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Original Article

Bacteriostatic effects of nanometer silver disinfectant on the biofilms in dental unit water lines

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KEYWORDS
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Abstract *Background/purpose:* Dental unit water lines (DUWLs) may be contaminated by aerobic bacteria in clinical settings and comprehensive disinfecting methods should be considered without delay. Herein, this study aims to investigate the timeliness and dynamic bacteriostatic effects of different forms of nanometer silver (NMS) disinfectant on bio-film in DUWLs.
Materials and methods: Bacterial DUWLs samples were respectively treated with different NMS forms, including liquid phase and solid phase at the concentrations of 0.25%, 0.5%, 1% and 2% and their bacteriostatic effects were observed at the 1st, 4th, 7th, 14th, ^{28th} day.
Results: The bacteriostatic effects of liquid phase NMS at all concentrations were unsatisfactory and the bacteriostatic rate was only 20% at the 1st day. However, there appeared massive bacteria growth at the 4th, 7th, 14th, 28th day. Comparatively, no bacteria growth was found at the 1st, 4th, 7th, 14th, ^{28th} day after sterilizing with different concentrations of solid phase NMS and the bacteriostatic rate was 100%.
Conclusion: Microbial contamination in DUWLs can be disinfected by different NMS forms, among which solid phase NMS is more bactericidal against bacteria bio-films, demonstrating significant roles of solid phase NMS in preventing DUWL contamination.
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Introduction

In recent years, Hospital infections have become an common issue in clinical settings and may cause serious health problems in some immune compromised patients, including aged and organ transplantation patients.[1,2] Due to the necessity of water use in the dental treatment process, contamination of the bio-films in the dental unit water lines (DUWLs) has also emerged as a significant worldwide health problem, which may also cause environmental pollution in dental practice.[3,4] Thus, it is of great necessity to find some effective disinfectants to prevent DUWLs contamination. Indeed, some disinfection agents, including chemical disinfectants, have been used for DUWLs contamination in dental clinics and some satisfactory outcomes have been also obtained.[5,6] Although chemical disinfection is considered to be the most effective method to control microbial contamination, its harmful effects both on the waterway pipelines and patients can not be ignored.[7] Worse still, biofilm formed in the pipelines may not be completely sterilized by most chemical disinfectants, causing secondary infection or damage to the waterway pipelines.[8] During the accidental contamination of oral microorganisms, biofilms caused by the bacteria in DUWLs were considered as a new contamination source. In addition, due to the small diameter of DUWL tube, bio-films were easily formed in the inner of DUWL, causing potential contamination in the immune compromised hosts.[9]

With the development of nanotechnology, antibacterial materials containing nanometer silver (NMS) are widely used as an effective treatment method of infectious diseases.[10,11] Recently, it has been reported that NMS disinfectants not only have better to bacteriostatic effects when being used to wash the waterway pipelines, but also can effectively control the formation of bio-films.[12] Numerous nanosilver solutions are available in dentistry to reduce the microbial contamination in DUWLs for a duration of time, suggesting the popularity of this practice.[13] However, it is still not clear whether this disinfectant is safe enough for clinical use. In this study, different concentrations of liquid phase and solid phase NMS were applied for DUWLs contamination in order to investigate the timeliness and dynamic bacteriostatic effects of different forms of NMS disinfectants, thus to further explore appropriate disinfection methods and dosage. Furthermore, this disinfectant may provide more effective, safer and feasible disinfection methods for the regulation of DUWLs contamination, and may benefit both patients and medical staff.

Materials and methods

Ethical approval

Design of this study and management of the patient were in accordance with the Helsinki Declaration,[14] which was also approved by the Human Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Approval No: 20190131−3). All experiments were undertaken with the understanding and written consent of each subject and according to the above-mentioned principles. Written and signed informed consent was obtained from all patients or their legal custodies in their native language.

Sample collection

Liquid phase and solid phase NMS at different concentrations were obtained from (Beyotime Institute of Biotechnology, Jiangsu, China). Liquid samples were collected after oral care in the stomatological department and they were placed into 10 ml aseptic collecting tube (Beyotime), which were then mixed with liquid phase and solid phase NMS (Beyotime) at different concentrations within 1 h. Total bacteria number in the samples was counted under a time-phase microscope (Leica Microsystems, Wetzlar, Germany).

Sample culture

DUWLs samples from outpatient oral care patients were cultured in accordance with the germiculture method under strict aseptic conditions. Briefly, solid phase NMS were dissolved in Dulbecco's modififfied eagle medium (DMEM, Thermo Fisher Scientiffic, Waltham, MA, USA). Oral liquid samples were treated with liquid phase and solid phase NMS at the concentrations of 0.25%, 0.5%, 1%, 2% and maintained at 37 °C in the incubator. Dynamic bacteriostatic effects of different forms of NMS were observed at the 1st, 4th, 7th, 14th, ^{28th} day after sterilization. Bacterial colonies were counted under a time-phase microscope using bacterial counters (Beyotime). The GB5749-2006 Sanitary Standard for Drinking Water was used as reference in this study, which is qualified when the total bacteria number was not more than 100 cfu/ml.[15]

Statistical analysis

Statistical comparisons of parameters between groups were made by χ^2 test. $P < 0.05$ was considered statistically significant. Data analysis was conducted using SPSS 20.2 Software.

Results

Bacteriostatic effects of liquid phase of NMS on DUWLs

It was shown that bacteriostatic effects of liquid phase of NMS on the bio-films in DUWLs were far from unsatisfactory, which was only 20% at the 1st day. Although the number of bacteria was unlikely to count under the microscope, it was clear that there appeared vast number of bacteria growth at the 4th, 7th, 14th, 28th day, and the liquid phase of NMS lost their bacteriostatic roles at later times. Most importantly, the bacteriostatic effects of liquid phase of NMS on the contamination of DUWLs decreased with the prolongation of time, and there was no significant difference between different concentrations ($P > 0.05$) (shown in Table 1, Figs. 1, 2 and 3A).

Table 1 Comparison of antibacterial effects of two kinds of NMS on the reduction of contamination in DUWLs at different concentrations and different time points.

Concentration	Co-culture of DUWLs samples with liquid phase NMS					Co-culture of DUWLs samples with solid phase NMS				
	1st day	4th day	7th day	14th day	28th day	1st day	4th day	7th day	14th day	28th day
0.25%	20%	0	0	0	0	100%	100%	100%	100%	100%
0.5%	20%	0	0	0	0	100%	100%	100%	100%	100%
1%	20%	0	0	0	0	100%	100%	100%	100%	100%
2%	20%	0	0	0	0	100%	100%	100%	100%	100%

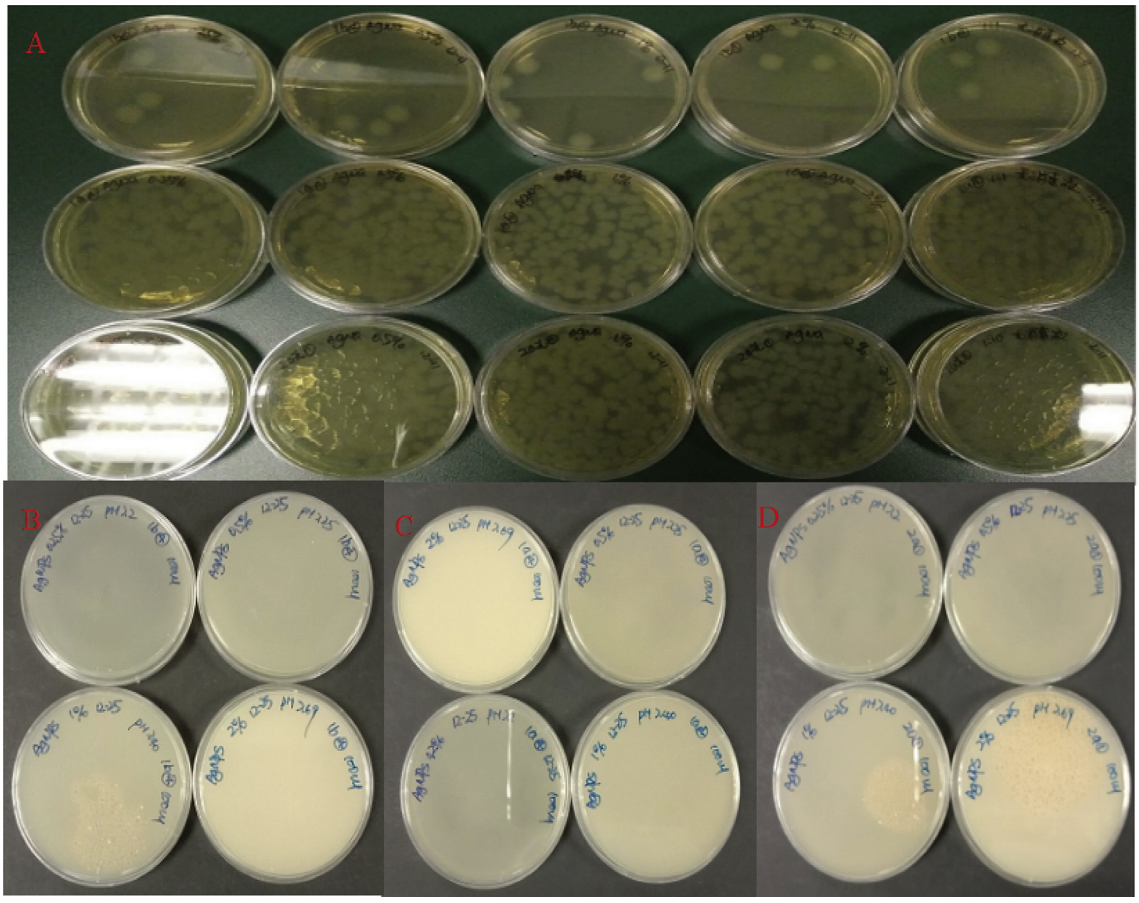

Figure 1 Morphological images of bacteria growth in two forms of NMS. (A) showing vast number bacteria growth after culturing the oral samples with liquid phase NMS. (B, C, D) demonstrating no bacteria growth after culturing oral samples with solid phase NMS.

Bacteriostatic effects of solid phase of NMS on DUWLs

Compared with the liquid phase of NMS, solid phase of NMS had strong bacteriostatic capacity to reduce the contamination in DUWLs. As anticipated, there was no bacteria growth at the 1st, 4th, 7th, 14th, 28th day of sterilizing with solid phase NMS, suggesting the long-term and safe anti-bacterial activity of solid phase NMS. There was significant difference in terms of bacteriostatic effects between different forms of NMS ($P < 0.001$). However, the differences between different concentrations of solid phase NMS was also not statistically significant ($P > 0.05$) (shown in Table 1, Figs. 1, 2 and 3B).

Discussion

DUWLs, as an important part of dental chair units, can play important roles in cooling and irrigating during dental treatment. Whereas, a variety of factors, including formation of bio-film, water pollution, back-suction and cross-infection of instruments, may contaminate DUWLs in dental clinics.[16,17] Bacteria flowing into the water may stay on the inner wall of DUWLs and further form bio-film through breeding in a humid environment, which is the main source of the continuous microbial contamination in the DUWLs.[18] Furthermore, the bacteria adhered to the inner wall and the formed bio-film are encircled by the extracellular matrix of biopolymer, which not only helps to enhance the

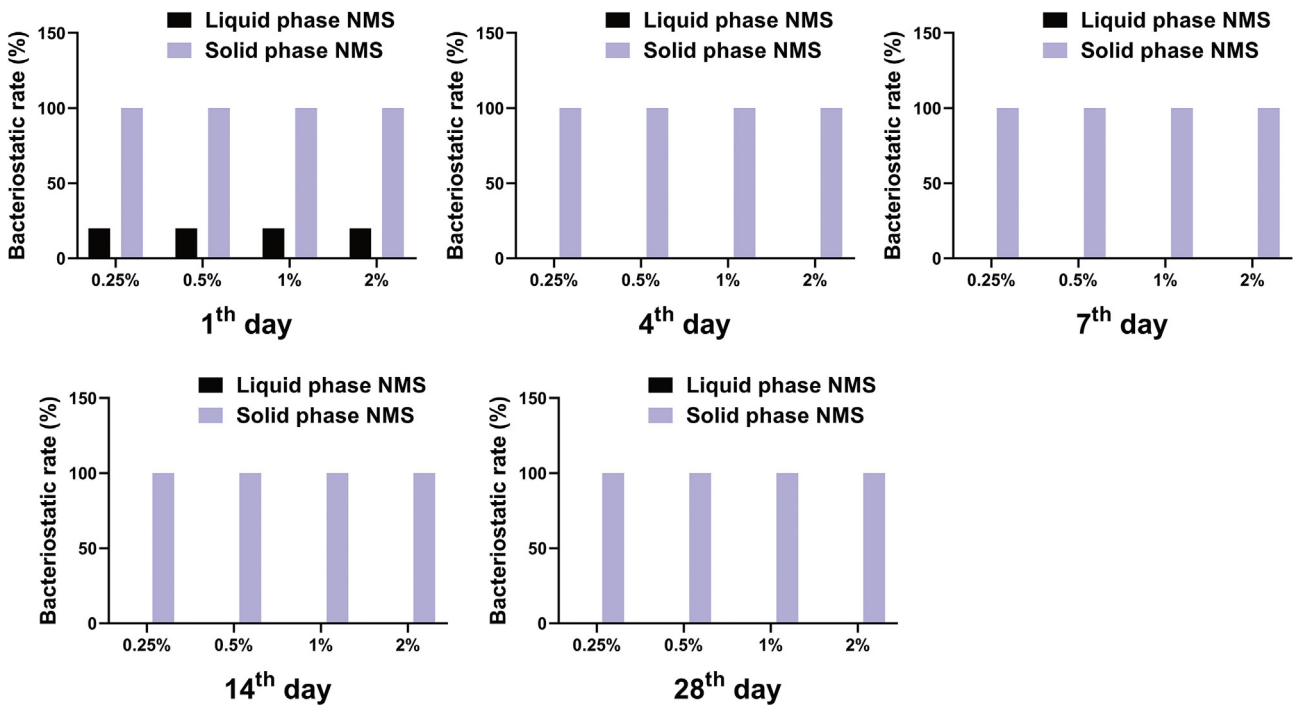

Figure 2 Antibacterial effects of two forms of NMS on DUWLs at different concentrations. There was significant difference in the bacteriostatic effects between different forms of NMS ($P < 0.01$). However, there was no statistical difference in the bacteriostatic effects between different concentrations of NMS ($P > 0.05$).

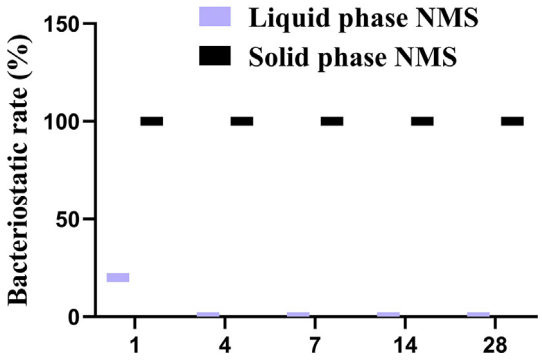

Figure 3 Antibacterial effects of two forms of NMS on DUWLs at different time points. There was no significant difference in the bacteriostatic effects of two forms of NMS at different time points ($P > 0.05$).

resistance of bacteria in bio-membrane against antibiotics and host immune defense, but also continues to become the main source of secondary contamination.[19] The most commonly used chemical disinfectants are as follows: hydrogen peroxide, hydrogen peroxide silver ion, sodium hypochlorite, chlorhexidine, micro-acidic electrolyzed water and so on.[20,6] However, long-term corrosion effects of some chemical disinfectants to the water channels and their further harm to the patients were ignored. According to the Consensus of American Air Force Association, it is clearly stated that sodium hypochlorite was not allowed to be used for disinfectants due to the blockage of water channels resulted from the long-term use of sodium hypochlorite disinfectants, thus largely reducing the life of equipment.[21]

NMS is a broad-spectrum antibacterial agent with high biological safety, which is also not easy to induce bacterial drug resistance, thus becoming the focus of antibacterial research fields.[22] Recent research results have shown that NMS has strong bactericidal effects on common bacteria, stubborn bacteria, drug-resistant bacteria and fungi, and can kill various harmful bacteria in a few minutes.[23,24] In addition, it has been shown that NMS also has exhibit powerful antibacterial activity to the colibacillus, green algae, salmonella, staphylococcus aureus, methicillin-resistant staphylococcus aureus, acinetobacter, candida albicans and candida albicans with the superiority of long-term effect, low toxicity, good bio-compatibility, thus, which is widely used in the clinical scenario in recent years.[25] Besides, NMS is also used in the process of wound closure, prosthesis, dental implantation and resin material, adhesive system.[26] In an artificial simulation experiment of waterway, the growth of bio-film was controlled by the NMS disinfectant. The results also showed that bacterial count declined to zero after disinfection with NMS, and that the bacterial concentration in the bio-film was obviously reduced by scanning electron microscope.[12] In another study, 0.1% NMS, 1.313% sodium hypochlorite and 0.9% sodium chloride were used to sterilize bio-membrane of enterococcus faecalis on the dentin surface. Comparatively, 0.1% NMS was detected to have a strong permeability and bactericidal effects on the enterococcus faecalis bio-film, whose effect was enhanced as disinfecting time extension and reached to the top after 24 h.[27]

In this current study, we analyzed antibacterial effects of two different forms of NMS disinfectants on DUWLs, and found that the antibacterial effect of liquid phase NMS was

not ideal. There still existed large amount of bacterial growth after co-culturing DUWLs samples with four different concentrations of liquid phase NMS, suggesting that effective components may be decomposed or some parts may be precipitated in the liquid phase NMS. Compared with liquid phase NMS, no bacteria growth was discovered at the different time points after co-culturing DUWLs samples with solid phase NMS, demonstrating its strong antibacterial effect. Thus, it is recommended for equipment manufacturers to cover the solid phase NMS onto the inner surface of water channel pipes for the purpose of preventing bio-film formation. If NMS was loaded into the nanometer tubes, NMS can control the releasing speed of silver, so that the long-term antibacterial effect may be achieved. Besides, propagation of bacteria on the surface of implants may also blocked, thus effectively preventing occurrence of peri-implantitis.[28] At the 28th day, there also existed few bacteria growth after culturing DUWLs samples with solid phase NMS, suggesting the bacteriostatic role of solid phase NMS rather than bactericidal roles. Most importantly, bacteria from DUWLs samples was cultured under aerobic environment and it may be unsuitable for other anaerobic bacteria growth. Thus, specific bacterial species available for solid phase NMS and disinfection effects of solid phase NMS in the water-path pipelines as well as its potential bacteriostatic effects on anaerobic bacteria still need to be further studied.

In summary, our data demonstrated that microbial contamination in DUWLs can be disinfected by different NMS forms. Among them, solid phase NMS is more efficient in preventing DUWL contamination. However, more studies are further needed to better illustrate the bactericidal effects of solid phase NMS on bacteria bio-films.

Declaration of Competing Interest

The authors declare that they have no competing interest.

Acknowledgements

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