Title: Enhanced production of water for hemodialysis using electrodeionization (EDI)

ABSTRACT

Water for hemodialysis (WFH), which is manufactured on-site in hemodialysis hospitals, is indispensable for hemodialysis therapy. Water treatment systems for manufacturing WFH primarily consist of membrane separation technologies: reverse osmosis (for chemical contaminants) and ultrafiltration (for microbial contaminants). To further improve the quality of WFH, we successfully apply adsorption technology, namely electrodeionization (EDI), after reverse osmosis to remove additional chemical contaminants and microbial contaminants from WFH. When the DC current was supplied to the EDI system, we found that single-stranded bacterial DNA could also be removed by the EDI, as well as viable bacteria and endotoxins.

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INTRODUCTION

The number of dialysis patients in Japan has increased every year and reached over 320,000 at the end of December 2014.[1] To compensate for this rise, the number of dialysis hospitals has reached 4,330 hospitals (Figure 1). The number of dialysis patients is equivalent to 0.25% of the total Japanese population and has the potential to increase until around 2020. This increase in the number of dialysis patients is caused by diabetes, which is also the worldwide tendency. Approximately 400,000 dialysis patients exist in China, and it is estimated that this will rise to 2,000,000 patients in the future. As a result, the frequency of chronic renal failure in foreign countries is also increasing rapidly.

For treatment, there are two types in dialysis therapy: in-center dialysis (treatment at dialysis hospitals) and home dialysis (treatment at the patient's home). In Japan, 97% of dialysis therapy is in-center dialysis, so we focus on in-center dialysis in this study. Blood purification methods during in-center dialysis are classified into hemodialysis (HD), hemodiafiltration (HDF), and hemofiltration (HF). HD uses diffusion, whereas HF uses filtration; HDF combines the two methods, utilizing both diffusion and filtration. At the end of December 2014, 255,000 patients were receiving HD in Japan, accounting for 80% of the patient population. Approximately 43,000 patients were receiving online HDF at the end of December 2014, as this increased after the 2012 medical service fee. In the future, there is a

possibility that online HDF will have increased to more than 100,000 patients. The therapy methods of HD and online HDF are shown in Figure 2. In the case of HD, material transfer occurs between the blood and the dialysis fluid through an ultrafiltration (UF) membrane called the dialyzer (Figure 3). HD is a blood purification method in which waste in the blood moves to the dialysis fluid side by diffusion. However, middle molecular substances (molecular weights of 500 Da–60,000 Da) are slow in material transfer speed because of their large molecular weight. Thus, there is a problem with HD in that these substances cannot be sufficiently removed from the blood of patients.

In contrast, online HDF uses both diffusion and filtration to remove middle molecular substances. In online HDF, dialysis fluid is injected directly into the blood of patients, as shown in Figure 2 and waste in the blood, including middle molecule substances, is removed by a UF membrane called the hemodiafilter. However, if some contaminants exist in the dialysis fluid, the patient's blood is directly polluted by them in online HDF. To prevent this, it is necessary to purify the dialysis fluid as much as possible in online HDF.[2]

Dialysis fluid is manufactured with the following procedure, as shown in Figure 4. Concentrates for hemodialysis are diluted with water for hemodialysis (WFH) to form the dialysis fluid in a machine called the central dialysis fluid delivery system (CDDS). Subsequently, the dialysis fluid is sent to patient monitoring equipment; thereafter, it passes through a UF membrane called the endotoxin retentive filter (ETRF), and it is finally sent to a dialyzer or hemodiafilter. In online HDF, purification of WFH is especially important. Relevantly, two ISO standards for hemodialysis and related therapies were established in September 2002: "ISO13958—Concentrates for hemodialysis and related therapies"[3] and "ISO13959—Water for hemodialysis and related therapies."[4] In April 2009, these standards were revised and two additional standards were established: "ISO11663—Quality of dialysis fluid for hemodialysis and related therapies"[5] and "ISO26722—Water treatment equipment for hemodialysis applications and related therapies."[6] In addition, "ISO23500—Guidance for the preparation and quality management of fluids for hemodialysis and related therapies"[7] was established in May 2011. These five standards, which were again revised in April 2014, contribute to the enhancement of purification of dialysis fluid. ISO13959 is the standard for WFH, in which requirements of chemical contaminants and microbial contaminants are

prescribed (Tables 1 and 2). If harmful contaminants are included in WFH, patients are subject to various adverse events. Microbial contaminants (bacteria, endotoxin) infiltrating the patient's blood would result in a decline of hematogenous functions, fever, and inflammation.[8]

To meet the limitations on chemical contaminants and microbial contaminants, many membrane technologies are used in water treatment systems for manufacturing WFH. Figure 4 shows an example of a water treatment system. A water treatment system generally consists of a cartridge filter, softener, activated carbon, a reverse osmosis (RO) module, an RO permeate storage tank, a UV disinfection device, and a UF module. The water treatment system installed in all hemodialysis hospitals in Japan is mainly composed of an RO membrane and a UF membrane; it is capable of producing 2–3 cubic meters of WFH per hour. The cartridge filter removes particles in raw water such as tap water. The softener exchanges divalent cations (e.g., Ca^{2+} and Mg^{2+}) for monovalent cations (e.g., Na^{+}). The activated carbon removes free and combined chlorine. The RO module removes chemical contaminants, and the UV disinfection device prevents bacterial regrowth in the RO permeate storage tank. The UF membrane removes some bacteria that cannot be disinfected by UV and removes endotoxins generated by the UV disinfection. The RO membrane is used to remove chemical contaminants and the UF membrane is used to remove bacteria and endotoxins. Although bacteria and endotoxins should also be removed by the RO membrane, it is known that these substances cannot be sufficiently removed by the spiral wound type RO module that is generally used in water treatment.[9]

In actual equipment, a hollow fiber type UF membrane is often installed after the RO permeate storage tank. WFH has to meet limits on both chemical contaminants and microbial contaminants under ISO13959. However, because there is no long-term experience in online HDF, it is necessary to note that some substances are not addressed by ISO13959. For example, it is desirable to remove as many chemical contaminants in the RO membrane as possible for online HDF. In addition, some microbial contaminants may not be removed by the UF membrane. For example, bacterial DNA can induce an endotoxin-like cytokine response if it enters the patient's body.[10–14] Shindler reported that bacterial DNA in dialysis fluid passes through dialyzers and remains in the patient's blood.[15] Because the molecular

weight of a single-stranded of bacterial DNA is very small (MWCO 1,250 Da), single-stranded bacterial DNA seems to pass through dialyzers. Therefore, insufficient removal of chemical contaminants and microbial contaminants presents the most significant problem of WFH and dialysis fluid.

Electrodeionization (EDI) technology is often used for the semiconductor industry. Some studies have reported on the use of EDI for hemodialysis therapy. A large volume of dialysis fluid is used during hemodialysis therapy and discharged continuously. Therefore, a system that recycles dialysis fluid via EDI was considered.[16,17] Another example is a study on the production of WFH.[18] By using EDI, the resistivity of WFH has become more than 10 MΩ ·cm , and the water quality has become equivalent to that of water for injection.

The goal of this study is to remove trace contaminants that exists in RO permeate. Primarily, EDI removes chemical contaminants, which are the trace elements in the RO permeate. However, we have previously confirmed through experiments that microbial contaminants can be removed as well.[19,20,21] In the present study, we investigate the performance of EDI for removing trace chemical contaminants and microbial contaminants. Ultimately, we apply this outcome to a full-scale water treatment system.

MATERIALS AND METHODS

Materials

Equipment

An EDI system was installed after the RO membrane of a general water treatment system for WFH. EDI can remove trace elements that cannot be removed by the RO membrane. The removal ability of the combined RO plus EDI system was estimated using three types of equipment (Figures 5, 6, and 7): one bench-scale setup, one pilot-scale setup, and four full-scale setups. Table 3 summarizes the specifications of the equipment used in the three setups. The equipment of bench-scale setup is small, and the setup does not have an RO permeate storage tank. As it is very simple, it is easy to acquire correct data. The equipment of the pilot-scale setup has the same electrode surface area as the equipment of the full-scale setup. The region that controls separation in the EDI system is called a cell. The number of cells differs between the equipment of the pilot-scale setup and the full-scale setup. In the equipment of the pilot-scale setup, we can confirm the influence of the DC current, because

the DC current has a wide range of adjustment. The equipment of the full-scale setup was designed for practical use based on the results of the bench-scale and pilot-scale setups. In the equipment of the full-scale setup, it is possible to manufacture 2 cubic meters of WFH per hour—enough to simultaneously supply WFH to approximately 60 patients. The removal ability for chemical contaminants and microbial contaminants was checked using three types of equipment. The equipment of the full-scale setup has already been installed in 27 hospitals in Japan—in Chiba-ken, Ibaraki-ken, Hokkaido, etc.—and has actually been used there for hemodialysis therapy.

EDI stack

Characteristics

EDI comprises two water treatment technologies: ion-exchange resins and electrodialysis (ED). EDI is different from ED as the ion-exchange resins are filled in a cartridge called the EDI stack. The EDI stacks installed in the EDI system were products of SnowPure LLC (San Clemente, CA; Figure 8). The ion-exchange resins in the EDI system are mixed bed type of anion-exchange resins and cation-exchange resins; in addition to being utilized as ion-exchangers, they are used as an ion transfer method. Accordingly, ions can move in EDI under low current even if the feed water has low conductivity. In addition, chemicals are not required to regenerate the resins.

Removal of inorganic ions

In EDI, ions are removed by the ion-exchange membranes and ion-exchange resins. The ion-exchange resins (i.e., anion-exchange resins and cation-exchange resins) are sandwiched between the anion-exchange membranes and the cation-exchange membranes. This chamber, which is filled with the ion-exchange resins, forms an EDI desalination chamber. An EDI concentration chamber then forms between the EDI desalination chambers. The EDI desalination and EDI concentration chambers, as shown in Figure 9, are placed between two electrodes that supply a DC voltage; each electrode (cathode and anode) is in an electrode chamber. The RO permeate is sent to three chambers in the EDI system, becoming EDI desalinated water, EDI concentrated water, and EDI electrode water. The EDI concentrated water is adjusted to have approximately 10% of the water content of the EDI desalinated water. First, inorganic ions are removed from the RO permeate via ion-exchange reactions in

the EDI desalination chambers. Anions are exchanged with hydroxide ions (OH^-) in the reaction of the anion-exchange resins, and cations are exchanged with hydrogen ions (H^+) in the reaction of the cation-exchange resins. Next, ions adsorbed on the ion-exchange resins are attracted to the electrodes by the DC voltage. Cations are attracted to a cathode (−), and anions are attracted to an anode (+). Anion-exchange membranes are permeable to anions but not to cations. Cation-exchange membranes are permeable to cations and but not to anions. Ion-exchange membranes are not water-permeable.

The removal mechanism of inorganic ions by EDI is as follows.

1. Inorganic ions are adsorbed on the ion-exchange resins and are removed from the RO permeate.
2. Ion-exchange resins are continuously regenerated by electricity. Chemical regeneration is not required.
3. Inorganic ions move on the resin surface, and pass through ion-exchange membranes. Finally, they enter the EDI concentration chambers; from which, they are discharged.
4. As a result, desalinated water is continuously manufactured in the EDI desalination chambers.

Regeneration by water-splitting phenomenon

Water is split into H^+ and OH^- by the DC voltage; this is a water-splitting phenomenon. The ion-exchange resins are continuously regenerated by the H^+ and OH^- produced by this process. As a result, desalinated water can be continuously manufactured. This water-splitting phenomenon also plays an important in controlling bacteria regrowth. Strong acid and strong base layers of high concentration form on the surface of ion-exchange resins. Viable bacteria that interact with these strong acid and strong base layers are killed.[22,23]

Methods

Measurement method of chemical contaminants

ISO13959 specifies 22 chemical contaminants. We measured their concentrations by the measurement method specified in ISO13959. Although silica and total organic carbon are not included in the 22 items, they were also measured at the same time.

Measurement method of microbial contaminants

ISO13959 specifies viable bacteria and endotoxins as microbial contaminants. ISO13959 also specifies the measurement method for viable bacteria and endotoxins. We counted the number of viable bacteria by using culture and fluorescent direct counting methods. We used R2A agar medium, which cultivates heterotrophic bacteria, in the culture method that is specified in ISO13959. Cultivation is necessary because WFH contains little nutrition for bacteria. In contrast, the fluorescent direct counting method does not require time for colony formation like the culture method. Bacteria are directly dyed using a fluorescence stain, and the number of bacteria is counted by using a dedicated measuring device. Therefore, results are quickly available in approximately 10 min. Furthermore, even bacteria that cannot be cultivated with the culture method can be counted with the fluorescent direct counting method. Thus, the fluorescent direct counting method shortens the measurement time, and the exact number of bacteria can be obtained. In addition, the fluorescent direct counting method counts not only the number of viable bacteria but also the number of dead bacteria.

Viable bacteria

Culture method (Milliflex plus pump; Merck Millipore Co.,Japan)

Viable bacteria were cultured from each sample on a membrane filter (pore size 0.45 μm) using R2A agar medium. Each sample (10–100 mL) was vacuumed with a vacuum pump and filtered with a membrane filter. After that, the membrane filter was set on the culture medium and it was cultivated at 20–25 °C for 1 week; the number of colonies of viable bacteria was counted thereafter.

Fluorescent direct counting method (Bioplorer; KOYO SANGYO Co., Ltd Japan)

The fluorescent direct counting method counts the number of bacteria stained with fluorescent dye. Viable bacteria were counted using carboxyfluorescein diacetate (6-CFDA) as an index of the intracellular enzyme activity (esterase activity). Dead bacteria were counted using PI (Propidium iodide) for staining the nucleic acid directly. Each sample (1–10 mL) was dropped on the filter (pore size 0.4 μm), which is set in the funnel, and were vacuumed and filtered by the vacuum pump. After filtration, the filter was treated with a fluorescence reagent that reacts to bacteria on the filter. After the reaction, the filter was set in a dedicated measuring device. The number of bacteria was counted by the PC connected to the dedicated measuring device.

Endotoxin

We measured endotoxins using a Limulus Amebocyte Lysate (LAL) test. The principle of a Limulus test is that endotoxin reacts with the solidified factor (LAL) extracted from the horseshoe crab. When LAL reacts to an endotoxin, the LAL reagent solidifies into a gel. In the measurement, LAL was injected into a test tube filled with a 0.3 mL sample. After injection, the test tubes were stirred for 10 s. After stirring, the test tube was set in a dedicated measuring device (turbidimetric analysis: *Toxinometer mini; Wako Pure Chemical Industries, Ltd., Japan*). As the reaction developed, the transmission factor gradually fell. When the transmission factor became 92%, we presumed that the reaction had ended. In addition, some samples were measured by colorimetry *(Endospecy; SEIKAGAKU Co,. Japan)*.

Measurement of bacterial DNA

Criteria for bacterial DNA have not yet been set in the criteria for microbial contaminants of WFH. Therefore, bacterial DNA has rarely been measured in WFH. In addition, among bacterial DNA, the molecular weight of single-stranded bacterial DNA is very small (molecular weight of approximately 1,250 Da).[15] Therefore, it was necessary to measure single-stranded bacterial DNA. In addition, measurement is very difficult because only trace amounts of bacterial DNA exist in WFH. To estimate the removal ability of EDI for bacterial DNA, we had to develop a measurement method to quantify trace amounts of single-stranded bacterial DNA in WFH.

We used a fluorescent staining reagent *(Quant-iT™ OliGreen® ssDNA Assay Kit; Thermo Fisher Scientific K.K, Japan)* for quantification of single-stranded bacterial DNA.[24] The sensitivity of quantification of DAPI (4',6-diamidino-2-phenylindole) is 10 ng/mL, whereas the sensitivity of quantification of OliGreen is 1 ng/mL. However, the concentration of bacterial DNA in WFH is very low—below 1 ng/mL. Therefore, it was necessary to concentrate the sample before measurement, and we measured the concentration of bacterial DNA in the concentrated sample. The steps used for concentration and measurement are as follows.

1. Set 1 g of each sample in a centrifugal concentration machine *(WKN-PV-1200; WAKEN BTECH Co., Ltd. Japan)*, and concentrate each sample via vacuum concentration for 3 hours at 2000 rpm.
2. Obtain a 200 ng/mL oligonucleotide solution using an oligonucleotide standard and a

buffer.

3. Obtain various concentrations of oligonucleotide solution for the calibration curve using the 200 ng/mL oligonucleotide solution and a buffer.
4. Adjust OliGreen solution using an OliGreen reagent and a buffer.
5. Add 100 µL of each concentrated sample and each concentration of oligonucleotide solution for the calibration curve to each slot of the micro-plate.
6. Add 100 µL of OliGreen solution to each slot of the microplate.
7. Shield the microplate for 3 min.
8. Set the microplate into the fluorescence analysis device (*SpectraMax Gemini XPS; Molecular Devices, LLC)* and measure the fluorescence of each sample by the defined measurement condition.
9. Determine the concentration of each concentrated samples using the calibration curve.
10. Calculate the concentration of single-stranded bacterial DNA in the samples using the determined value and the concentration rate.

RESULTS AND DISCUSSION

Chemical contaminants

By using EDI, the resistivity of WFH has become more than 10 MΩ·cm.[18] However, this study focused on the removal of trace chemical contaminants by EDI. The removal ability of EDI for chemical contaminants was checked in the equipment of the full-scale setups installed in two hospitals located in Hokkaido. In these case, the water treatment system had already been confirmed to meet the criteria of chemical contaminants at the RO permeate stage (Tables 4 and 5). However, some chemical contaminants, such as nitric acid, are poorly rejected by the RO membrane. In the case of treating raw water with high concentrations of nitric acid, it may be difficult to meet the criterion with only the RO membrane; however, nitric acid is well removed by EDI. It would be possible to improve the total removal ability for inorganic ions by using both RO and EDI. Moreover, although silica is not included in the 22 items specified in ISO13959, it was further removed by EDI. After treating with only the RO membrane, the conductivity of the RO permeate was approximately 0.5–1 mS/m. In contrast, the conductivity after treating with RO and EDI was 0.006–0.01 mS/m and the

concentration of inorganic ions was reduced by at least 99% (Table 6). In the future, new inorganic substances may be determined to have a negative impact on patients. In particular, there is a need for caution in online HDF therapy.

Microbial contaminants

Removal and desorption of bacteria

We previously reported on the relation between the DC current and removal ability for viable bacteria.[19] The number of viable bacteria was counted using the fluorescent direct counting method. As the DC current increased, the removal ratio of viable bacteria also increased (Figure 10). Because viable bacteria have a negative charge, if the DC current value becomes higher, they should be strongly attracted to the anode. In addition, dead bacteria were adsorbed onto the ion-exchange resins or ion-exchange membranes during EDI. When the DC voltage supplied to the EDI system was switched off, the viable bacteria and dead bacteria retained in the EDI desalination chambers were rapidly released.[19]

The removal ability of EDI for viable bacteria was checked using the equipment of the bench-scale setup and the full-scale setup installed in the hospital located in Chiba-ken. The removal ratio of viable bacteria was 95% in the equipment of the bench-scale setup. Additionally, in the equipment of the full-scale setup, there were no viable bacteria per 100 ml of sample at the RO permeate stage (Table 7). This result was under 1/10,000 of the criterion for viable bacteria specified in ISO13959. However, EDI initially has very strong adsorption ability, but its adsorption ability gradually decreases during operation.

Next, by using the equipment of the full-scale setup, we investigated the number of viable bacteria in the EDI desalinated water after switching off the unit. Desorption completed over 30 min after the start of desorption (Figure 11). In the full scale setup, a desorption time of approximately 30 min is necessary. This slow desorption rate of viable bacteria may be due to regrowth of viable bacteria in the EDI desalination chamber. Although the regrowth of viable bacteria is restrained by water desorption phenomenon, there is a possibility that regrowth can partially progress.

Removal and desorption of endotoxins

We previously reported on the relation between the DC current and removal ability for endotoxins.[19] Endotoxins were measured via turbidimetry of the LAL test. As the DC

current increased, the removal ratio of endotoxins also increased (Figure 12). As with viable bacteria, endotoxins have a negative charge, so they should be strongly attracted to the anode.[19]

The removal ability of EDI for endotoxins was checked using the equipment of the bench-scale setup, the pilot-scale setup and the full-scale setups installed in the hospitals located in Chiba-ken and Ibaraki-ken. The removal ratio of endotoxins was 98% in the equipment of the bench-scale setup. In the equipment of the full-scale setup installed in the hospital located in Ibaraki-ken, there was less than 0.001 EU/ml at the RO permeate stage (Table 8). This result meets the criterion for endotoxins specified in ISO13959. Because viable bacteria release endotoxins upon death, if viable bacteria were killed by EDI, the number of endotoxins in the EDI desalinated water should rise. However, because the number of endotoxins in the EDI desalinated water decreased, most of the viable bacteria were not killed during EDI.

Next, we investigated whether endotoxins passed through the ion-exchange membrane or remained in the EDI desalination chamber using the equipment of the pilot-scale setup. Although inorganic ions can pass through the ion-exchange membrane, effectively no water can pass through the ion-exchange membrane. However, the molecular weights of endotoxins are quite big compared with inorganic ions, restricting their ability to pass through the ion-exchange membrane. Therefore, we measured the concentration of endotoxins in the EDI concentrated water. Because the volume of the EDI concentrated water becomes approximately 10% of the volume of the EDI desalinated water, if all substances in the raw water pass through the ion-exchange membranes, the concentration of these substances in the EDI concentrated water becomes approximately 10 times that in the RO permeate. When the DC current value was 6 A, the number of endotoxins in the EDI concentrated water did not increase to 10 times that in the RO permeate (Figure 13). From this result, we confirmed that endotoxins cannot pass through the ion-exchange membrane.

When the DC voltage supplied to the EDI system was switched off, the endotoxins retained in the EDI desalination chamber were rapidly released.[19] At this stage, we investigated the release rate of endotoxins from the EDI desalination chamber. The equipment of the full-scale setup installed in the hospital located in Chiba-ken was switched off and the endotoxins in

the EDI desalinated water were measured by colorimetry of the LAL test. It was confirmed that desorption from the EDI system completed after only 2 min, as shown in Figure 14. Compared to viable bacteria, the desorption rate of endotoxins was confirmed to be very fast.

Quantitative analysis of bacterial DNA and removal by EDI

We succeeded to quantify trace amount of single-stranded bacterial DNA in WFH by concentrating the samples. We checked the removal ability of EDI for single-stranded bacterial DNA using the equipment of the bench-scale setup and the full-scale setups installed in the hospitals located in Chiba-ken and Ibaraki-ken (Table 9). In the full-scale setups, the concentrations of single-stranded bacterial DNA in the raw water and RO permeate were 5–7 ng/mL and 0.5 ng/mL, respectively. The concentration of single-stranded bacterial DNA in the EDI desalinated water was less than 0.5 ng/mL. From the results of these experiments, we confirmed that single-stranded bacterial DNA could also be removed by EDI. Because bacterial DNA is also negatively charged, it is likely attracted to the anode and retained in the EDI desalination chamber. As UF membranes cannot effectively remove single-stranded bacterial DNA, EDI has a big advantage in that it can also remove bacterial DNA.

Regeneration of EDI

The ion-exchange resins are continuously regenerated by supplying the DC voltage to the EDI system, and inorganic ions are continuously discharged from the EDI concentration chamber. Additionally, when the DC voltage to the EDI system was switched off, high concentrations of bacteria and endotoxins were immediately released into the EDI desalinated water. Therefore, viable bacteria and endotoxins must be periodically released from the EDI desalination chamber. WFH needs to be manufactured in accordance at the time of hemodialysis therapy. Thus, the operating time of WFH depends on the time of hemodialysis therapy and preparation. For this reason, the desorption time is decided in each case and the EDI desalination chamber should be perfectly regenerated in the desorption time. Specifically, leaving the EDI system unpowered for 30 min seems necessary to purge the EDI desalination chamber of bacteria. However, because 30 min is long, it is desirable to

inhibit the regrowth of viable bacteria by periodic chemical or hot water disinfection,

Resistivity

As shown in Figure 15, when the DC current is raised, the resistivity of the EDI desalinated water falls owing to excessive water splitting. While maintaining high resistivity, we must set an appropriate current value that still allows for the removal of bacteria and endotoxins.

CONCLUSION

In this study, we investigated the application of EDI to the production of WFH based on the knowledge that EDI could remove both viable bacteria and endotoxins. As part of this study, water treatment systems that meet the criteria for chemical contaminants of WFH were installed in two hospitals in Hokkaido. However, inorganic ions, which may adversely affect patients, are not considered in these criteria. In particular, more stringent water quality is required in online HDF. In addition to microbial contaminants addressed in ISO standards, we could quantify trace amounts of single-stranded bacterial DNA in WFH using a fluorescence stain reagent (OliGreen). UF membranes cannot effectively remove single-stranded bacterial DNA because its molecular weight is very small; however, we found that EDI can remove single-stranded bacterial DNA. Similar to endotoxins, bacterial DNA remains in the EDI desalination chamber.

Thus, we were able to confirm the effectiveness of the water treatment system established by adding EDI after RO. In the near future, RO plus EDI systems have the potential to become a standard water treatment system for WFH. This work also provokes multiple opportunities for future work. First, EDI initially has very strong adsorption ability, but its adsorption gradually ability decreases during operation. Therefore, it remains necessary to evaluate its adsorption ability after becoming stable. Second, our experience suggests it is necessary to enhance quantification sensitivity for single-stranded bacterial DNA. Finally, the DC current used for EDI must be optimized, maximizing both the resistivity of the EDI desalinated water and the removal ability of EDI for the chemical contaminants and microbial contaminants.

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References

1. Masakane, I. (2014) An overview of regular dialysis treatment in Japan as of 31 December 2014. Japanese Society for Dialysis Therapy.

2. Kawanishi, H.; Masakane, I.; and Tomo, T. (2009) The New Standard of Fluids for Hemodialysis in Japan. *Blood Purif.*, 27(1): 5–10.

3. ISO13958: Concentrates for hemodialysis and related therapies, 2014

4. ISO13959: Water hemodialysis and related therapies, 2014.

5. ISO11663: Quality of dialysis fluid for hemodialysis and related therapies, 2014.

6. ISO26722: Water treatment equipment for hemodialysis applications and related therapies, 2014.

7. ISO23500: Guidance for the preparation and quality management of fluids for hemodialysis and related therapies, 2011.

8. Schindler. R. (2009) Clinical Effect of Purification of Dialysis Fluids, Evidence and Experience. *Blood Purif.*, 27 (1): 20–22

9. Ase, T.; Ohkouchi, Y. (2016) Behavior of the bacteria which exist in permeate of RO modules formed like spiral wound. The 50th Annual Conference of Japan Society on Water Environment (proceeding), 102.

10. Navarro, M D.; Carracedo, J.; Ramirez, R.; Madueno, J A.; Merino, A.; Rodriguez, M.; Martin-Malo, A.; and Ajama, P. (2007) Bacterial DNA prolongs the survival of inflamed mononuclear cells in haemodialysis patients. *Nephrol. Dial. Transplant.*, 22: 3580–3585.

11. Handelman, G J.; Megdal, P A.; and Handelman, S K. (2009) Bacterial DNA in Water and Dialysate:Detection and Significance for Patient Outcomes. *Blood Purif.*, 27: 81–85.

12. Bossola, M.; Sanguinetti, M.; Scribano, D.; Zuppi, C., Giungi, S.; Luciani, G.; Torelli, R.; Posteraro, B.; Fadda, G.; and Tazza, L. (2009) Circulating Bacterial-Derived DNA Fragments and Markers of Inflammation in Chronic Hemodialysis Patients. *Clin. J. Am. Soc. Nephrol.,* 4

(2): 379–385.

13. Kwan, B.C.; Chow, K.; Ma, T.K.; Cheng, P.M.; Leung, C.; Li, P. K. and Szeto, C. (2013) Effect of Using Ultrapure Dialysate for Hemodialysis on the Level of Circulating Bacterial Fragment in Renal Failure Patients. *Nephron Clin. Pract.*, 123: 246–253.

14. Hazzah, W.A.; Hashish, M.H.; El-Koraie, A.F.; Ashour, M.S. and Abbass, A.A. (2015) Circulating Bacterial DNA Fragments in Chronic Hemodialysis Patients. *Saudi J. Kidney Dis. Transpl.*, 26 (6): 1300–1304.

15. Schindler, R.; Beck, W.; Deppisch, R.; Aussieker, M.; Wilde, A.; Goel, H.; and Frei, U. (2004) Short Bacterial DNA Fragments: Detection in Dialysate and Induction of Cytokines. *J. Am. Soc. Nephrol.*, 15 (12): 3207–3214.

16. Ding, Y.S.; Lo, Y.; Miller, J.J.; Rohde, J.; Karoor, S.; and Dupont, S. (2014) Hemodialysis and peritoneal dialysis systems having electrodeionization capabilities. *U.S. Patent No. 8,858,792*.

17. Lin, R.; Ding, Y.S.; White, J.M.; Chen, Y.; Lo Y.; Miller, J.; and Rohde, J. (2015) Hemodialysis and peritoneal dialysis systems having electrodialysis and electrodeionization capabilities. *U.S. Patent No. 9,005,440.*

18. Lv, W.; Huang, G.; Liu, Y.; Chen, X.; and Wang, S. (2007) Design of a hemodialysis water treatment system with RO-EDI. *Technol. Water Treat.* , 33: 52-54.

19. Ase, T.; Harada, N.; Otomo, T.; Watabe, T.; Sato, T.; and Takemura, T. (2011) Removal of Viable Bacteria and Endotoxins by Electro Deionization(EDI). *Biocontrol Sci.*, 16 (3): 109–115.

20. Ase, T.; Otomo, T.; Noguchi, T.; Onoue, M.; Kuwabara, T. and Sato, T. (2013) Study on Relation Between Disinfection and Inactivation Endotoxin Mechanisms and Water Dissociation phenomenon of Electro De-ionization(EDI) Method as Complement Technology in Production of Undergo Dialysis Water. *J. Antibact. Antifung. Agents*, 41 (12): 647–657.

21. Katai, Y,; Miyamoto, D,; Sasai, R. and Sato, T. (2016) Relation between the Disinfection and Endotoxin Inactivation Effects of the Electro De-ionization(EDI) Method and the Water Splitting Phenomenon. *J. Antibact. Antifung. Agents*, 44 (1): 3–7.

22. Sato, T.; Tanaka, T.; Iwata, Y.; Goto, S. and Suzuki, T. (1986) Disinfection of E.coli by Using Surface Characteristics of H^+ and OH^- Form Ion-Exchange Resins II. *Electrochemistry*,

54: 269–273.

23. Ganzi, G.C. and Parise, P.L. (1990) The Production of Pharmaceutical Grades of Water Using Continuous Deionization Post-Reverse Osmosis. *J. Parenter. Sci. Technol.*, 44 (4): 231–241.

24. Glorieux, G.; Schepers, E.; Schindler, R.; Lemke, H.; Verbeke, F.; A., Lameire, N. and Vanholder, R.(2009) A novel bio-assay increases the detection yield of microbiological impurity of dialysis fluid, in comparison to the LAL-test. *Nephrol. Dial. Transplant.*, 24: 548–554.

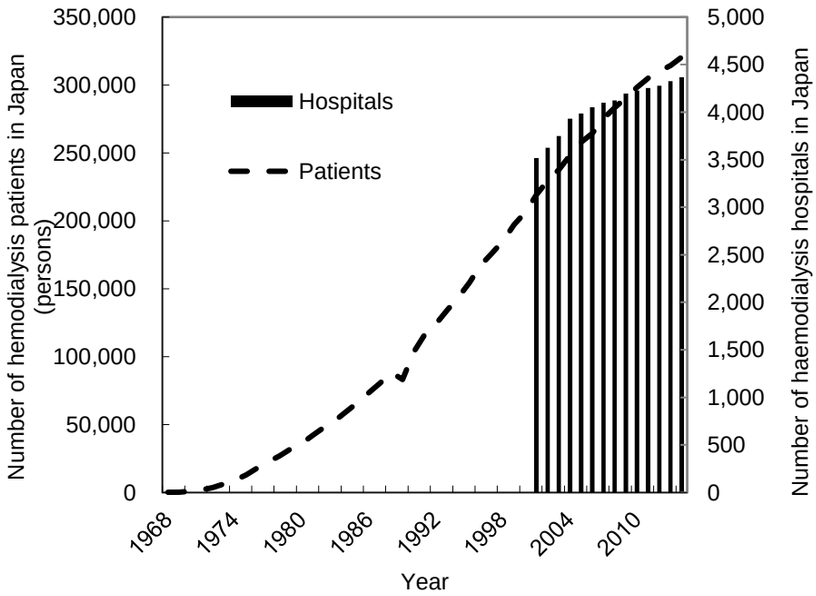

Figure 1. Hemodialysis patients and hospitals in Japan (we do not have the data of hospitals before

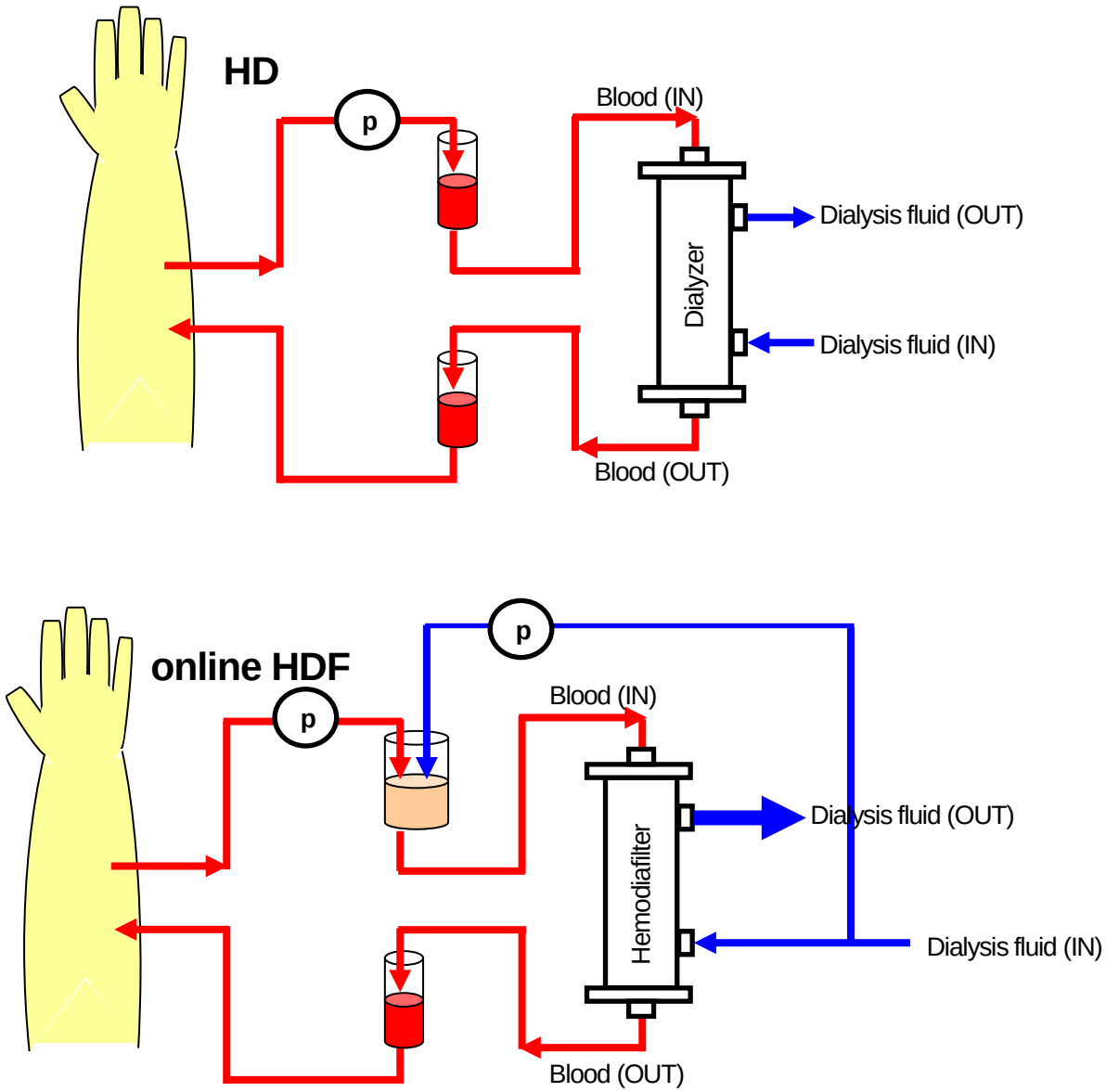

Figure 2. The difference between HD and online HDF

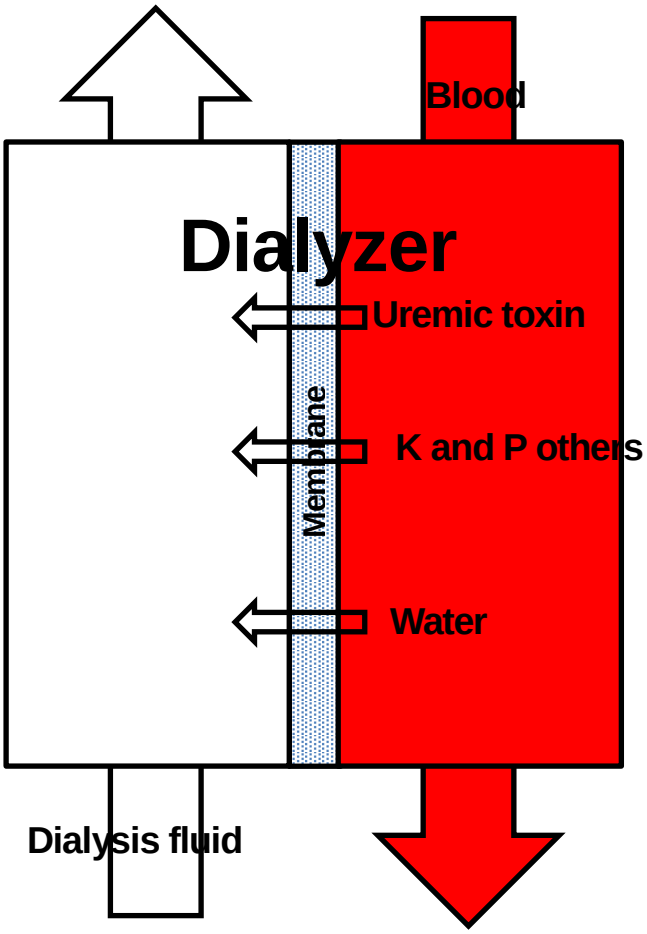

Figure 3. Principle of HD therapy

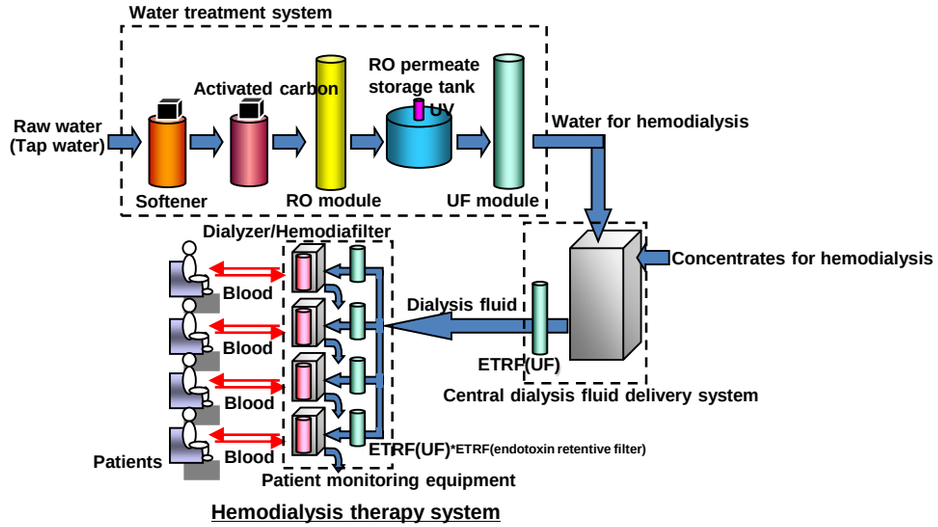

Figure 4. Water treatment system in HD therapy

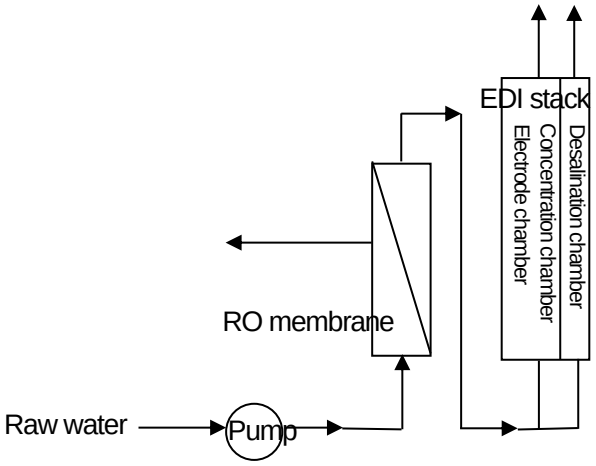

Figure 5. Bench scale equipment

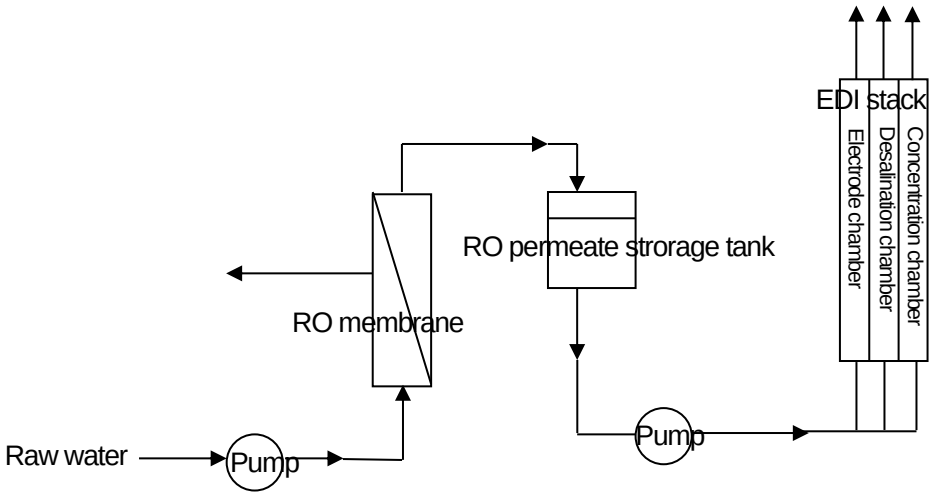

Figure 6. Pilot scale equipment

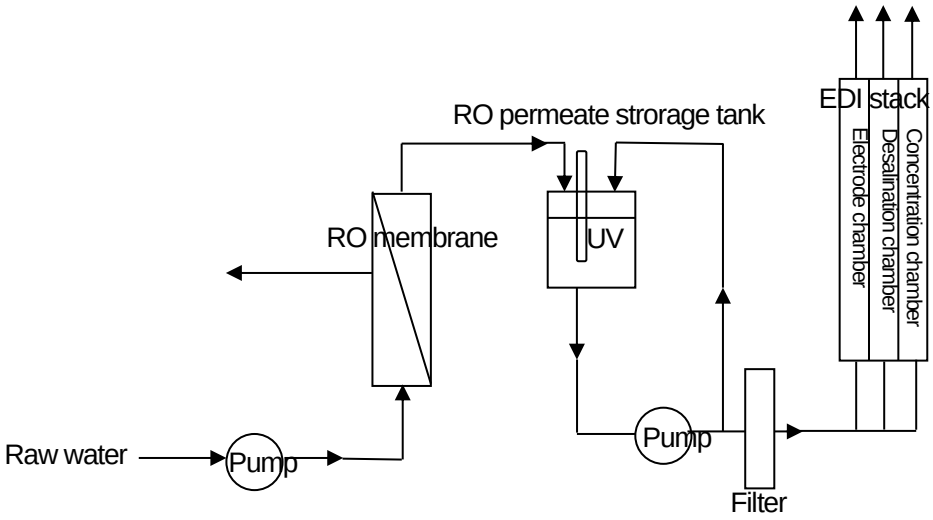

Figure 7. Full scale equipment

(Left:XL-100, Center:Zap-10, Right:XL-500)

Figure 8. EDI stack photo

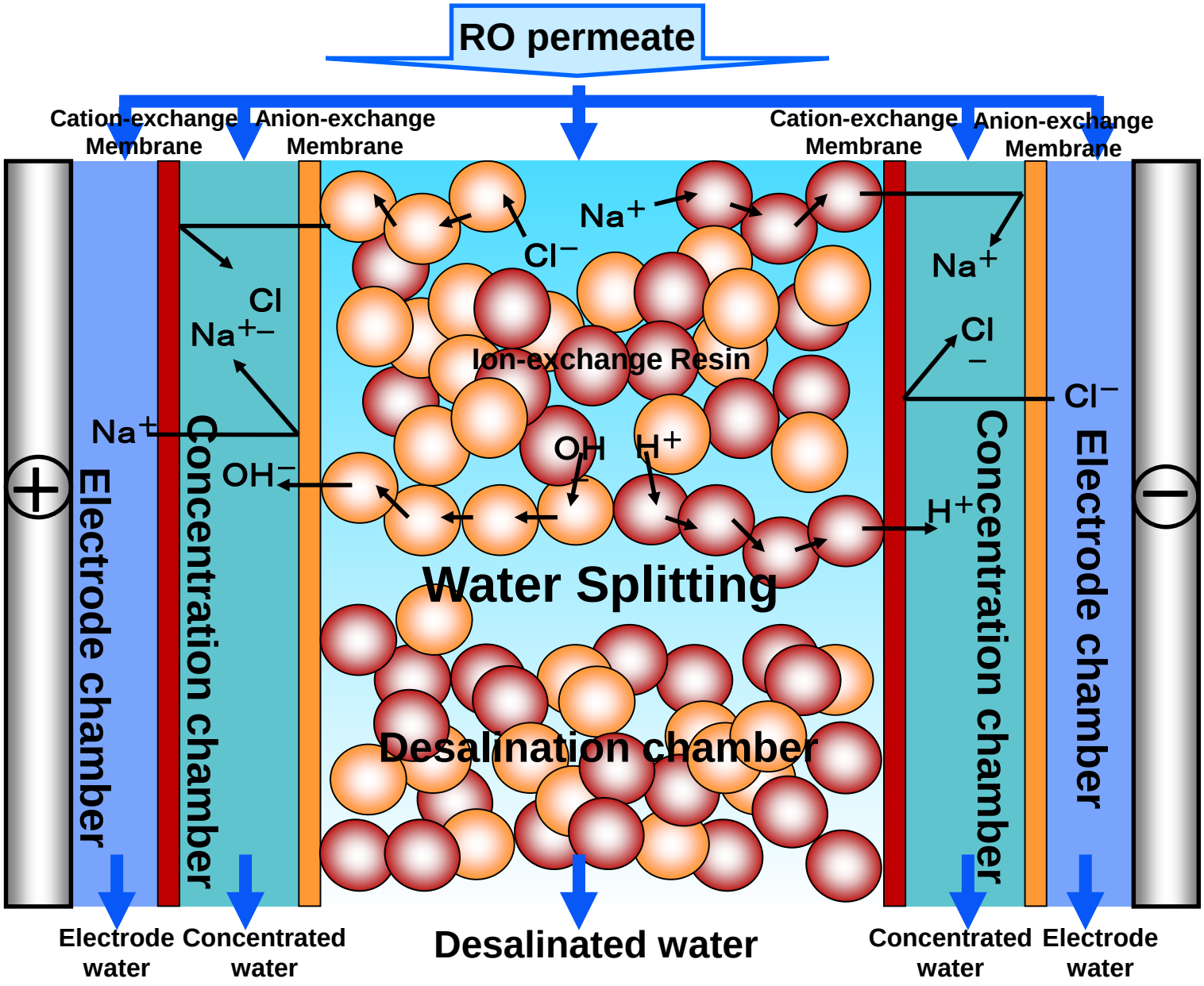

Figure 9. EDI stack

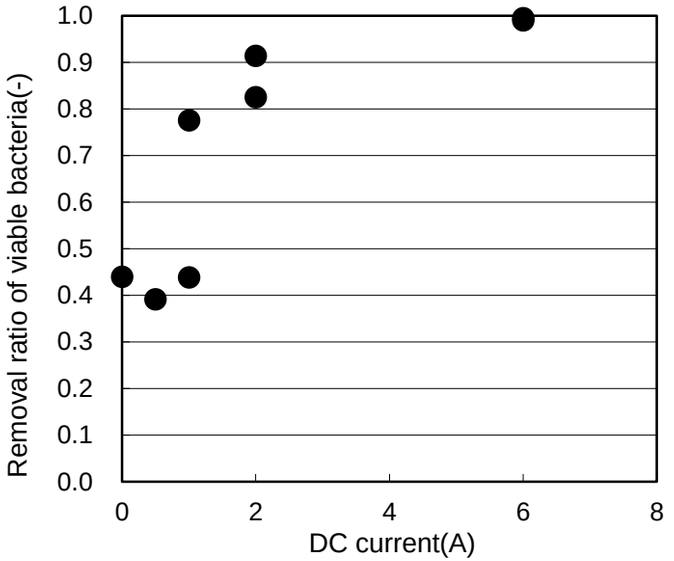

Figure 10. Effect of the DC current on bacteria removal (Source: Reference 16, p.112)

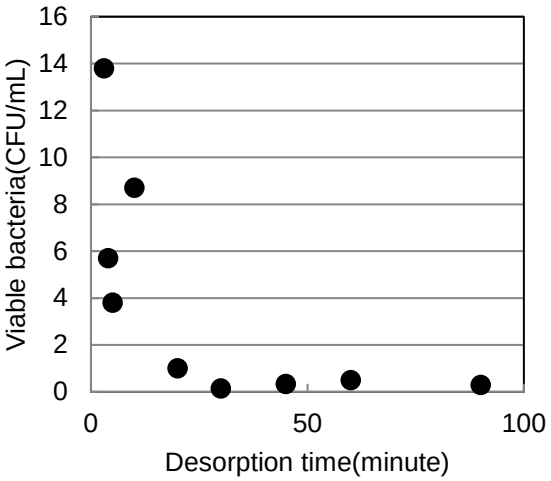

Figure 11. Desorption of viable bacteria from the EDI desalination chamber

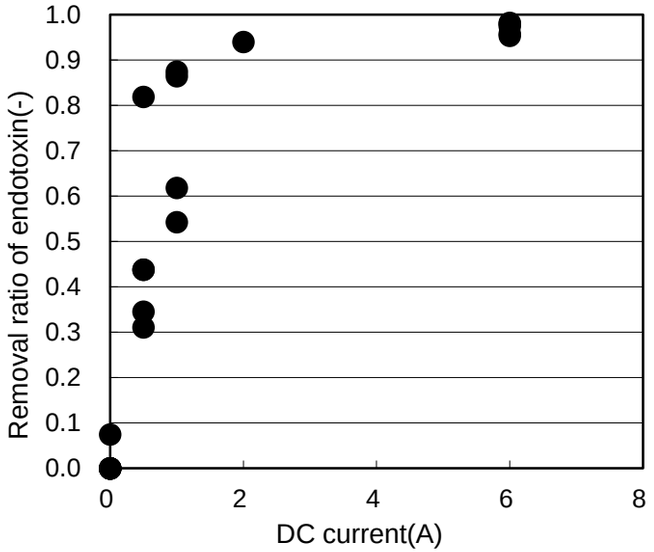

Figure 12. Effect of the DC current on endotoxin removal
(Source: Reference 16, p.111)

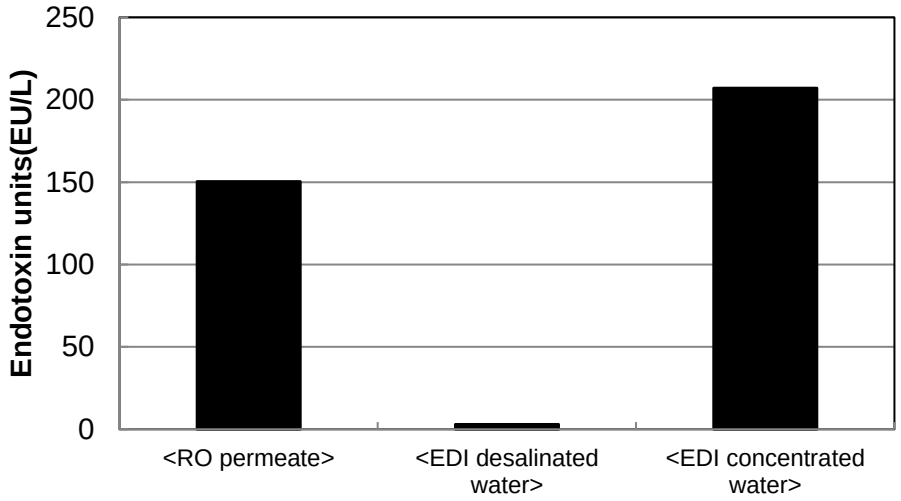

Figure 13. Endotoxins in the RO permeate,EDI desalinated water and EDI cocentrated water(DC current 6A)

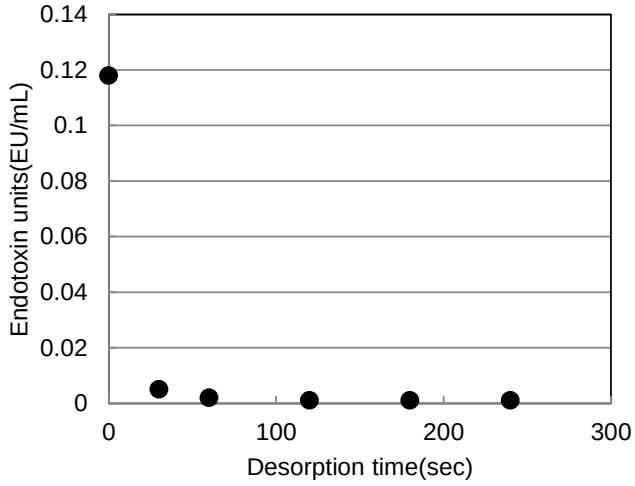

Figure 14. Desorption of endotoxins from the EDI desalination chamber

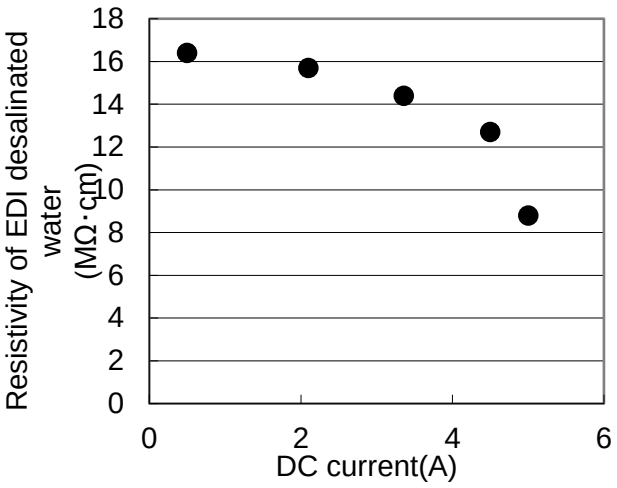

Figure 15. Resistivity of EDI desalinated

Table 1. Requirments of chemical contaminants

Contaminants	Maximum concentration (mg/l)
Contaminants with documented toxicity in hemodialysis	
Aluminum	0.01
Total chlorine	0.10
Copper	0.10
Fluoride	0.20
Lead	0.005
Nitrate (as N)	2.0
Sulfate	100
Zinc	0.10
Electrolytes normally included in dialysis fluid	
Calcium	2
Magnesium	4
Potassium	8
Sodium	70
Trace elements in dialysis water	
Antimony	0.006
Arsenic	0.005
Barium	0.1
Beryllium	0.004
Cadmium	0.001
Chromium	0.014
Mercury	0.0002
Selenium	0.09
Silver	0.005
Thallium	0.002

Table 2. Requirments of microbial contamainants

	Microbiological requirements	Action level
Viable bacteria (CFU/ml)	<100	<50
Endotoxin (EU/ml)	<0.25	<0.125

Table 3. Specification of equipment

Equipment	Type of EDI stack	Size of EDI stack (W × D × H)	Electrode surface area (cm^2)	Number of cells	Maximum flow (L/hr)
Bench scale	Zap-10	7 cm × 7 cm × 25 cm	31.2	3	10
Pilot scale	XL-100	22 cm × 17 cm × 52 cm	458	5	150
Full scale	XL-500	22 cm × 37 cm × 52 cm	458	48	2,300

Table 4. Chemical analysis of A hospital

Contaminants	Raw water (mg/l)	RO Permeate (mg/l)	EDI desalinated water (mg/l)
Aluminum	<0.01	<0.01	<0.01
Total chlorine	0.2	<0.05	<0.05
Copper	<0.1	<0.1	<0.1
Fluoride	0.08	<0.08	<0.08
Lead	<0.001	<0.001	<0.001
Nitrate(as N)	0.4	<0.1	<0.1
Sulfate	31.9	0.2	0.2
Zinc	<0.1	<0.1	<0.1
Calcium	0.2	<0.1	<0.1
Magnesium	<0.1	<0.1	<0.1
Potassium	2.5	<0.1	<0.1
Sodium	52.8	0.8	<0.1
Antimony	<0.0015	<0.0015	<0.0015
Arsenic	0.004	<0.001	<0.001
Barium	<0.1	<0.1	<0.1
Beryllium	<0.0004	<0.0004	<0.0004
Cadmium	<0.001	<0.001	<0.001
Chromium	<0.005	<0.005	<0.005
Mercury	<0.00005	<0.00005	<0.00005
Selenium	<0.001	<0.001	<0.001
Silver	<0.005	<0.005	<0.005
Thallium	<0.002	<0.002	<0.002
Silica	26.4	0.75	<0.01
TOC	0.5	<0.3	<0.3

Table 5. Chemical analysis of B hospital

Contaminants	Raw water (mg/l)	RO Permeate (mg/l)	EDI desalinated water (mg/l)
Aluminum	<0.01	<0.01	<0.01
Total chlorine	0.2	<0.05	<0.05
Copper	<0.1	<0.1	<0.1
Fluoride	0.11	<0.08	<0.08
Lead	<0.001	<0.001	<0.001
Nitrate(as N)	<0.1	<0.1	<0.1
Sulfate	2.1	0.2	0.2
Zinc	<0.1	<0.1	<0.1
Calcium	0.2	<0.1	<0.1
Magnesium	<0.1	<0.1	<0.1
Potassium	1.5	<0.1	<0.1
Sodium	56.0	0.7	<0.1
Antimony	<0.0015	<0.0015	<0.0015
Arsenic	0.002	<0.001	<0.001
Barium	<0.1	<0.1	<0.1
Beryllium	<0.0004	<0.0004	<0.0004
Cadmium	<0.001	<0.001	<0.001
Chromium	<0.005	<0.005	<0.005
Mercury	<0.00005	<0.00005	<0.00005
Selenium	<0.001	<0.001	<0.001
Silver	<0.005	<0.005	<0.005
Thallium	<0.002	<0.002	<0.002
Silica	45.1	1.10	0.12
TOC	0.8	<0.3	<0.3

Table 6. Conductivity of RO permeate and EDI desalinated water

	Conductivity (mS/m)	
	RO permeate	EDI desalinated water
Full scale	0.506	0.0055

Table 7. Removal of viable bacteria by EDI

	Raw water (CFU/ml)	RO Permeate (CFU/ml)	EDI desalinated water (CFU/ml)
Bench scale	No data	2	0.1
Full scale	286	<0.01	<0.01

Table 8. Removal of endotoxins by EDI

	RO Permeate (EU/ml)	EDI desalinated water (EU/ml)
Bench scale	0.131	0.002
Pilot scale	0.016	0.002
Full scale	<0.001	<0.001

Table 9. Removal of bacterial DNA by EDI

	Raw water (ng/ml)	RO permeate (ng/ml)	EDI desalinated water (ng/ml)	Quantitative lower limit (ng/ml)
Bench scale	3.0	5.9	0.3	0.1
Full scale (Chiba-ken)	5.6	0.5	0.3	0.1
Full scale (Ibaraki-ken)	7.3	0.5	N.D.	0.5