—Original—

Use of Ultrasonic Cleansing in Managing the Couplers of Dialyzer Systems

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Abstract

Dialysis-related complications have become a major concern as the number of patients receiving long-term maintenance dialysis increases. One cause of complications is contamination of the dialysis fluid. When dialysis fluid contaminated by bacteria or endotoxin (ET) or both has been used for a long time, cytokine production *in vivo* is enhanced and can lead to such complications as dialysis amyloidosis. The rate of dialysis-related complications might be reduced with a hemopurification method that uses a large amount of dialysis fluid as a substitution fluid (on-line hemodiafiltration) or an efficient dialyzer with enhanced internal filtration in which the dialysis fluid returns to the body as a replacement fluid; however, at the same time, there is an increased risk of ET entering the body because the dialysis fluid might be contaminated. Therefore, the dialysis fluid must be made aseptic, and the dialysis fluid line must be properly managed to prevent contamination of the dialysis fluid. A half-opened line is at great risk of contamination by living microbes, which can grow in dead spaces and where the flow of dialysis fluid is interrupted. The management of couplers is an important measure for maintaining cleanliness at the end of the dialysis fluid flow. We attempted to separate and regularly clean the main body of the coupler with ultrasonic equipment as a method of managing the conventional coupler. Using improved types of coupler, the water quality of the postcoupler flow was maintained at a level as high as that of the precoupler flow for the duration of the evaluation period without separate cleansing being done. Although separate once-a-week cleansing of the conventional coupler was able to keep ET values less than the detection limit, viable cell counts were unstable. On the other hand, twice-a-week ultrasonic cleansing eliminated almost all viable cells. No definite difference in ET values or viable cell counts was found between the cleansing groups, and ultrasonic cleansing was able, by itself, to provide a sufficient cleansing effect. We conclude that ultrasonic cleansing of conventional couplers is a useful method for maintaining the water quality of the postcoupler flow because the cleansing of the coupler twice or more a week is sufficient to keep the water quality of the postcoupler flow as high as that of the precoupler flow.
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Key words: endotoxin, dialysis fluid purification, dialyzer coupler, ultrasonic cleansing method

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Introduction

Dialysis-related complications have become a major concern in Japan as the number of patients receiving long-term dialysis has increased to more than 290,000. Such complications lower patients' quality of life and interfere with social rehabilitation. One cause of complications is contamination of the dialysis fluid. Long-term use of dialysis fluid contaminated with viable cells or endotoxin (ET) or both increases *in vivo* cytokine production[1,2], which then increases the risk of such complications as dialysis-related amyloidosis and malnutrition-inflammation-atherosclerosis syndrome[3]. The risk of dialysis-related complications might be reduced with a high-performance dialyzer with enhanced internal filtration, in which the dialysis fluid perfuses into the body, or with a hemopurification method, in which a large volume of dialysis fluid is used as the substitution fluid; however, at the same time, the risk of ET entering the body from contaminated dialysis fluid is increased. Therefore, the dialysis fluid must be aseptic.

To reduce the risk of dialysis fluid contamination, the dialysis fluid line must be properly managed. A half-opened line is at great risk for contamination by living microbes, which can then grow in dead spaces and where the flow of dialysis fluid is interrupted. Important measures to ensure the cleanliness of the end of the dialysis fluid flow include management of couplers and installation of an ET-retentive filter (ETRF). Even if water quality standards for ultrapure dialysis fluid (viable cell count, <0.1 CFU/mL; ET value, <0.001 EU/mL) are achieved through installation of an ETRF, cross-contamination can occur through open handling at lower-flow postcoupler sites when the dialyzer or bypass connector is uncoupled[4]. Moreover, with a conventional coupler, some parts, including inlaying O-rings, cannot be cleaned with the cleansing/disinfection process of the central dialysis fluid delivery system; therefore, such parts can be important sources of contamination.

Although the coupler has been modified so that it contains no uncleanable parts, we attempted to improve the method of managing the conventional coupler by separating and regularly cleansing its main body with ultrasonic equipment. In ultrasonic cleansing, contaminants attached to the target object are broken away by the shockwave generated with the bursting of cavitation bubbles, which are produced in the fluid by ultrasonic waves[5,6]. The cavitation bubbles can reach every part of the target object, and the shockwaves produced can break microbial cell membranes and, therefore, have a microbicidal effect[5,6].

In the present study, we used an ultrasonic device to clean O-rings and corrugated parts of a conventional coupler, and the cleanliness of both conventional couplers and improved couplers was evaluated.

Materials and Methods

Subjects

The evaluation was performed in 2 facilities, our corporate member facilities A and B. For this study, we used 6 sets of a commonly used plastic coupler as conventional couplers and used 2 sets each of Nipro's H-coupler (Nipro Corp., Osaka, Japan) and Nikkiso's Clean-coupler (Nikkiso Co., Ltd., Tokyo, Japan) as improved couplers. The conventional coupler seals both the dialyzer and the bypass connector with the same O-ring, and this sealing structure has been found to interfere with the cleansing/disinfection process of the central dialysis-fluid delivery system (**Fig. 1**). As an improved coupler, Nipro's H-coupler excludes the O-ring as a contamination source, and the part connecting the dialyzer to the bypass connector is made of silicone rubber to function as air packing. Nikkiso's Clean-coupler introduces a double O-ring system in which the coupler and its bypass connector each have an O-ring. The O-ring on the coupler serves as the sealing element when the dialyzer is connected, and the O-ring on the bypass-connector serves as the sealing element and provides space for the in-coupler O-ring area for effective washing during the cleansing/disinfection process of the central dialysis-fluid delivery system (**Fig. 2**). Each coupler was newly installed and evaluated for 24 weeks. The

Fixing ball

Connecting position of dialyzer
(bypass connector)
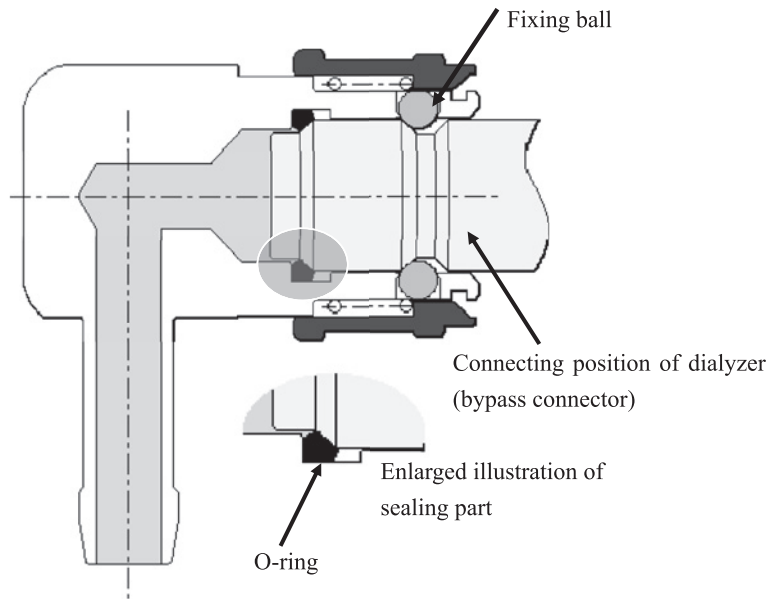
Enlarged illustration of
sealing part

O-ring

Fig. 1 Conventional coupler specification (manufacturer's document:
modified in part)
Widely used polypropylene coupler
This coupler seals both the dialyzer and bypass-connector with
the same O-ring, and this sealing structure has been found to
have the problem of insufficient washing during the cleansing/
disinfection process of the central dialysis-fluid delivery system.

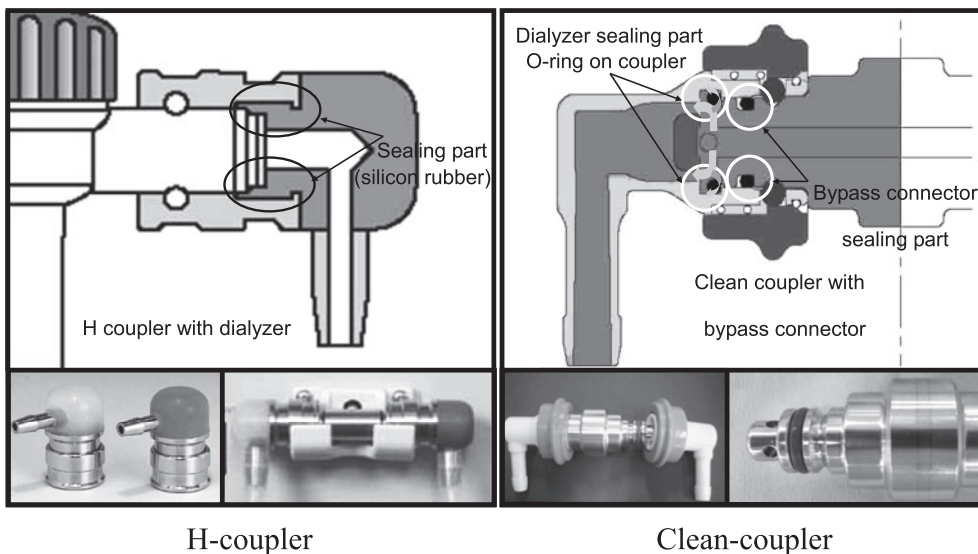H-coupler   Clean-coupler

Fig. 2 Specifications of improved couplers (manufacturer's document: modified in part)
The H-coupler excludes the O-ring as a contamination source, and the part connecting the
dialyzer to the bypass connector is made of silicone rubber to function as air packing.
The Clean-coupler introduces a double O-ring system in which the coupler and its bypass-
connector each have 1 O-ring. The O-ring on the coupler serves as the sealing element when
the dialyzer is connected, and the O-ring on the bypass connector serves as the sealing element
and provides some space for the in-coupler O-ring area for sufficient washing during the
cleansing/disinfection process of the central dialysis-fluid delivery system.

connections of the dialyzer and the coupler are shown in **Figure 3**. Both facilities A and B provide

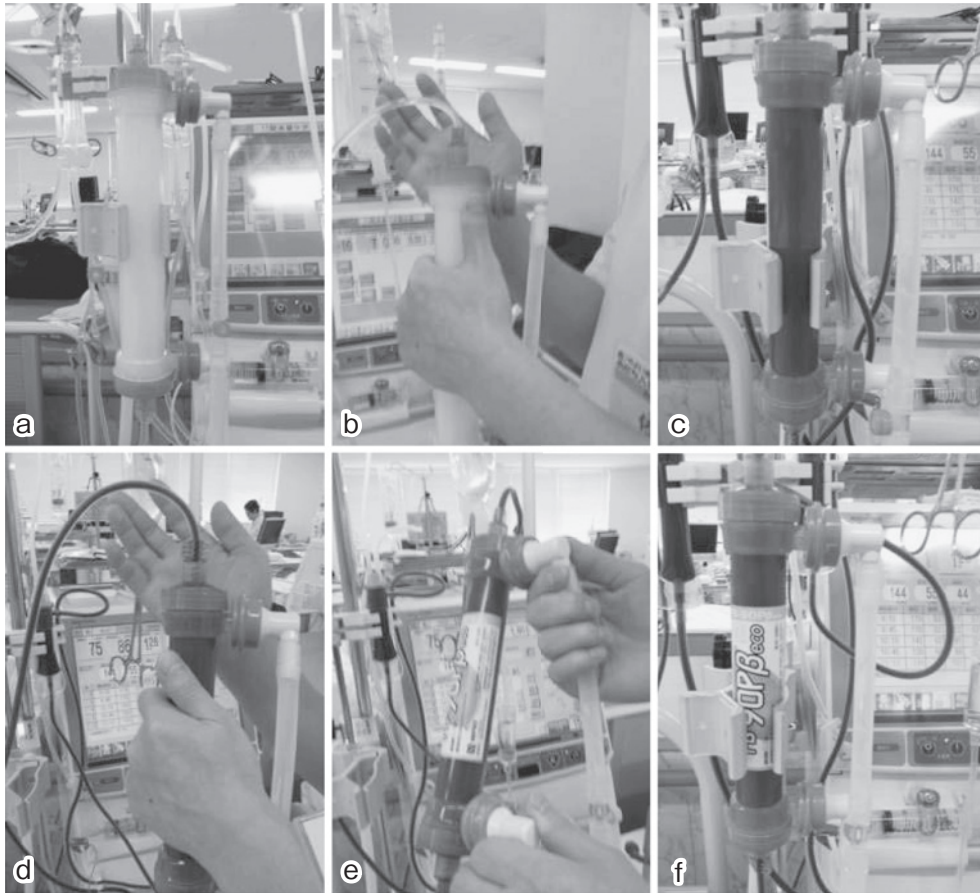hemodialysis and (off-line) hemodiafiltration treatments, have the same conditions of water quality

Fig. 3   Connection of dialyzer and coupler
a: Connection of the dialyzer and coupler
b: Air removal from the dialyzer
c: Immediately after the start of dialysis
d: Confirmation of air removal
e: Inversion of the dialyzer
f: During dialysis treatment
If the interior of the coupler is heavily contaminated, some viable cells adhering to the inner surface of the coupler can be transferred to the dialysis fluid by the shaking load during the air-removal process or inversion of the dialyzer.

management, and use the same type of conventional coupler.

Methods

The ultrasonic cleansing equipment was prepared after being washed once before use by changing the tap water in the reservoir without the addition of a disinfectant solution (**Fig. 4**). The conventional couplers, including a bypass connector, were divided into 3 groups on the basis of cleansing method: group A (2 sets), ultrasonic cleansing only; group B (2 sets), 50 ppm sodium hypochlorite disinfection after ultrasonic cleansing; and group C (2 sets), hydrothermal (>80℃ reverse-osmosis water)

disinfection after ultrasonic cleansing. The duration of ultrasonic cleansing was 20 minutes, the disinfection time was 10 minutes, and the rinsing time with reverse-osmosis water was 10 minutes. For the first 16 weeks of the evaluation period, the cleansing was done weekly, and from the week 17 through the week 24, cleansing was done twice weekly. The ultrasonic cleansing equipment used was Tamano's Pearl-Cleaner TS-510 H (high-frequency output: 500 W, 40 kHz) (Tamano Co., Ltd., Tokyo, Japan) for facility A, and Tamano's Pearl-Cleaner TS-320H (high-frequency output: 300 W, 28 kHz) for facility B. The improved couplers were placed in group D (H-coupler, 2 sets) and group E
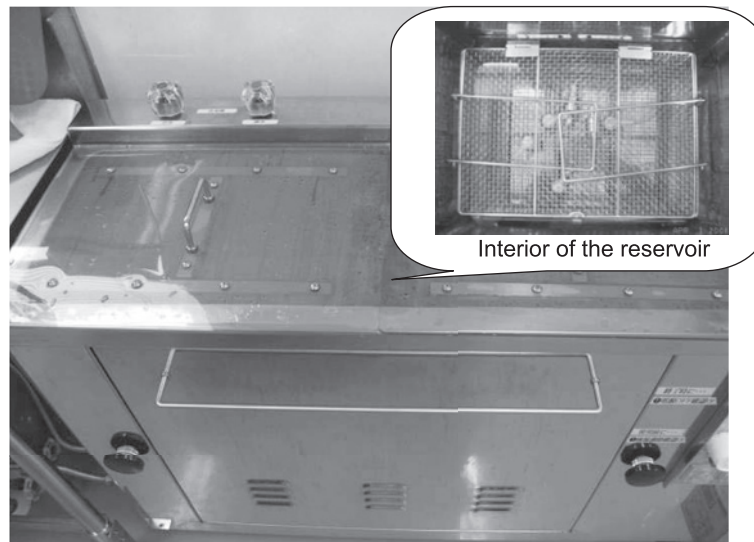
Fig. 4   The ultrasonic cleansing equipment used for this study
Tamano's Pearl-Cleaner TS-320H (high-frequency output: 300 W 28 kHz)

(Clean-coupler, 2 sets). In contrast to the conventional couplers, the improved couplers were not subjected to separate regulatory cleansing and were cleaned only with the cleansing/disinfection process of the central dialysis-fluid delivery system. In addition, all couplers were wiped with alcohol swabs when the bypass connector was connected.

To evaluate the cleansing level of the coupler, ET values and viable cell counts were determined at the precoupler site and the postcoupler site. To verify the cleansing interval and the cleansing effect, samples for analysis were obtained before cleansing (before the start of the dialysis session on the day of ultrasonic cleansing) and after cleansing (before the start of the dialysis session on the day after ultrasonic cleansing). To obtain samples for analysis, the bypass connector was connected to the coupler, and the fluid line was flushed continuously for at least 5 minutes. The sampling ports (Nikkiso's EF-P) prepared in the precoupler site and the postcoupler sites were wiped with an alcohol swab, and the sampling was done in a retrograde fashion from postcoupler sites to precoupler sites. The coupler was swiveled about 10 times. Meanwhile, for the postcoupler sampling site, the load to the coupler under normal conditions was simulated by applying a rotational load to the coupler, and then the sampling was done **(Fig. 5)**. The ET values were measured with nephelometry and the Limulus test

(Toxinometer MT-358; Wako Pure Chemical Industries, Ltd., Osaka, Japan), and the viable cell counts were determined with the membrane filter method (37-mm Quality monitor M-TGE Broth; Pall Corporation, Port Washington, NY, USA) with a filtrate volume of 10 mL, which was incubated for 7 days at room temperature ($25℃ \pm 1℃$).

At the end of the evaluation period (week 24), the inner surface of the coupler (O-ring or corresponding parts) was wiped with a sterile cotton swab, which was then applied to R2A agar culture medium (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and incubated for 7 days. Moreover, the inner surfaces of the couplers that had been used for 24 weeks were examined with a scanning electron microscope (SEM) (JMS-6360LA; JEOL Ltd., Tokyo, Japan) and compared with those of unused couplers **(Fig. 6)**. The accelerating voltage of examination after Au-Pd alloy vapor deposition was 15 kV.

Results

During the evaluation period, ET values were less than the detection limit at precoupler sites in all groups, and the viable cell counts indicated the absence of colonization. For the improved couplers, results similar to those for the precoupler sites were obtained for the postcoupler sites. The ET value transition at the postcoupler site of the conventional
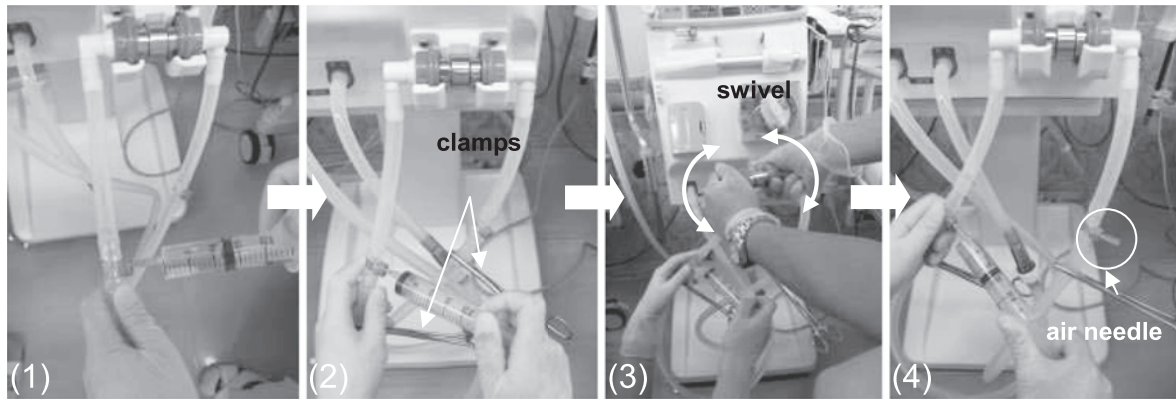
Fig. 5   Sampling method at postcoupler sites
(1) The fluid line is continuously flushed for more than 5 minutes and pumped several times.
(2) Flushing is stopped, and the post-EF-P site is closed off with clamps.
(3) The coupler is swiveled about 10 times.
(4) An air needle is inserted into the fluid line, and all fluid (15 to 20 mL) is collected.
Method provided by: (Healthcare Corporation) Showakai Kanagawa Gijutsu Kenkyukai
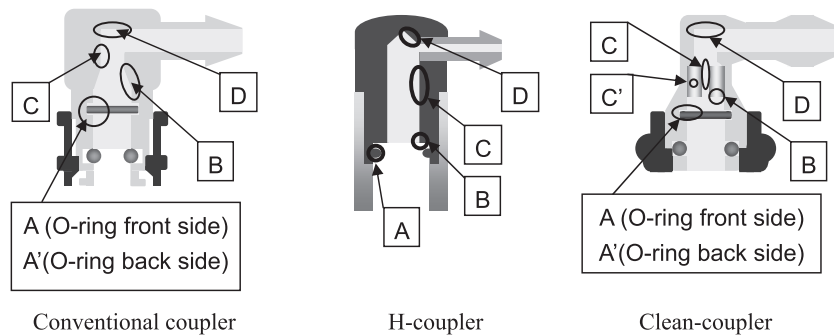Water Quality Control Division

Fig. 6   SEM examination site of coupler inner-surface

coupler is shown in **Figure 7**. Some of the ET values were high in the first week between coupler installation and cleansing; however, after regular cleansing started, all values were less than the detection limit. Changes in viable cell counts are shown in **Figure 8**. As with ET levels, some viable cell counts were high in the first week after coupler installation, and even after the cleansing was done on a weekly basis, the viable cell counts were unstable. After the cleansing frequency was changed to twice weekly from the week 17 of the study period, little or no colonization was found. The viable cell culture results with the membrane filter method are shown in **Table 1**.

To reexamine the cleansing interval, daily samples were obtained during the period when the cleansing was done on a weekly basis and cell counts were unstable. The colonization was confirmed about 3 days after cleansing. Therefore, by changing the cleansing frequency to twice weekly and by setting the cleansing intervals to 3 days or 4 days with a no-dialysis day, nearly no viable cells were found. No colonization on the agar medium was found in any group, as the results of culture test done at the end of the evaluation period (week 24) (**Table 2**).

The SEM images of the inner surfaces of conventional couplers (**Fig. 9**) showed a definite difference between unused and used couplers, except that a rough O-ring was found at examination sites A and A' in group B. In contrast, many materials were attached to the surface of the H-coupler, which was an improved coupler that was not separately cleansed, and the surface was found to be changed when compared with the surface of

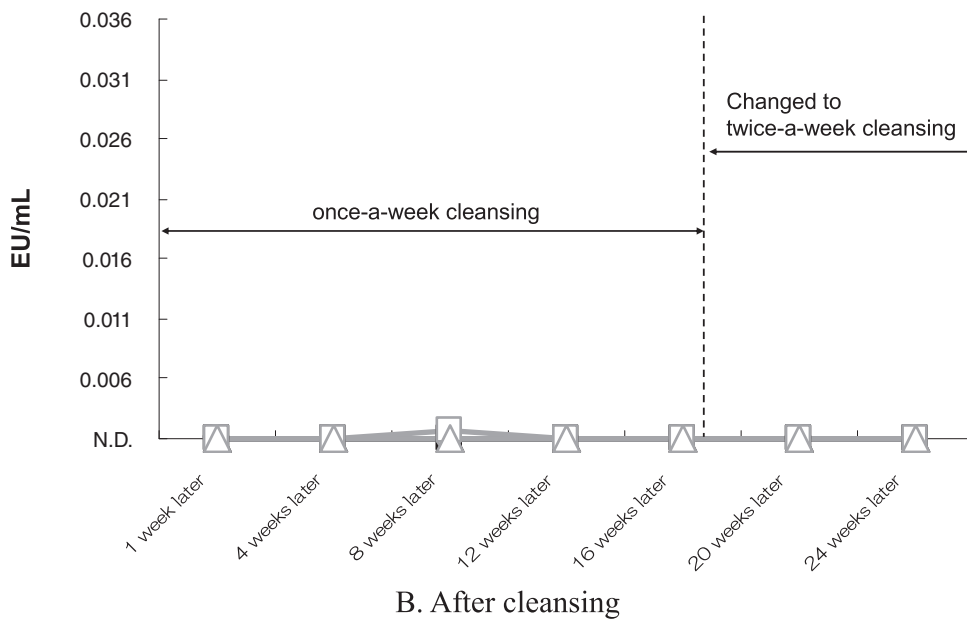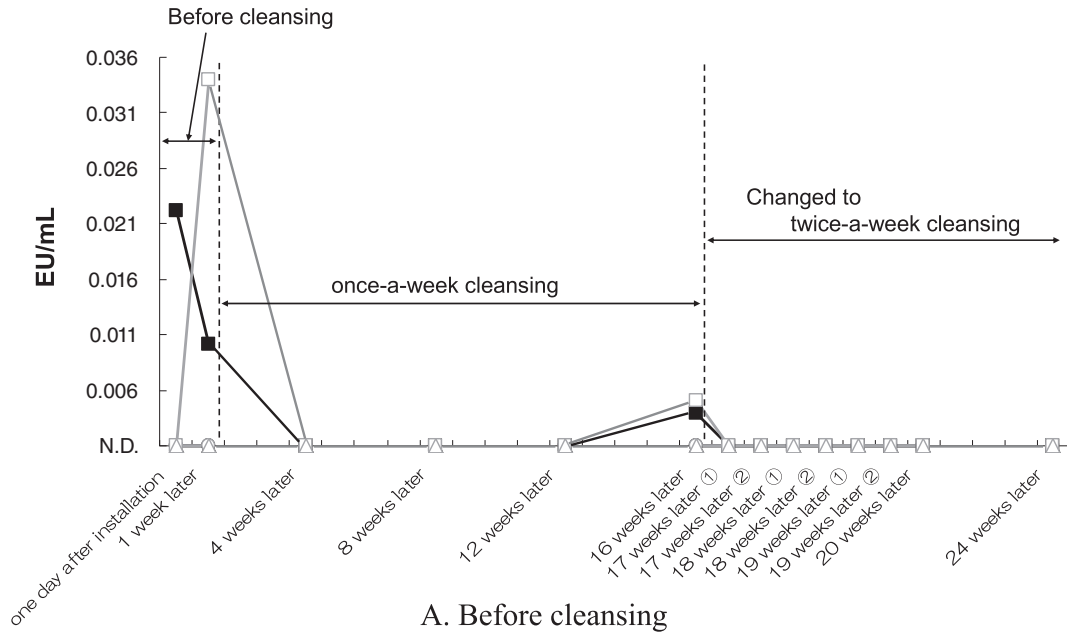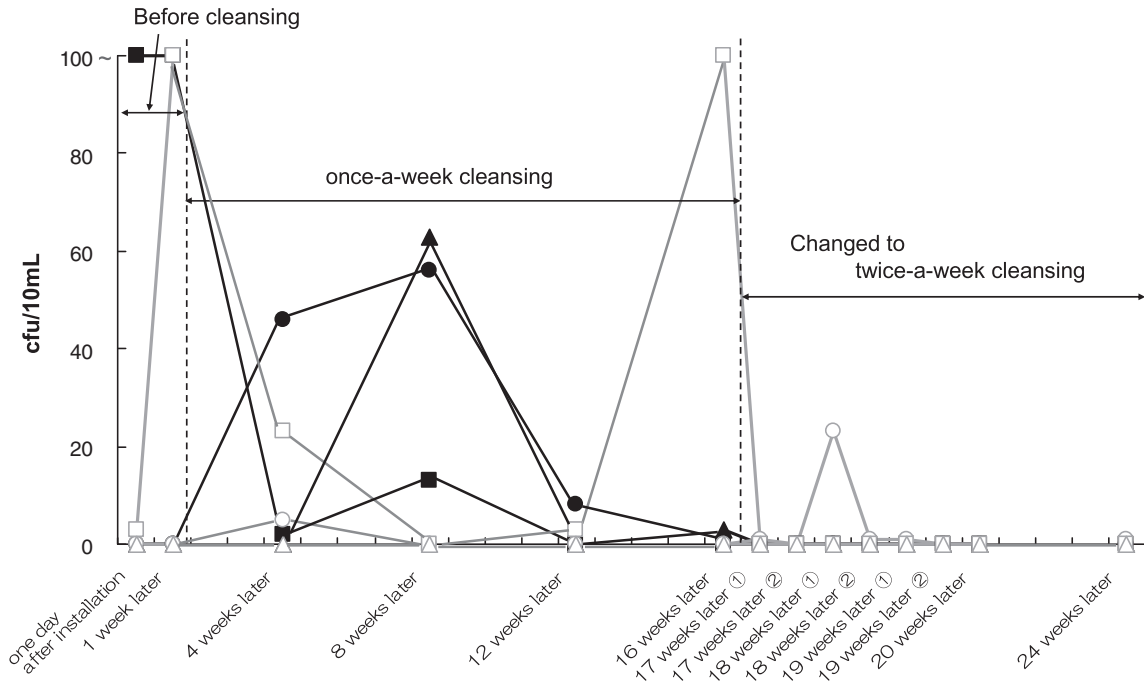A. Before cleansing

B. After cleansing

Fig. 7 Changes in ET levels at postcoupler sites
group A: (n=2) (1) ⚫—, (2) ○—
group B: (n=2) (1) ■—, (2) □—
group C: (n=2) (1) ▲—, (2) △—

The cleansing frequency was once a week until week 16. During the first few months of the evaluation period, we found that the viable cell counts could not be sufficiently suppressed with weekly cleansing. After reexamination of the cleansing interval, the cleansing frequency was changed to twice weekly from week 17. Therefore, after week 17, to evaluate the sufficiency of the cleansing frequency, sampling was done for every cleansing for 3 weeks (represented as ① and ② on the graph).

The values after cleansing were sufficiently stable; therefore, sampling was done every 4 weeks during the evaluation period.

Some values less than the detection limit overlap and lie behind other data in the graphs and are not seen on the surface of the graphs.

A. Before cleansing
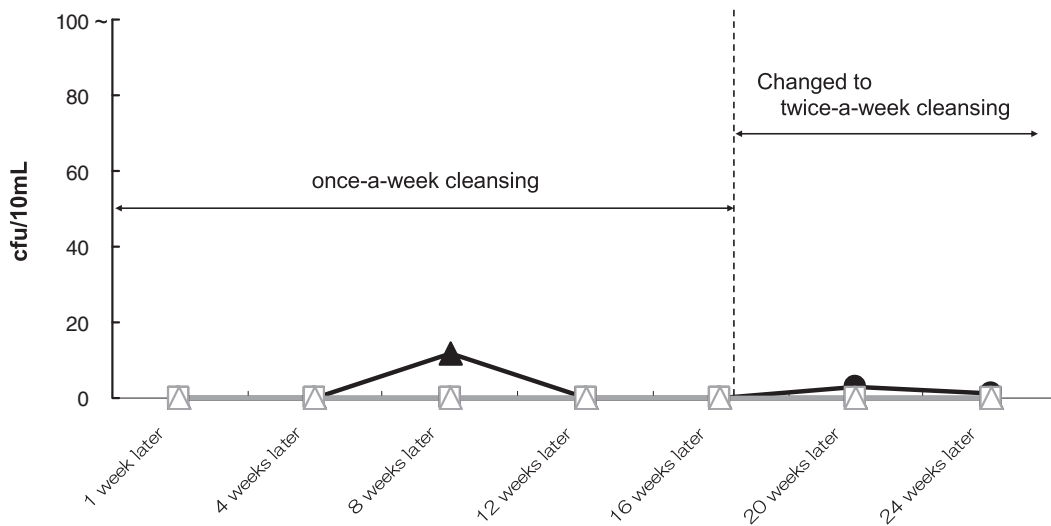
B. After cleansing

Fig. 8 Changes in viable cell count transition at postcoupler sites
Performed and shown in the same manner as in Figure 7.

an unused coupler. No change was found on the surface of the Clean-coupler on SEM examination, but a change in color to brown was visually confirmed **(Fig. 10, 11)**.

Discussion

For dialysis fluid purification, the international standard formulation has been under review by the International Organization for Standardization[7], and in Japan, a committee of the Scientific Academy of the Japanese Society for Dialysis Therapy presented its recommendations as the dialysis water quality standard and blood purification device performance evaluation standard in 2008[8]. These standards state that the infusing dialysis fluid should be nearly sterile and that its ET value should be less than the detection limit; therefore, it is necessary to avoid

Table 1 Viable cell culture results[a] with the membrane filter method

once-a-week cleansing	1 day later	2 days later	3 days later	4 days later
group A (1)[b]	—	—	+	+ + +
group B (2)[b]	—	+	—	+ +
twice-a-week cleansing	1 day later	2 days later	cleansing day (before cleansing)	1 day later
group A (1)	—	—	+	—
group B (2)	—	—	—	—

　—, no colonization; +, <10 cfu/10 mL; + +, <100 cfu/10 mL; + + +, >100 cfu/10 mL

7days' incubation

[a] For the postcoupler sampling site (sampling was done before the start of the dialysis session)

[b] To reexamine the cleansing interval, the two couplers: group A (1) and group B (2) (in Fig. 8A); which viable cell count was conspicuously high, were selected for sampling.

Table 2 Viable culture results[a] with R2A agar medium

Viable cell count (7 days' incubation)	Coupler group									
	group A		group B		group C		group D		group E	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Exit site	—	—	—	—	—	—	—	—	—	—
Returning site	—	—	—	—	—	—	—	—	—	—

　—, no colonization

[a] At the end of the evaluation period (the inner surface of the coupler was wiped with a sterile cotton swab, which was then applied to R2A agar culture medium)
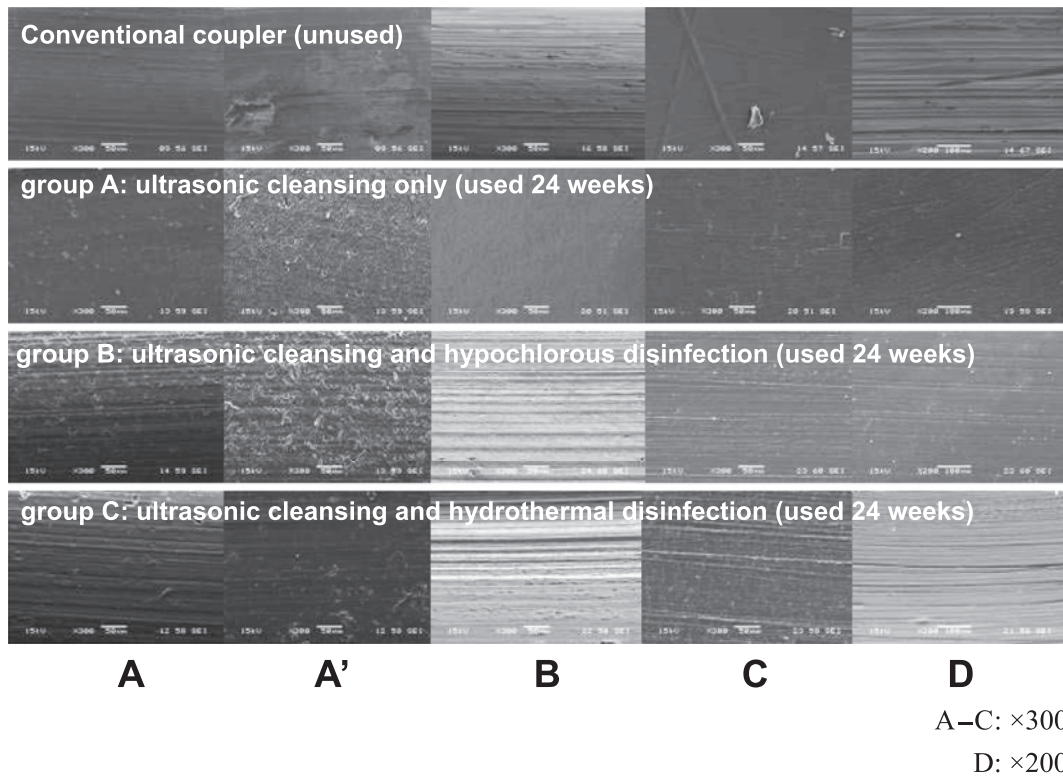

A−C: ×300

D: ×200

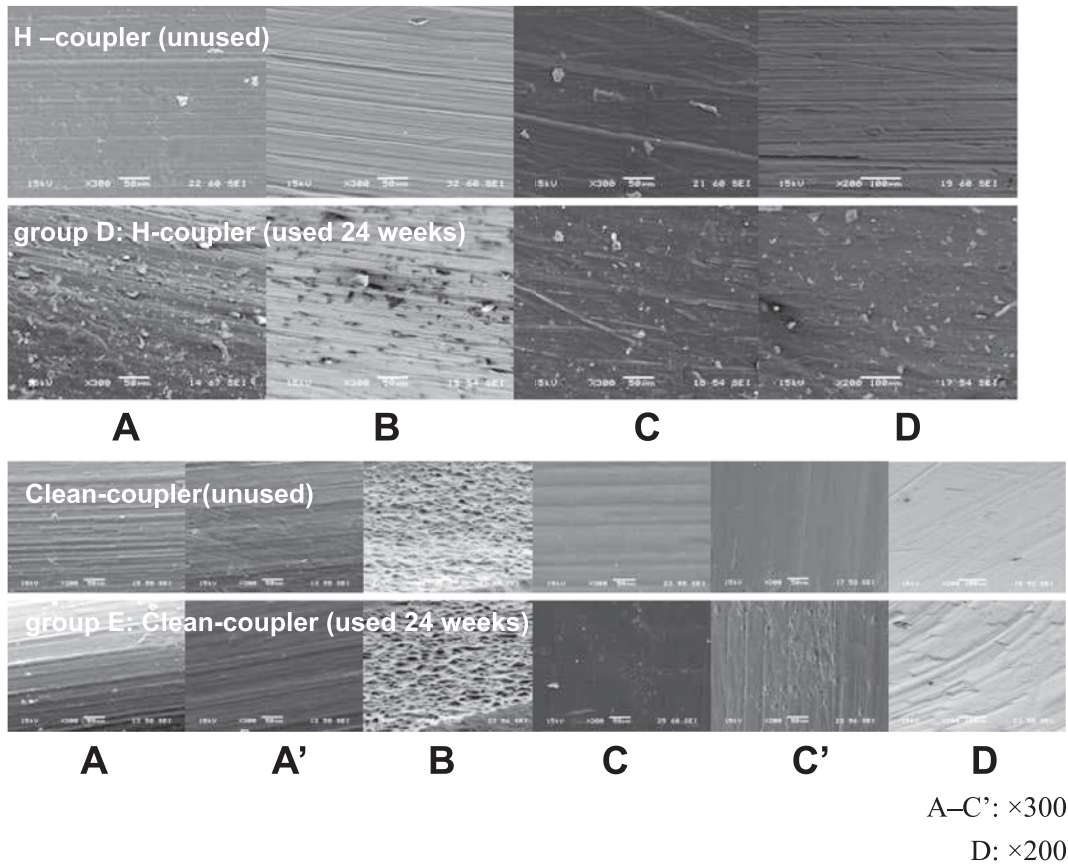Fig. 9 SEM examination of the inner surfaces of conventional couplers

H –coupler (unused)

group D: H-coupler (used 24 weeks)

A B C D

Clean-coupler(unused)

group E: Clean-coupler (used 24 weeks)

A A' B C C' D

A–C': ×300
D: ×200

Fig. 10   SEM examination of the inner surfaces of improved couplers

group A   group B   group C

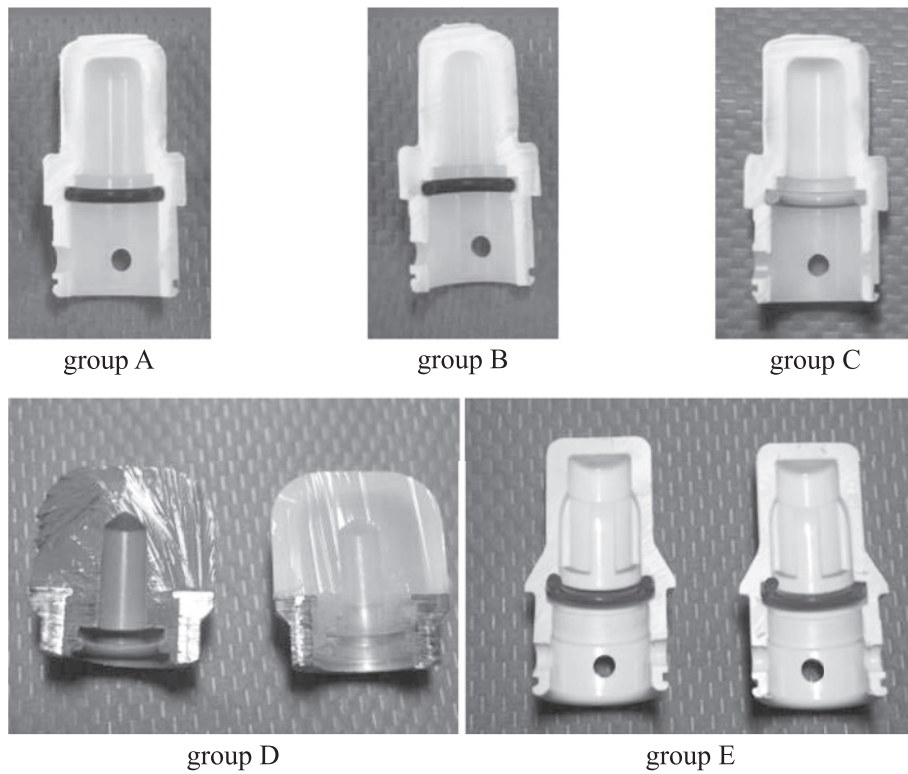group D   group E

Fig. 11   Appearance of the inner surfaces of couplers (used 24 weeks)

tertiary contamination from the sites, including couplers, that can contaminate the post-ETRF fluid. Although acute reactions, such as pyrexia, hypotension, and shock, to large quantities of ETs have long been recognized, chronic reactions to ETs are less well understood[9]. Furthermore, even an extremely low concentration of ETs in dialysis fluid (0.00231 EU/mL) can induce cytokine production[10,11]. Repetitive, long-term exposure to such extremely low ET concentrations can also induce various dialysis-related complications, such as anemia (erythropoiesis-stimulating agent hyporeactivity), amyloidosis (enhanced beta 2-microglobulin generation), malnutrition (depressed albumin synthesis in the liver and protein hypercatabolism), and arteriosclerosis (endothelial dysfunction by oxygen radicals)[9].

With a conventional coupler, some parts, such as an inlaying O-ring, cannot be cleaned by the cleansing/disinfection process of the central dialysis-fluid delivery system; therefore, these parts are important sources of contamination if they are not cleaned in some other way. Indeed, in our facilities, both ET values and viable cell counts were high before the introduction of ultrasonic cleansing. Some facilities have suggested that effective cleansing methods are the use of a weakly acidic cleansing solution, hydrothermal disinfection, and replacement of inlaying O-rings; however, these methods require both time and effort. Moreover, although improved couplers decrease the risk of dialysis fluid contamination[12], they still require cleansing/disinfection. With the improved coupler, the postcoupler water quality was maintained at a level equal to the precoupler water quality for the duration of the evaluation period without separate cleansing being done. Although the ET value of the conventional coupler was kept to less than the detection limit with once-a-week separate cleansing, viable cell counts were unstable. On the other hand, with twice-a-week ultrasonic cleansing, almost all viable cells were eliminated. No definite difference in ET values or viable cell counts was found between the cleansing groups, and ultrasonic cleansing by itself provided a sufficient cleansing effect. Because the ultrasonic cleansing equipment can be used after

being washed only once by changing the tap water in the reservoir, preparation time is less. However, the device must be checked regularly because time-dependent component degradation can cause mechanical dysfunction[13].

The cost of an improved coupler is significantly higher than that of a conventional coupler. With conventional couplers, every method we tried was performed at a similarly low cost. From this point of view, the ultrasonic cleansing method should prove useful.

On SEM images of conventional couplers, no definite difference was seen between the surfaces of used and unused couplers except that rough O-rings were found in group B, in which ultrasonic cleansing was followed by sodium hypochlorite disinfection. The chemical solution used for group B can cause component degradation of the coupler and is a possible reason for the rough O-rings in group B. In contrast, no separate cleansing was done for improved couplers, many materials were attached to the surface of the H-coupler, and a change in color of the Clean-coupler to brown was visually confirmed. Therefore, further study, including a review of evaluation methods, is required.

Conclusion

Ultrasonic cleansing of conventional couplers is a useful method for managing the water quality of the postcoupler flow because cleansing the couplers 2 or more times a week is sufficient to keep the water quality of the postcoupler flow as high as that of the precoupler flow.

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