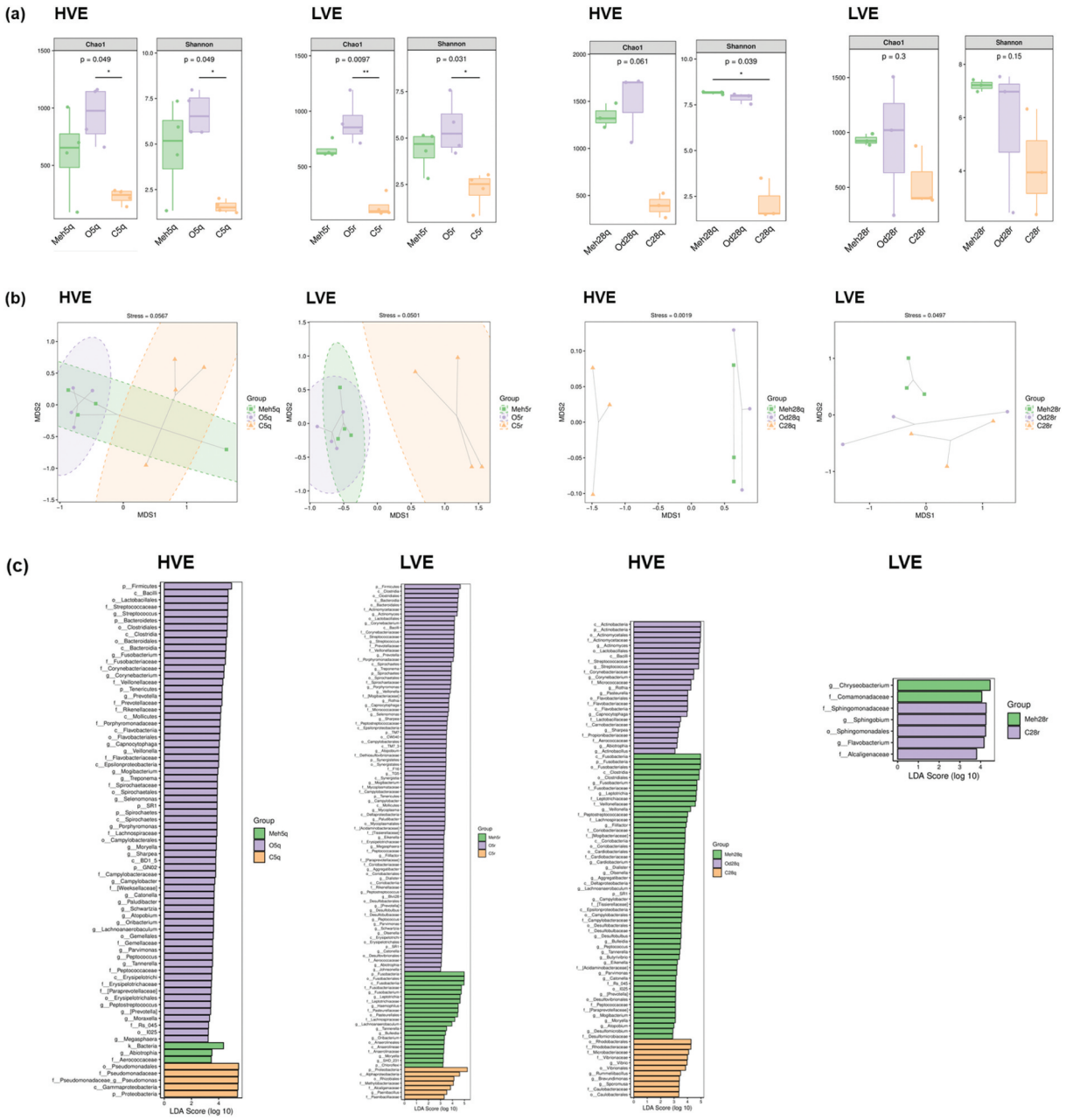

Figure 2. Gene sequencing results for biofilms of pipelines. (a) α-diversity: Chao1 index (abundance) and Shannon index (diversity) of biofilms in pipelines under different disinfection methods. (b) β-diversity: graph of NMDS analysis results. (c) LEfSe (LDA effect size) analysis: means of finding bacteria that differ between groups. *Meh5q and Meh5r (control group), HVE and LVE pipelines disinfected by 1:100 multi-enzyme detergent plus 3% H_2O_2 for 5 days. O5q and O5r (experimental group), HVE and LVE pipelines disinfected by original solution of double-chain quaternary ammonium salt disinfectant for 5 days. C5q and C5r (control group), HVE and LVE pipelines disinfected by chlorine disinfectant (500mg/L available chlorine) for 5 days. Meh28q and Meh28r (control group), HVE and LVE pipelines disinfected by 1:100 multi-enzyme detergent plus 3% H_2O_2 for 28 days. Od28q and Od28r (experimental group), HVE and LVE pipelines disinfected by double-chain quaternary ammonium salt disinfectant diluent (1:50) for 28 days. C28q and C28r (control group), HVE and LVE pipelines disinfected by chlorine disinfectant (500mg/L available chlorine) for 28 days.

As shown in Figure 5a, after soaking in the disinfectants for 3 months, the appearance of the metal sheets has changed. In group C4, except for the stainless-steel sheet, other metal sheets treated with chlorine-containing disinfectant have different degrees of corrosion. Under the metallographic microscope, it is found that the chlorine-containing disinfectant has a strong corrosion effect on the copper sheet, the metal structure has been damaged evenly and in a large amount, the copper sheet after hydrogen peroxide treatment is mostly corroded, while the double-chain quaternary ammonium salt disinfectant including the original solution and diluent has a slight corrosion effect on the copper sheet, and only local fine structure has been damaged. Four groups of disinfectants on stainless steel sheet show no corrosion effect. In contrast, pure aluminum and aluminum alloys disinfected with double-chain

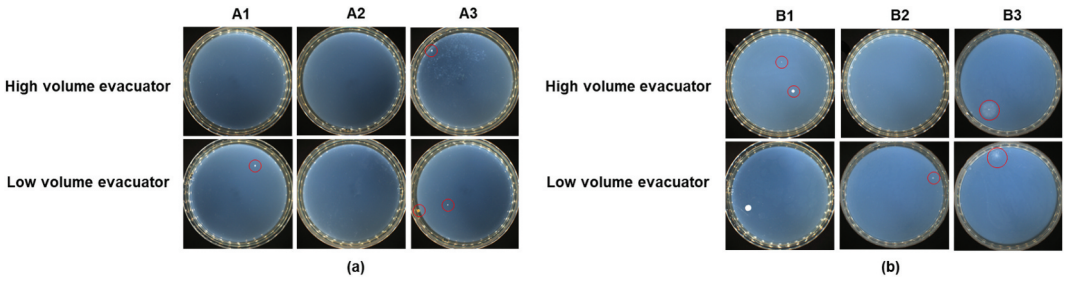

Figure 3. Influence of different disinfection methods on air quality. (a) Air samples taken in pipes disinfected by stock double-chain quaternary ammonium salt disinfectant for 5 days. (b) Air samples taken in pipes disinfected by double-chain quaternary ammonium salt disinfectant dilution for 4 weeks.

Table 2. Bacterial culture results of biofilms and eluates from pipelines with different disinfection methods.

	Biofilm		Eluate	
Groups	HVE	LVE	HVE	LVE
A1	*Lysinibacillus sphaericus*	*Pseudomonas aeruginosa*, *Lysinibacillus sphaericus*	None	*Enterobacter aerogenes*, *Comamonas testosterone* (resistant to gentamicin), *Citrobacter frazier*, *Pseudomonas aeruginosa*
A2	*Lysinibacillus sphaericus*	*Brevibacillus parabrevis*	None	None
A3	*Lysinibacillus sphaericus*	*Lysinibacillus boronitolerans*, *Brevibacillus agri*	None	*Pseudomonas putida*
B1	*Lysinibacillus macrolides*, *Paenibacillus ehimensis*	*Escherichia coli*, *Klebsiella aerogenes*	*Stenotrophomonas maltophilia*	*Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*
B2	*Lysinibacillus fusiformis*, *Pseudomonas putida*	*Pseudomonas aeruginosa*, *Brevibacillus agri*	*Pseudomonas putida*	*Pseudomonas aeruginosa*
B3	*Klebsiella pneumoniae*, *Paenibacillus ehimensis*	*Brevibacillus agri*, *Staphylococcus aureus*, *Paenibacillus ehimensis*	*Pseudomonas aeruginosa*, *Pseudomonas putida*	*Pseudomonas putida*

A1 (control group, n = 5), 1:100 multi-enzyme detergent plus 3% H_2O_2, 5 days. A2 (experimental group, n = 5), original solution of double-chain quaternary ammonium salt disinfectant, 5 days. A3 (experimental control group, n = 5), chlorine disinfectant (500 mg/L available chlorine), 5 days. B1 (control group, n = 5), 1:100 multi-enzyme detergent plus 3% H_2O_2, 4 weeks. B2 (experimental group, n = 5), double-chain quaternary ammonium salt disinfectant diluent (1:50), 4 weeks. B3 (control group, n = 5), chlorine disinfectant (500 mg/L available chlorine), 4 weeks.

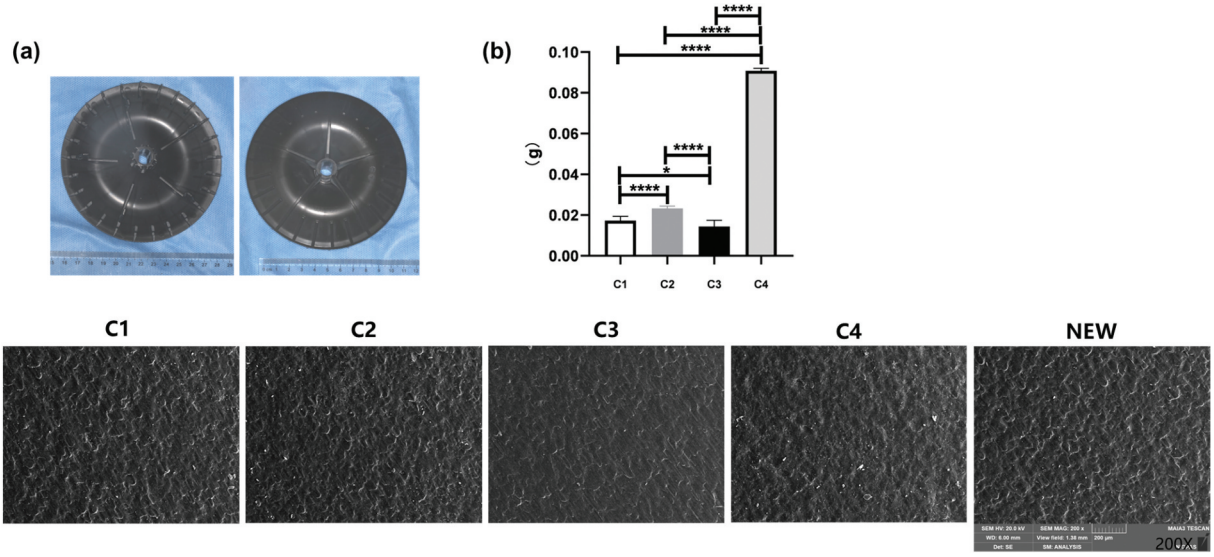

Figure 4. Corrosion effects of different disinfectants on the pipeline fittings. (a) A new pipeline fitting attached to the negative pressure system called moisture separation cover (made of PP-TD30). (b) Weights of corrosion effects of different disinfectants on moisture separation covers. (c) SEM images of the new and disinfected moisture separation covers. One asterisk indicates a significant difference from all other groups ($p < 0.05$), four asterisks indicate a significant difference from all other groups ($p < 0.0001$).

Table 3. Weights of corrosion effects of different disinfectants on moisture separation covers.

Groups	Corrosion in negative pressure systems (g)
C1 1:100 Multi-Enzyme Cleaners+3%H_2O_2	0.0173±0.0019
C2 Double-chain quaternary ammonium salt disinfectant stock solution	0.0233±0.0011
C3 Double-chain quaternary ammonium salt disinfectant diluent (1: 50)	0.0144±0.0029
C4 Chlorine disinfectant (500mg/L available chlorine)	0.0908±0.0011

Groups C1 and C4 are control groups, groups C2 and C3 are experimental groups.

quaternary ammonium salt disinfectant have more complete and rich metal structure, while chlorine-containing disinfectant has stronger metal corrosiveness and more metal structure damage (Figure 5b).

Discussion

The ideal method [16] for cleaning and disinfecting negative pressure pipelines should meet the following requirements: (1) removal and killing of planktonic microorganisms in the pipelines effectively; (2) decomposition of proteins on the inner wall of the pipelines effectively; (3) removal of biofilm on the inner wall of the pipelines and prevention of biofilm generation effectively; (4) low froth, which may avoid blockage of the negative pressure pipelines and gas backflow; (5) no or little corrosive effect on the pipeline fittings; (6) low cost, easy to operate and maintain good compliance.

Nevertheless, many disinfectants cannot meet the requirements of biofilm removal and may at risk of causing cross-infection, occupational exposure of doctors and nurses, pipeline blockage, gas backflow, corrosion of the accessories of devices, and accelerate the loss of DCUs. Therefore, there is a need to find a disinfectant that is more efficient in removing biofilm and is friendly to pipeline fittings to clean and disinfect pipelines, in order to control cross-infection in dental practice and to solve the problem of the difficult disinfection of DCUs.

By observing the effects of different intervention measures on the number of plankton in the negative pressure pipelines of DCUs, the explorations of this study are threefold: 1) The influence on plaque biofilm structure, microbial diversity and multi-drug resistant bacteria on the inner wall of the negative pressure suction pipelines of DCUs. 2) The effects of different cleaning and disinfection methods on the removal of biofilm. 3) The prevention of biofilm generation in the negative pressure pipelines of DCUs. The data show that less bacterial growth is detected in the eluates of evacuators treated by double-chain quaternary ammonium salt disinfectants compare with the other two disinfectants, of which the species, number and structure of bacteria in the biofilms are all reduced and destroyed. The results demonstrate that the effect of double-chain quaternary ammonium salt disinfectants is more significant than hydrogen peroxide and chlorinated disinfectants in biofilm removal and inhibition of biofilm formation in this study. While in the pipelines disinfected by double-chain quaternary ammonium salt disinfectants, multi-drug resistant bacteria can hardly be detected, indicating their capacity of multi-drug resistant bacteria of planktonic microbial and biofilm. As for the effect of double-chain quaternary ammonium salt disinfectants on air quality, no or little bacterial colonies are detected in the pipelines disinfected by double-chain quaternary ammonium salt disinfectants, while the pipelines disinfected by other two disinfectants have more obvious bacterial colonies. This shows that double-chain quaternary ammonium salt disinfectants can significantly improve air quality in the clinics.

In our study, compared with hydrogen peroxide disinfectants, double-chain quaternary ammonium salt disinfectants cause less clogging in the suction pipelines and spittoon down water pipelines, avoiding the gas reflux. Compared to chlorine-containing disinfectants, the removal of biofilm effect of double-chain quaternary ammonium salt disinfectants is more significant, and less corrosive effect to fittings, greatly prolongating the service life of pipelines.

It was mentioned in previous research [17] that although QACs are lethal to a wide variety of organisms, including vegetative cells of gram-positive and gram-negative bacteria, fungi, parasites (e.g. *Leishmania major, Plasmodia falciparum*), and lipophilic (enveloped) viruses, they are generally not considered sporicidal (e.g. *Bacillus subtilis, Clostridium sporogenes*), tuberculocidal (e.g. *Mycobacterium tuberculosis, Mycobacterium bovis*) or virucidal against hydrophilic (non-enveloped) viruses (e.g. *Coxsackievirus, Rhinovirus*). This may explain why the high detection rate of *Lysinibacillus* and *Brevibacillus* in appeared in biofilms and eluates. As far as the presence of *Pseudomonas aeruginosa* is concerned, the resistance to quaternary ammonium disinfectants is indeed an urgent problem which was mentioned in previous studies [17,18]. Additionally, the study [19] illustrated its unsatisfactory performance in this area. Our team will also focus on this aspect of research next, after all, this is a problem that cannot be ignored. Emmanuel [20] clarified that an interconnection between the oral and gut microbiomes has been demonstrated, so it is not surprising that samples of pipelines of DCUs in periodontology found enterobacteria in this study. Recently, many

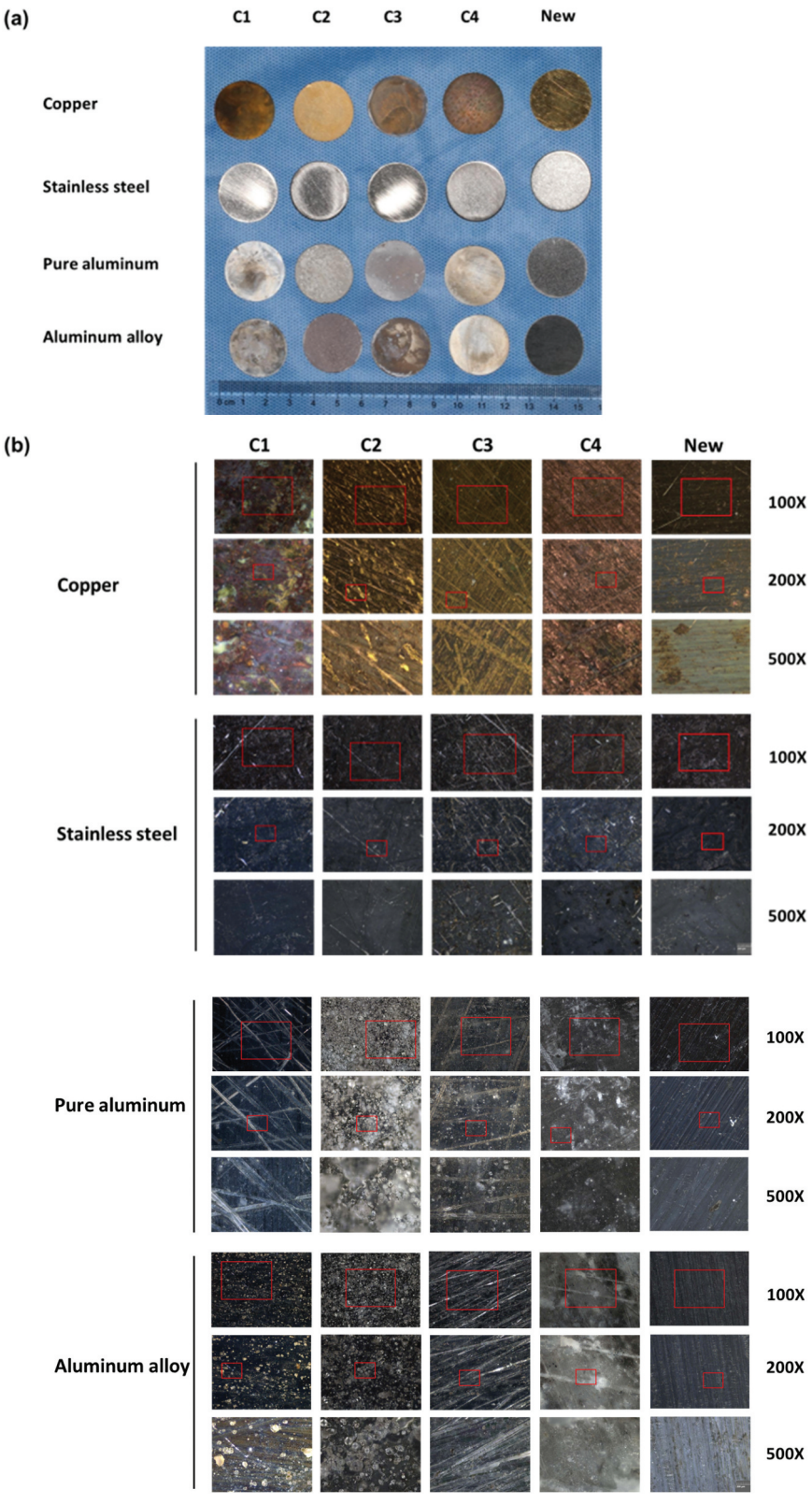

Figure 5. Observation of metal sheets immersed in different disinfectants. (a) Appearance of new metal sheets and metal sheets soaked in four disinfectants for 3 months. (b) Surface of the metal sheets observed under SEM. *C1 (control group), 1:100 multi-enzyme cleaners+3% H_2O_2, 3 months. C2 (experimental group), double-chain quaternary ammonium salt disinfectant stock solution, 3 months. C3 (experimental group), double-chain quaternary ammonium salt disinfectant diluent (1:50), 3 months. C4 (control group), chlorine disinfectant (500mg/L available chlorine), 3 months. The area in the red box of the image is magnified to be observe with a larger field of view.

researches [21] have proved that the human body is a complex connected body, and there are many intestinal flora in the oral cavity. It was found that the long-term DCU leaked due to corrosion of negative pressure pipeline fittings, and opportunistic pathogenic bacteria such as *Pseudomonas aeruginosa* were isolated at the interface of the corroded pipeline, with a positive rate of up to 61% [1]. Becoming

a possible source of cross infection in oral diagnosis and treatment. *Pseudomonads* are ubiquitous in pipelines, metals, and water. However, they can also be found in oral cavities, as clarified in the study [22].

Quaternary ammonium compounds (QACs), commonly used in cleaning, disinfecting, and personal care products, have recently gained worldwide attention due to the massive use of disinfectants during the COVID-19 pandemic [23]. Nevertheless, the clinical application of double-chain quaternary ammonium salt disinfectant in dental is not general. Although the double-chain quaternary ammonium salt disinfectant belongs to the fourth generation of new quaternary ammonium salt disinfectant with surface activity, and it has many unique advantages compared with the previous traditional quaternary ammonium salt disinfectants, its shortcomings and potential menace to be evaluated still cannot be ignored. It has been mentioned in Boyce's review [24] that although toxicity tests have been conducted extensively in industries and regulated in various legislations, the risks of exposure to multiple types of QAC are still fragmentary, likewise for long-term exposure in health effects. Also, the mass load of QACs at a WWTP has increased by 331% during COVID-19 compared to before COVID-19 in Alygizakis's research [25], and this was a wake-up call. Apart from this, there are growing concerns about environmental exposure associated with such disinfectants, particularly to aquatic and benthic animals and humans [26]. The contamination of QACs in the environment is a forefront challenge in mediating the spread of antimicrobial resistance (AMR), and hence there is an urgent need to conduct a robust risk assessment to evaluate both human and aquatic health risks associated with QACs and AMR [27].

In summary, double-chain quaternary ammonium salt disinfectant has the effect of removing biofilm and preventing the formation of biofilm, reduces the adverse effect of negative pressure suction system on air pollution in the clinics in dental treatment, and has a minimal corrosive effect on pipeline fittings of DCUs. Owing to these properties of double-chain quaternary ammonium salt disinfectant, it can be used as an alternative option for cleaning and disinfecting the negative pressure suction pipelines of DCUs to control cross infection during dental treatment, and to reduce the air contamination in the treatment unit and decrease the potential occupational exposure of dental workers. However, the limitation of this study is the small sample size and the lack of studies on drug resistance. It is known from the literature [21] that bacteria are able to build up resistance to quaternary ammonium compounds quite quickly. This aspect should be discussed and has been taken into account in our following studies. The specific biocides and doses used to control a planktonic population are significantly different from the specific biocides and doses used to eliminate a particular biofilm matrix [28]. Care must be taken in choosing any chemical disinfectant introduced into the system. Moreover, handy treatment essentially decreases the time allocated for disinfection procedures and increases the cost-effectiveness are more likely to achieve the desired compliance.

Conclusion

Disinfection of negative pressure suction pipeline in DCUs plays a vital role in inhaling aerosols and fluids generated during dental care. In this study, the double-chain quaternary ammonium salt disinfectant has significant bactericidal ability and anti-biofilm effect, and is less corrosive to pipeline fittings. This means that the double-chain quaternary ammonium salt disinfectant is expected to be a promising disinfectant for negative pressure suction system to control cross infection during dental care.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Supported by: National Natural Science Foundation of China [82101018]; Jiangsu Province Capability Improvement Project through Science, Technology and Education-Jiangsu Provincial Research Hospital Cultivation Unit [YJXYYJSDW4], Jiangsu Provincial Medical Innovation Center [CXZX202227].

References

[1] O'Donnell MJ, Tuttlebee CM, Falkiner FR, et al. Bacterial contamination of dental chair units in a modern dental hospital caused by leakage from suction system hoses containing extensive biofilm. J Hosp Infect. 2005;59(4):348–360. doi: 10.1016/j.jhin.2004.10.001

[2] Harrel SK, Molinari J. Aerosols and splatter in dentistry. J Am Dent Assoc. 2004;135(4):429–437. doi: 10.14219/jada.archive.2004.0207

[3] Mann GLB, Campbell TL, CRAWFORD JJ, et al. Backflow in low-volume suction lines: the impact of pressure changes. J Am Dent Assoc. 1996;127(5):611–615. doi: 10.14219/jada.archive.1996.0273

[4] Watson CM, Whitehouse RLS. Possibility of cross-contamination between dental patients by means of the saliva ejector. J Am Dent Assoc. 1993;124(4):77–80. doi: 10.14219/jada.archive.1993.0080

[5] Choudhary S, Durkin MJ, Stoeckel DC, et al. A comparison of aerosol mitigation strategies and aerosol persistence in dental environments. Dentistry And Oral Medicine. 2021. doi: 10.1101/2021.07.30.21261399

[6] Harrel SK, Barnes JB, Rivera-Hidalgo F, et al. Reduction of aerosols produced by ultrasonic sealers. J Periodontol. 1996;67(1):28–32. doi: 10.1902/jop.1996.67.1.28
[7] Allison JR, Dowson C, Jakubovics NS, et al. Waterline disinfectants reduce dental bioaerosols: a multitracer validation. J Dent Res. 2022;101(10):1198–1204. doi: 10.1177/00220345221093522
[8] Kruger TIM, Herzog S, Mellmann A, et al. Impact of chlorine dioxide on pathogenic waterborne microorganisms occurring in dental chair units. Microorganisms. 2023;11(5):1123. doi: 10.3390/microorganisms11051123
[9] Boyle MA, O'Donnell MJ, Russell RJ, et al. Overcoming the problem of residual microbial contamination in dental suction units left by conventional disinfection using novel single component suction handpieces in combination with automated flood disinfection. J Dent. 2015;43(10):1268–1279. doi: 10.1016/j.jdent.2015.07.018
[10] Gungor ND, Kadaifciler DG, Peker OÖ, et al. Investigation of the bacterial load and antibiotic susceptibility of dental units. Environ Monit Assess. 2014;186(3):1847–1853. doi: 10.1007/s10661-013-3498-3
[11] O'Donnell MJ, Shore AC, Coleman DC, et al. A novel automated waterline cleaning system that facilitates effective and consistent control of microbial biofilm contamination of dental chair unit waterlines: a one-year study. J Dent. 2006;34(9):648–661. doi: 10.1016/j.jdent.2005.12.006
[12] Kostakioti M, Hadjifrangiskou M, Hultgren SJ, et al. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the post antibiotic era. Cold Spring Harb Perspect Med. 2013;3(4):a010306–a010306. doi: 10.1101/cshperspect.a010306
[13] Davies D. Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov. 2003;2(2):114–122. doi: 10.1038/nrd1008
[14] Linger JB, Molinari JA, FORBES WC, et al. Evaluation of a hydrogen peroxide disinfectant for dental unit waterlines. J Am Dent Assoc. 2001;132(9):1287–1291. doi: 10.14219/jada.archive.2001.0374
[15] Li Y, Li B, Guo X, et al. Applications of quaternary ammonium compounds in the prevention and treatment of oral diseases: state-of-the-art and future directions. J Dent. 2023;137:104678. doi: 10.1016/j.jdent.2023.104678
[16] Maciel Monteiro R, Oliveira VC, Galo R, et al. Protocol with non-toxic chemicals to control biofilm in dental unit waterlines: physical, chemical, mechanical and biological perspective. Biofouling. 2022;38(6):628–642.
[17] Jiao Y, Niu LN, Ma S, et al. Quaternary ammonium-based biomedical materials: state-of-the-art, toxicological aspects and antimicrobial resistance. Prog Polym Sci. 2017;71:53–90. doi: 10.1016/j.progpolymsci.2017.03.001
[18] Tabata A, Nagamune H, Maeda T, et al. Correlation between resistance of *Pseudomonas aeruginosa* to quaternary ammonium compounds and expression of outer membrane protein OprR. Antimicrob Agents Chemother. 2003;47(7):2093–2099. doi: 10.1128/AAC.47.7.2093-2099.2003
[19] Lineback CB, Nkemngong CA, Wu ST, et al. Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms than quaternary ammonium compounds. Antimicrob Resist Infect Control. 2018;7(1):154. doi: 10.1186/s13756-018-0447-5
[20] Albuquerque-Souza E, Sahingur SE. Periodontitis, chronic liver diseases, and the emerging oral-gut-liver axis. Periodontol 2000. 2022;89(1):125–141. doi: 10.1111/prd.12427
[21] Kapila YL. Oral health's inextricable connection to systemic health: special populations bring to bear multimodal relationships and factors connecting periodontal disease to systemic diseases and conditions. Periodontol 2000. 2021;87(1):11–16. doi: 10.1111/prd.12398
[22] Leao I, de Carvalho TB, Henriques V, et al. Pseudomonadota in the oral cavity: a glimpse into the environment-human nexus. Appl Microbiol Biotechnol. 2023;107(2–3):517–534. doi: 10.1007/s00253-022-12333-y
[23] Arnold WA, Blum A, Branyan J, et al. Quaternary ammonium compounds: a chemical class of emerging concern. Environ Sci Technol. 2023;57(20):7645–7665. doi: 10.1021/acs.est.2c08244
[24] Boyce JM. Quaternary ammonium disinfectants and antiseptics: tolerance, resistance and potential impact on antibiotic resistance. Antimicrob Resist Infect Control. 2023;12(1):32. doi: 10.1186/s13756-023-01241-z
[25] Alygizakis N, Galani A, Rousis NI, et al. Change in the chemical content of untreated wastewater of Athens, Greece under COVID-19 pandemic. Sci Total Environ. 2021 Dec 10;799:149230. Epub 2021 Jul 30. PMID: 34364275; PMCID: PMC8321698. doi: 10.1016/j.scitotenv.2021.149230
[26] Zheng G, Schreder E, Sathyanarayana S, et al. The first detection of quaternary ammonium compounds in breast milk: implications for early-life exposure. J Expo Sci Environ Epidemiol. 2022;32(5):682–688. doi: 10.1038/s41370-022-00439-4
[27] Mohapatra S, Yutao L, Goh SG, et al. Quaternary ammonium compounds of emerging concern: classification, occurrence, fate, toxicity and antimicrobial resistance. J Hazard Mater. 2023;445:130393. doi: 10.1016/j.jhazmat.2022.130393
[28] Yoshimura T, Chiba N, Matsuoka K, et al. Supra-long chain surfactants with double or triple quaternary ammonium headgroups. J Colloid Interface Sci. 2012;374(1):157–163. doi: 10.1016/j.jcis.2012.01.033