Renal Replacement Therapy

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Effects of metal corrosion in the pump of a dialysis machine on the sterility of the terminal dialysate by spike-and-recovery testing with bacteria

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Abstract

Background Dialysis units have been concerned that the corroded metal parts of pumps used in hemodialysis might not allow sterility of the pump to be ensured due to bacterial contamination, even after cleaning and disinfection are performed after dialysis treatment. The purpose of this study was to clarify the effectiveness of the cleaning/disinfection process in eliminating bacterial contamination of the dialysate in pumps with and without metal corrosion.

Methods A suspension of *Pseudomonas aeruginosa* [10 colony-forming unit (CFU)/mL] was introduced into pumps without or with corrosion of the metal parts, and the flow in the dialysis circuit was stopped for 6, 12, or 18 h. Then, after cleaning and disinfection of the circuit with a sodium-hypochlorite-containing reagent, the amounts of live bacteria in the terminal dialysate and on the surface of the metal parts of the pump were counted.

Results Irrespective of the presence or absence of metal corrosion, bacteria were detected, even after cleaning and disinfection, on the surfaces of the pump parts that had been left in contact with the bacterial suspension for more than 12 h. However, on the surfaces of the pump parts showing metal corrosion, the bacterial numbers increased dramatically after 18 h of flow stoppage time following introduction of bacteria, and bacteria were even detected in the terminal dialysate despite cleaning/disinfection of the pump.

Conclusions Corrosion of the metal parts used in pumps used for dialysis increases the risk of bacterial contamination of not only the pump parts and flow path of the dialysis machine but also the terminal dialysate, even if cleaning/disinfection is performed. For sterility assurance of the dialysis circuit and dialysate during routine use, it is necessary to eliminate corrosion of the metal parts of dialysis pumps during scheduled maintenance.

Keywords Bacterial contamination, Sterility, Dialysate, Metal corrosion, *Pseudomonas aeruginosa*

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Background

Dialysis performed with good-quality dialysate improves various clinical conditions in dialysis patients [1, 2]. Including the international organization for standardization (ISO) [3], The Japanese Society for Dialysis Therapy and the Japanese Association of Clinical Engineers have proposed many updated guidelines for management and validation of dialysates [4–6]. To ensure the good quality of the dialysate, validation and management of the entire dialysate manufacturing process, including the dialysis water treatment system, central dialysis fluid delivery system (CDDS), dialysis machine, and piping to drainage of the dialysate, are necessary. Various cleaning and disinfection methods have been studied, including the use of sodium hypochlorite, acetic acid, peracetic acid, and hot citric acid solution. High temperatures and high concentrations of these agents are required for high efficacy in removing biofilm [7–10]. However, disinfecting CDDS with hot water can be expensive. The manufacturer's instruction manuals recommend cleaning/disinfecting with sodium hypochlorite, which is effective against biofilm-forming bacteria [10]. However, prolonged use of sodium hypochlorite can cause metal corrosion. Previous studies have shown that metal corrosion can lead to increased bacterial contamination [11, 12]. However, there is limited research on the effectiveness of disinfection and cleaning for maintaining dialysate sterility when faced with different flow stoppage times and varying levels of bacterial contamination in the presence or absence of metal corrosion in the pump parts.

Herein, we focused on the relationship between metal corrosion on the metal parts and the risk of bacterial contamination in the dialysis machines. According to a previous study, numerous bacteria were isolated from corroded metal parts of dialysis machines during clinical inspections, although no bacterial contamination was observed in the terminal dialysate samples [11]. We considered metal corrosion to be an important risk factor for bacterial contamination of the dialysate. When pump parts polluted artificially by immersion in water containing *Pseudomonas aeruginosa* for 15 h were assembled into a pump in a CDDS line, bacterial contamination was found in the terminal dialysate from pumps assembled using corroded metal parts, but not in that from pumps assembled using parts without metal corrosion [12]. Based on these observations, we believe that it would be difficult to ensure adequate sterility of the terminal dialysate using the conventional cleaning and disinfection program when using dialysis pumps assembled using metal parts showing corrosion. That being said, it is also important to develop optimal cleaning and disinfection methods to prevent contamination of dialysate lines and the terminal dialysate, irrespective of the presence or absence of corrosion on the metal parts of dialysis pumps.

The objective of this study was to evaluate the effectiveness of cleaning and disinfection on the sterility of a dialysate line and terminal dialysate following exposure of the dialysis circuit to a bacterial suspension for various lengths of time of flow stoppage. We propose an appropriate cleaning and disinfection program using a sodium-hypochlorite-containing reagent approved by the manufacturer for maintaining optimal sterility of the dialysate and pumps, on the basis of the results of simulation experiments conducted using pumps artificially polluted with *P. aeruginosa*.

Methods

Experimental circuit

The experimental circuit used to verify the relationship between bacterial contamination of the pump and risk of contamination of the terminal dialysate simulated a part of the dialysis machine configuration (Fig. 1). An endotoxin retentive filter (ETRF; Nipro, Osaka, Japan) was installed ahead of the pump to avoid contamination from the environment.

The Iwaki Magnet Gear Pump MDG-R2 (Iwaki Co., Tokyo, Japan) was used as the degassing pump in the dialysis machine (Fig. 2). The metal parts, the front plate and gear case, of the pump were made of stainless steel SUS316. Unused new parts were used as parts without metal corrosion. Parts used routinely in an ordinary clinical environment for 10 months were corroded, and were used as parts showing metal corrosion (Fig. 3).

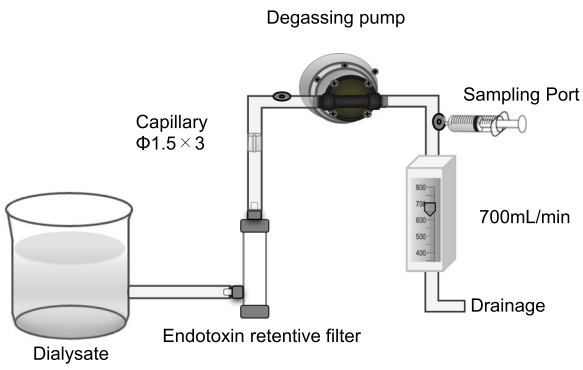

Fig. 1 Experimental circuit. To verify the bacterial contamination level of the pump and the terminal dialysate, an ETRF was installed ahead of the pump to avoid contamination from the environment. Dialysate was introduced into the circuit at a flow rate of 700 mL/min, and the terminal dialysate samples were collected from the sample port distal to the pump

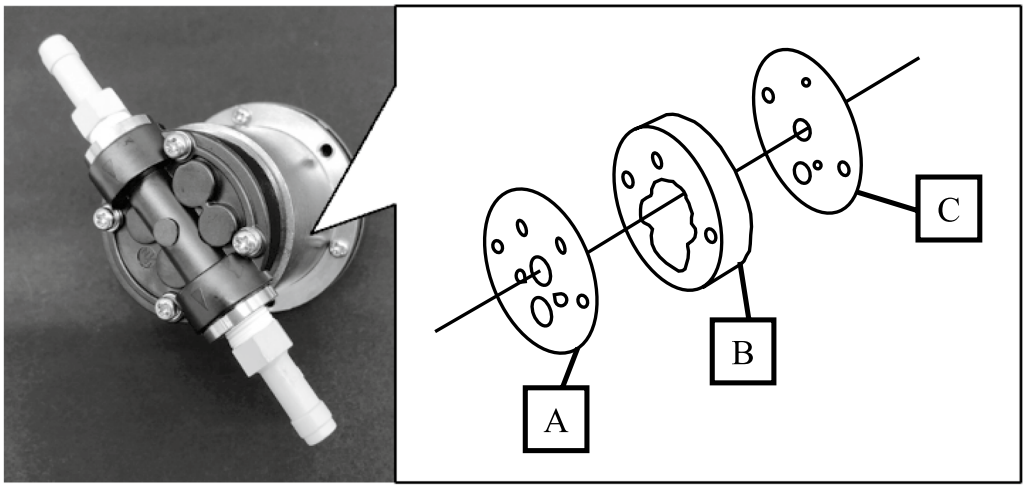

Fig. 2 IWAKI Magnet Gear Pump Model MDG-R2. **a** Front plate, **b** gear case, and **c** rear plate

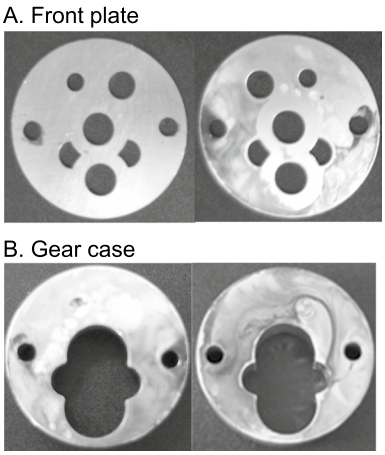

Fig. 3 Metal corrosion on the used magnet gear pump parts made by SUS 316. (**a**) Front plate and (**b**) gear case. Photos on the left and right represent the front and rear sides, respectively. "Used parts" refers to parts of a pump that had been used routinely in a clinical environment for 10 months. The corrosion area of the used metal parts was 4.02 cm^2 for the front plate, and 6.97 cm^2 for the gear case

Table 1 Composition of EverClean-500

Category	Property
Principal component	Sodium hydroxide, silicate salt, carboxylic acid metal chelating agent
Liquid properties	Alkaline
Appearance	Yellowish clear liquid
Odor	Odorless
pH	11.8 ± 0.2 (100-fold dilution)

EverClean-500 is a detergency enhancer added to sodium hypochlorite. The disinfection fluid contains 800 ppm sodium hypochlorite and 0.2%(v/v) EverClean-500

Spike-and-recovery testing of the pump and dialysis line using a bacterial suspension

The capacity of the cleaning/disinfection process used to assure sterility of the dialysate line and terminal dialysate was examined by spike-and-recovery testing using a bacterial suspension. The experimental condition was set to simulate bacterial contamination of the dialysate occurring after the morning pre-cleaning and the contaminated dialysate being left in the terminal dialysis machine for certain lengths of time.

The strain of *P. aeruginosa* used for the experiment was isolated from the surface of the corroded metal part of a dialysis machine that was routinely used in a hospital dialysis center (Sapporo, Japan). The bacteria were cultured in nutrient broth (Premedia, Kyokuto Pharmaceutical, Tokyo, Japan).

The *P. aeruginosa* suspension at a final concentration of about 10 colony-forming unit (CFU)/mL used for the experiment was prepared using dialysate Kindaly 3E (Fuso Pharmaceutical Industry, Osaka, Japan) sterilized by filtration using a Sartorius Syringe Filter 17598 K with a pore size of 0.45 μm (Sartorius Japan, Tokyo, Japan). The contaminated dialysate was introduced into an ethylene-oxide-gas-sterilized dialysis machine with or without corroded metal parts, and circulated for 25 min. Then, the flow in the pump was stopped, and the bacterial suspension left in place for 6, 12, or 18 h to allow the *P. aeruginosa* cells to adhere to the metal parts. The temperature of the dialysate was kept at 32 °C by calculating the average internal temperature of 17 dialysis machines after stoppage of the dialysate flow. The disinfection fluid contains 800 ppm sodium hypochlorite and 0.2%(v/v) EverClean-500 (Amtec, Osaka, Japan), as shown in

Table 1 and Fig. 4. Thereafter, the pumps were disassembled, the metal parts were removed, and specimens of the terminal dialysate were collected.

Bacterial culture of the terminal dialysate

The terminal dialysate was filtered through a 47 mm membrane filter with a pore size of 0.45 μm (Advantech Toyo, Tokyo, Japan). Then, the filter was placed in a nutrient agar medium (Eiken Chemical, Tokyo, Japan) and cultured at 37 °C for 48 h. The terminal dialysates were sampled, and the live bacterial counts in the samples were measured. The minimum detection sensitivity was 0.05 CFU/mL, 0.1 CFU/mL, and 0.5 CFU/mL at 6 h, 12 h, and 18 h of flow stoppage time, respectively. Then, after cleaning and disinfection, the minimum detection sensitivity of terminal dialysates was 0.05 CFU/mL. Nalidixic acid cetrimide (NAC) ager (Eiken Chemical) was used for identifying *P. aeruginosa*.

Recovery of bacteria from the metal parts of the dialysis pump

To determine bacterial contamination of the metal parts, the pump head was removed after or without being subjected to the cleaning/disinfection process, and incubated in fresh dialysate for 32 °C for 24 h for the enrichment culture. After incubation, the pump heads were disassembled as aseptically as possible on a clean bench, and the metal parts were immersed in a sterile physiological saline solution. The bacteria adhering to the metal parts were released by ultrasonic treatment using an ultrasonic homogenizer US-50 (Japan Precision Machinery, Tokyo, Japan). The probe tips of the homogenizer were sterilized with alcohol and flame, and inserted into the dips of the metal parts. Ultrasonic irradiation was performed at a rated power of 50 W at 28 kHz for 5 s. The resulting dip fluids were cultured on nutrient agar (Eiken Chemical) at 37 °C for 48 h. The minimum detection sensitivity was 1 CFU.

Statistical analysis

Statistical analysis was performed using the Pharmaco Basic software (Scientist, Tokyo, Japan). The Bonferroni's test or Wilcoxon's test was used to evaluate the differences in the numbers of bacteria isolated from the samples. The Holm's test was used to evaluate the differences in the detection times of bacteria from the samples. $p < 0.05$ was considered indicative of a statistically significant difference.

Results

Metal corrosion of used metal parts

The metal parts of a disassembled dialysis pump that had been used routinely in an ordinary clinical environment for 10 months were removed. The corroded areas of the metal parts were determined to be 4.02 cm^2 on the front plate and 6.97 cm^2 on the gear case (Fig. 3). The area of metal corrosion was larger on the gear case than on the front plate, because the metal-to-metal contact area is larger in the gear case than in other metal parts of a dialysis pump.

Recovery of the spiked bacteria in the terminal dialysate

We measured the numbers of spiked bacteria in the terminal dialysate before the lines were subjected to the cleaning and disinfection process (Fig. 5). The counts of bacteria in the terminal dialysate increased as the flow stoppage time increased. The bacterial counts in the terminal dialysate collected from the pump without corrosion of the metal parts were 0.003 ± 0.005, 6.4 ± 8.7, and 97.3 ± 93.8 CFU/mL for flow stoppage times of 6, 12, and 18 h, respectively. The bacterial counts in the terminal dialysate collected from the pump showing corrosion of the metal parts were 2.1 ± 6.5, 18.9 ± 13.1, and 147.1 ± 98.2 CFU/mL for flow stoppage times of 6, 12, and 18 h, respectively. The results indicated that corrosion of the metal parts of the pump was associated with an increased rate of bacterial contamination compared with that on the metal parts of the pump not showing corrosion. When the numbers of bacteria were measured after cleaning and disinfection of the dialysis circuit with a sodium-hypochlorite-containing reagent, no bacteria were observed in the terminal dialysate collected after flow stoppage times of 6 and 12 h, irrespective of the presence/absence of metal corrosion. However, after a flow stoppage time of 18 h, even after cleaning/disinfection, bacteria were detected in the dialysate specimens collected from the pump showing metal corrosion (one out of six experiments), but not in dialysate specimens

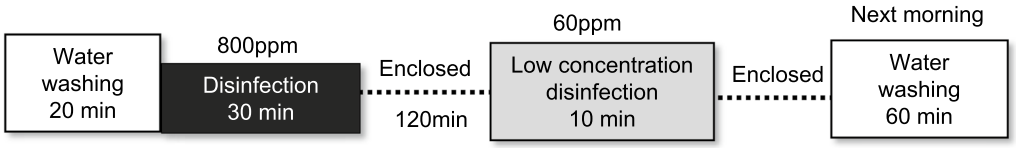

Fig. 4 Cleaning and disinfection program. Sodium hypochlorite solution supplemented with EverClean-500 was used as the disinfectant, and a single pass at 800 ppm was used for the cleaning program. Then, the system was left filled overnight with the disinfectant at 60 ppm. Finally, the circuit was washed with water the following morning

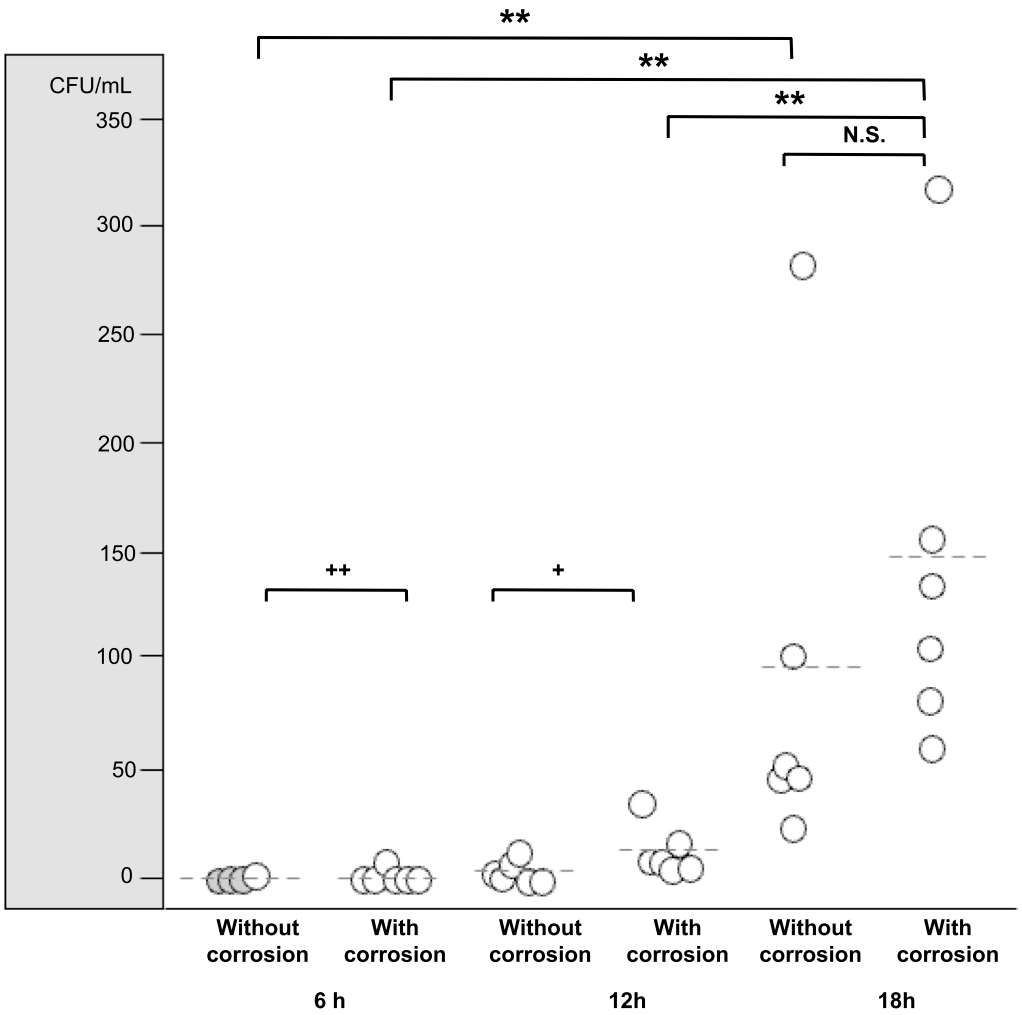

Fig. 5 Effects of flow stoppage time and presence of metal corrosion on the sterility of the terminal dialysate before cleaning and disinfection. Dialysate spiked with *P. aeruginosa* (10 CFU/mL) was introduced into the experimental circuit (Fig. 1), and the circuit flow was stopped for the indicated periods. The terminal dialysates were sampled, and the live bacterial counts in the samples were measured. The minimum detection sensitivity is 0.05 CFU/mL, 0.1 CFU/mL, and 0.5 CFU/mL at 6 h, 12 h, and 18 h of flow stoppage time, respectively. ● indicates not detected; ○ indicates detected; and the dashed lines indicate mean value; $^{**}p < 0.01$ (Bonferroni's test); ++ $p < 0.01$; and + $p < 0.05$. NS, not significant (Wilcoxon's test)

collected from the pump not showing corrosion of the metal parts (Fig. 6).

Detection of spiked bacteria on the metal parts of the dialysis pump

We measured the counts of live bacteria isolated from the metal parts of the dialysis pumps (Fig. 7). No bacteria were observed after cleaning and disinfection on the metal parts of the pump after a flow stoppage time of 6 h. After a flow stoppage time of 12 h, even after cleaning and disinfection, bacteria were detected at 0.2 ± 0.4 and 0.7 ± 1.6 CFU on the front plate of the pump not showing and showing metal corrosion, respectively. About three times more live bacteria (but not significant) were observed on the front plate of the pump showing metal corrosion compared with that of the pump not showing metal corrosion. Whereas no bacteria were detected on the gear case of the pump not showing metal corrosion, bacteria were detected at 14 ± 32 CFU on the gear cases showing metal corrosion. After a flow stoppage time of 18 h, even after cleaning and disinfection, live bacteria were detected at 560 ± 443 and 693 ± 609 CFU from the front plate and gear case of the pump without metal corrosion, respectively. In contrast, live bacteria were detected at 1004 ± 1090 and 894 ± 1037 CFU from the gear case of the pumps without and with metal corrosion, respectively. Therefore, the count of contaminated bacteria in the flow path was markedly higher after a flow

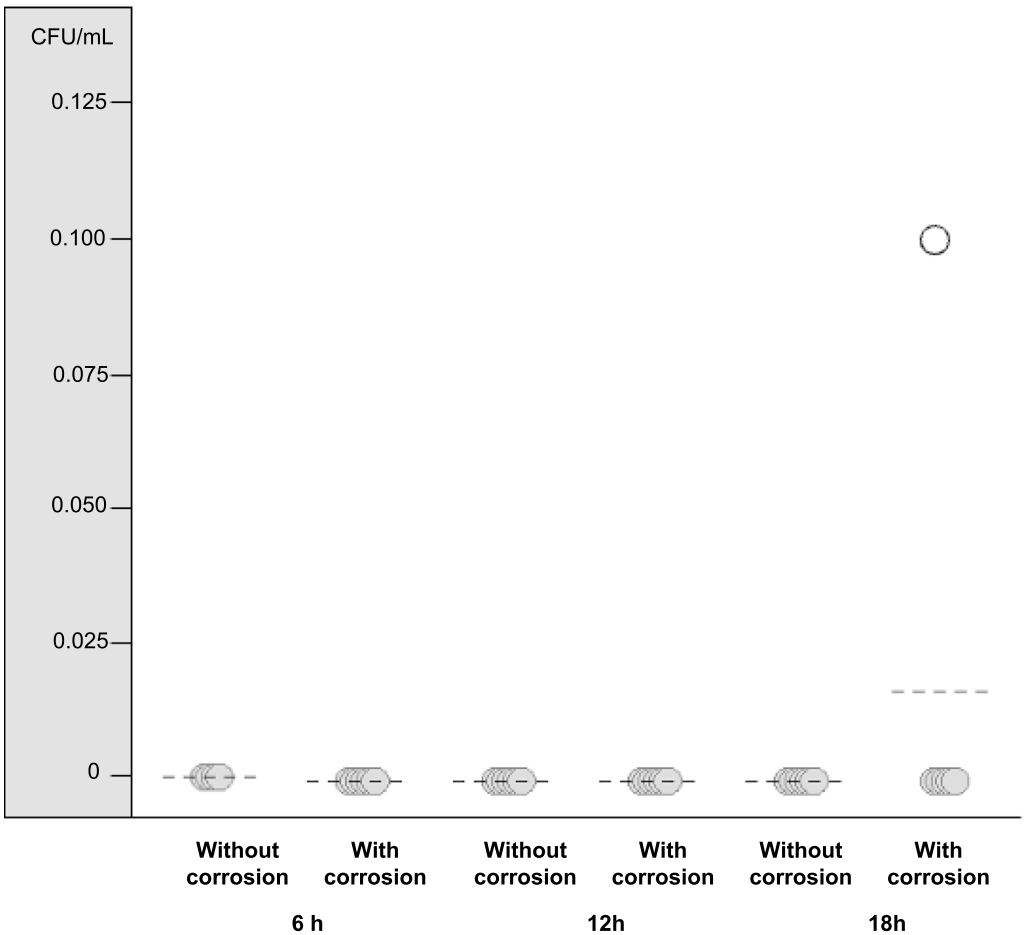

Fig. 6 Effects of flow stoppage time and presence of metal corrosion on the sterility of the terminal dialysate after cleaning and disinfection. Dialysate spiked with *P. aeruginosa* (10 CFU/mL) was introduced into the experimental circuit (Fig. 1), and the circuit flow was stopped for the indicated periods. Then, after cleaning and disinfection (Fig. 4), the terminal dialysates were sampled, and the live bacterial counts in the samples were measured. The minimum detection sensitivity is 0.05 CFU/mL. ● indicates not detected; ○ indicates detected; and the dashed lines indicate mean value

stoppage time of 18 h compared with that after 12 h, and the increase in count was more pronounced in the presence of corrosion of metal parts of the pump. The frequency of detection of bacteria on the metal parts was also higher after a flow stoppage time of 18 h than that after a flow stoppage time of 12 h (Table 2).

Discussion

To the best of our knowledge, this was the first study conducted to evaluate influence of metal corrosion in dialysis machines on the sterility of dialysate lines and terminal dialysates by spike-and-recovery testing using bacteria. The results of the study indicated that the sterility of the terminal dialysate can be guaranteed by conventional cleaning/disinfection with a sodium-hypochlorite-containing cleaning agent (Table 1), even if the contaminated dialysate is pumped on the day of treatment, if the flow in the machine has been stopped for less than 12 h. However, bacteria were occasionally detected in the terminal dialysate if the flow had been stopped for 18 h or more.

Our results suggest that the sterility of pump parts, regardless of the presence or absence of metal corrosion, cannot be assured, even after cleaning and disinfection, when the flow in the pump line is stopped for more than 12 h (Fig. 6). This suggests that bacteria adhere to and grow on the surfaces of the metal parts of the pumps. In particular, corrosion of metal surfaces in the pump was associated with an increased rate of bacterial contamination of the terminal dialysate. More bacteria were detected in the gear case than on the front plate because the gear case is located at the center of the pump, an area that is relatively poorly accessible to cleaning/disinfecting agents. Furthermore, the corrosion area was larger on the gear case than on the front plate (Fig. 2). In general, pumps cannot be routinely disassembled for checking the

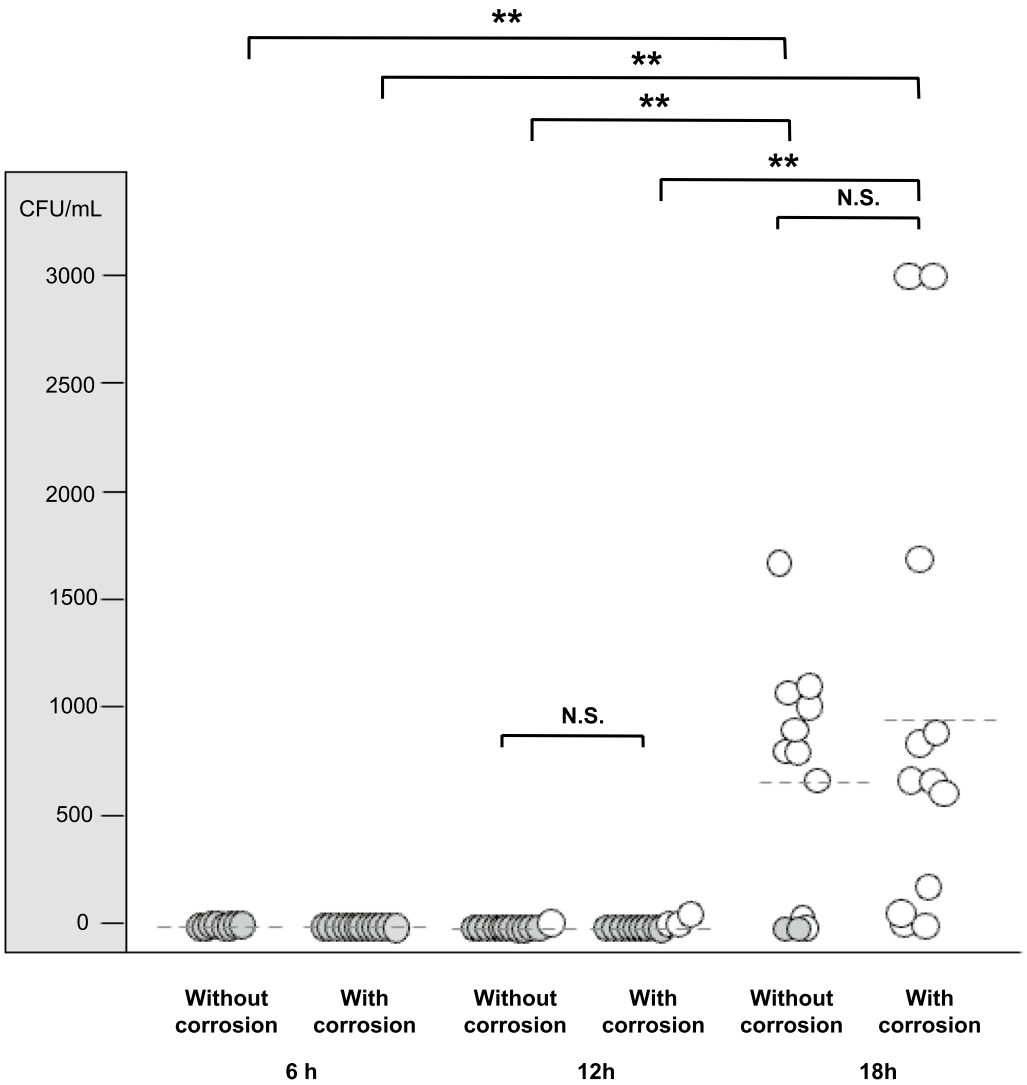

Fig. 7 Effects of flow stoppage time and presence of metal corrosion on the risk of bacterial contamination of the metal parts of the dialysis pump. Dialysate spiked with *P. aeruginosa* (10 CFU/mL) was introduced into the experimental circuit (Fig. 1), and the circuit flow was stopped for the indicated periods. Then, after cleaning and disinfection, as shown in Fig. 4, the pump was disassembled, the bacteria on the metal parts were released by ultrasonic exposure, and the released bacteria were counted. The minimum detection sensitivity is 1 CFU. ● indicates not detected; ○ indicates detected; and the dashed lines indicate mean value. ** $p < 0.01$ (Bonferroni's test). NS, not significant (Wilcoxon's test)

sterility; therefore, the sterility of the terminal dialysate cannot be assured, because the sterility of the entire dialysate line cannot be checked every time.

Biofilm formation is a probable mechanism of bacterial colonization of the metal parts of a pump. It is considered to increase the risk of bacterial contamination of dialysate lines. It is important to avoid biofilm formation in the dialysate line to the best extent possible [13]. Therefore, early cleaning and disinfection of the machine piping after treatment, and filling the stopped line with cleaning and disinfecting agents is important to avoid bacterial growth and biofilm formation [14]. Because bacterial adhesion increases on the rough surfaces of the metal parts [15], elimination of metal corrosion is needed to suppress bacterial adhesion to the metal parts of pump. It is difficult to completely remove a biofilm after it is already formed [16]. Disassembly, cleaning, and disinfection of the pump would be required for complete removal of the bacteria adhering to the pump. Such maintenance should be performed as aseptically as possible to prevent contamination from the environment.

In a previous study, bacterial contamination was observed on the corroded metal parts of a dialysis pump during clinical inspection, although no bacteria were

Table 2 Effects of flow stoppage time and the presence of metal corrosion on number of detection times from the metal parts

Metal corrosion	Flow stoppage time (h)	Detected times	Not detected times	*p*-Value (12 h versus 18 h)
Absent	12	1	11	< 0.01
	18	10	2	
Present	12	3	9	< 0.01
	18	12	0	

Dialysate spiked with *P. aeruginosa* (10 CFU/mL) was introduced to the experimental circuit (Fig. 1), and stopped flow for indicated times. After the cleaning and disinfection as shown in Fig. 4, the dialysis pump was disassembled, and bacteria on 12 metal parts were released by ultrasonic exposure. Released live bacteria were detected. $^{**}p < 0.01$ (Holm's test)

observed in the terminal dialysate [11]. We consider the experimental setup in the present study to be a good simulation of the situation in clinical practice. A limitation of this study was that we examined only one strain of *P. aeruginosa* isolated from a hospital dialysis line. We have observed many species of glucose non-fermenting gram-negative rods (NFGNR), so-called heterotrophic bacteria, during clinical verifications [11]. The abilities for adhesion, including biofilm formation, differ among bacterial species and strains [17]. Therefore, spiking-and-recovery experiments using other bacteria might be required in the future.

Even if live bacterial contamination is avoided by cleaning and disinfection, contaminations derived from dead cells, such as endotoxin (ET) and bacterial DNA fragments (bDNA), could still pose a problem. ET is well known as a pyrogen, which is strong inducer of inflammatory reaction [18, 19]. bDNA also induces proinflammatory cytokines and inflammatory reactions [20, 21]. In peritoneal dialysis patients, the blood bDNA level is reported as a strong predictor of the development of cardiovascular disease [22]. Contamination of dialysate with bDNA is known to directly affect the prognosis of dialysis patients [21, 22]. In general, an ETRF is installed in dialysis machines to remove ET and bacteria in clinical practice [6]. However, a report described that ET leaks from polysulfone (PS) and polyester polymer alloy (PEPA) membranes with repeated cleaning and disinfection [23, 24]. Several reports have indicated that bDNA passes through dialyzers [25]. And bDNA could pass through the ETRF, and enter the bloodstream through the dialyzer. It is important to ensure optimal sterility of the dialysate before placing an ETRF. Negligible contaminations of ET and bDNA should be guaranteed in the terminal dialysate. However, there are few reports on the content of bDNA in the dialysate, and the guidelines of the Japanese Society for Dialysis Therapy and the Japanese Association of Clinical Engineering have not referred to the bDNA contamination [4–6]. The levels of bDNA in the dialysate in clinical practice are typically very low and often below the detection limit. Therefore, it is necessary to use a spike-and-recovery test to evaluate the removal performance of an ETRF. However, it is important to note that high levels of bDNA applied in the experiments may not accurately simulate the low levels of bDNA found in dialysates in clinical practice. Despite this limitation, we recommend verifying the performance of an ETRF to remove bDNA by spike-and-recovery testing using bDNA or bacteria.

Conclusions

Bacterial colonization rates of the metal parts were higher in pumps showing metal corrosion than in those not showing corrosion. The sterility of the terminal dialysate cannot be assured, even after cleaning and disinfection, when using pumps showing corrosion of the metal parts. Furthermore, irrespective of the presence or absence of metal corrosion, the sterility of the metal parts of a pump cannot be assured, even after cleaning and disinfection, if the flow in the pump has been stopped for 12 h or more. Since metal corrosion of the pump is associated with an increased risk of bacterial contamination of the terminal dialysate even after cleaning and disinfection with a sodium-hypochlorite-containing reagent, it is necessary to ensure removal of corrosion of the metal parts during periodic inspections.

Abbreviations

CDDS	Central dialysis fluid delivery system
CFU	Colony-forming unit
ETRF	Endotoxin retentive filter
NAC	Nalidixic acid cetrimide
NFGNR	Glucose non-fermenting gram-negative rods
ET	Endotoxin
bDNA	Bacterial DNA fragments
PS	Polysulfone
PEPA	Polyester polymer alloy

Acknowledgements

The authors would like to thank Dr. Kenichi Kokubo (Kitasato University, Sagamihara, Japan), Dr. Hiroki Yabe (Seirei Christopher University, Hamamatsu, Japan), Dr. Makoto Saito (Gunma Paz University, Takasaki, Japan), and members of the Department of Microbiology, Sapporo Medical University School of Medicine (Sapporo, Japan) for the valuable suggestions and discussions. The authors would also like to acknowledge the technical assistance for scientific writing provided by tutoring program of the Japanese Society for Technology of Blood Purification.

Author contributions

All authors contributed to the conception and design of the study, the critical reading of the article for important intellectual content, and the final approval of the article. MN and MO contributed to the collection and assembly of data. MN and S-iY contributed to the analysis and interpretation of the data, and also drafting of the article.

Funding

None.

Availability of data and materials
The datasets analyzed during this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 10 August 2023 Accepted: 14 January 2024
Published online: 02 February 2024

References

1. Schiffl H, Lang SM, Bergner A. Ultrapure dialysate reduces the dose of recombinant human erythropoietin. Nephron. 1999;83:278–9.
2. Masakane I. Review: clinical usefulness of ultrapure dialysate: recent evidence and perspectives. Ther Apher Dial. 2006;10:348–54.
3. ISO 23500–5:2019(en). 2019. Preparation and quality management of fluids for hemodialysis and related therapies. Part 5: Quality of dialysis fluid for hemodialysis and related therapies. Available from: https://www.iso.org/obp/ui/#iso:std:iso:23500:-5:ed-1:v1:en. Accessed 31 July 2023
4. Kawanishi H, Akiba T, Masakane I, Tomo T, Mineshima M, Kawasaki T, et al. Standard on microbiological management of fluids for hemodialysis and related therapies by the Japanese Society for Dialysis Therapy 2008. Ther Apher Dial. 2009;13:161–6.
5. Mineshima M, Kawanishi H, Ase T, Kawasaki T, Tomo T, Nakamoto H, et al. 2016 update Japanese Society for Dialysis Therapy Standard of fluids for hemodialysis and related therapies. Ren Replace Ther. 2018;4:15.
6. Masakane I, Kawanishi H, Mineshima M, Takemoto Y, Uchino J, Hoshino T, et al. 2011 JSDT standard on the management of endotoxin retentive filter for dialysis and related therapies. Ther Apher Dial. 2013;17:229–40.
7. Sakuma K, Uchiumi N, Sato S, Aida N, Ishimatsu T, Igoshi T, et al. Experience of using heat citric acid disinfection method in central dialysis fluid delivery system. J Artif Organs. 2010;13:145–50.
8. Isakozawa Y, Migita H, Takesawa S. Efficacy of biofilm removal from hemodialysis piping. Nephrourol Mon. 2016;8:e39332.
9. Tagaya M, Oda Y, Kimura A, Irifune R, Okano S, Muraktaka T, et al. An easy disinfection strategy for pipes connecting hemodialysis equipment. Int J Artif Organs. 2021;44:385–92.
10. Osono E, Honda K, Inoue Y, Ichimura K, Kamano C, Akimoto T, et al. Sodium hypochlorite is effective against biofilms in dialysis equipment. Biocontrol Sci. 2021;26:1–7.
11. Nakamura M, Okayama M, Kimura K, Shibata H, Hagiwara M, Nawa T, et al. Isolation several bacteria from the corrosion confirmed pump parts in the bedside console for hemodialysis therapy. J Jpn Assoc Clin Eng Technol. 2017;61:109–15 (**in Japanese**).
12. Nakamura M, Okayama M, Kimura K, Shibata H, Hagiwara S, Nawa T, et al. Influence of metal pump corrosion on the contamination of terminal dialysis fluid by *Pseudomonas aeruginosa*. J Jpn Soc Dial Ther. 2019;52:7–13 (**in Japanese**).
13. Smeets E, Kooman J, van der Sande F, Stobberingh E, Claessens P, Grave W, et al. Prevention of biofilm formation in dialysis water treatment systems. Kidney Int. 2003;63:1574–6.
14. Isakozawa Y, Takesawa S. A basic review of the biofilm component in the dialysate plumbing system. J Kyushu Univ Health Welf. 2008;9:93–8 (**in Japanese**).
15. Vanhaecke E, Remon JP, Moors N, Raes F, De Rudder D, Van Peteghem A. Kinetics of *Pseudomonas aeruginosa* adhesion to 304 and 316-L stainless steel: role of cell surface hydrophobicity. Appl Environ Microbiol. 1990;56:788–95.
16. Cappelli G, Ballestri M, Perrone S, Ciuffreda A, Inguaggiato P, Albertazzi A. Biofilms invade nephrology: effects in hemodialysis. Blood Purif. 2000;18:224–30.
17. Matsuoka T. Biology of biofilms. Microbes Environ. 1999;14:163–72.
18. Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA, et al. Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med. 1988;318:1481–6.
19. Giovanni P, Giuseppe G, Loreto G, Francesco PS. Clinical relevance of production in hemodialysis. Kidney Int. 2000;58(Suppl. 76):S104–11.
20. Schindler R, Beck W, Deppisch R, Aussieker M, Wilde A, Göhl H, et al. Short bacterial DNA fragments: detection in dialysate and induction of cytokines. J Am Soc Nephrol. 2004;15:3207–14.
21. Bossola M, Sansuinetti M, Scribano D, Zuppi C, Giungi S, Luciani G, et al. Circulating bacterial-derived DNA fragments and markers of inflammation in chronic hemodialysis patients. Clin J Am Soc Nephrol. 2009;4:379–85.
22. Szeto C-C, Kwan BC-H, Chow K-M, Kwok JS-S, Lai K-B, Cheng PM-S, et al. Circulating bacterial-derived DNA fragment level is a strong predictor of cardiovascular disease in peritoneal dialysis patients. PLoS ONE. 2015;10:e0125162.
23. Schepers E, Glorieux G, Eloot S, Hulko M, Boschetti-de-Fierro A, Beck W, et al. Assessment of the association between increasing membrane pore size and endotoxin permeability using a novel experimental dialysis simulation set-up. BMC Nephrol. 2018;19:1.
24. Nozaki H, Tange Y, Inade Y, Uchino T, Azama N. Leakage of endotoxins through the endotoxin retentive filter: an in vitro study. Blood Purif. 2022;51:831–9.
25. Hulko M, Dietrich V, Koch I, Gekeler A, Gebwert M, Beck W, et al. Pyrogen retention: comparison of the novel medium cut-off (MCO) membrane with other dialyzer membranes. Sci Rep. 2019;9:6791.

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