

DECONTAMINATION METHOD AND COMPOSITION THEREFOR

FIELD OF THE INVENTION

This invention relates to methods and compositions for carrying out decontamination procedures on apparatus and equipment that has been in contact with biological materials, in particular blood and blood products. More specifically, the invention relates to an improved procedure (which may be operated manually and/or automatically) for reprocessing used dialysers.

BACKGROUND OF THE INVENTION

Extracorporeal hemodialysis therapy provides today a means whereby patients with end stage renal failure can be sustained for prolonged periods by removing the body uremic toxins. The principle of hemodialysis involves the circulation of blood and of dialysis fluids within a dialysis machine on the opposite sides of a membrane device (called a "hemodialyser"). The hemodialyser permits the passage of metabolites which become elevated as the consequence of renal failure, but restricts the transfer of blood proteins and cells. The therapy is in general applied three times a week for a duration of four hours per session.

The hemodialyser plays a central role in hemodialysis by determining the ultrafiltration rate and the selective effect required for removal of uremic toxins. Hemodialysers vary from one another in many respects, in particular in respect of the material used to form the membrane. These materials have a wide range of chemical compositions, but generally, the nature of the material determines the blood-compatibility or biocompatibility, the membrane pore structure, the membrane sieving, and the membrane transport properties. The membranes are

in general made from regenerated cellulose, modified cellulose or synthetic (plastics) materials. The membrane may be constructed in a wide variety of configurations, but the most usually membrane hemodialysers are in the form of hollow fibers which are encased in tubular housing made with polycarbonate.

In order to form two compartment in the device, the ends of the fibers are embedded in polyurethane binding material. The fibre bundle is bonded at the two end of the housing and a skew cap manifold may be used.

Blood enters and leaves the device via manifolds which are designed to optimize both blood velocity and pressure drops at all points in the manifold thereby ensuring an even distribution of the blood in the fibre bundle.

Hemodialysis devices are in general intended for only a single use. However, with the increased health care service in renal replacement therapy, attempts have been made on economical grounds to adopt procedures for reusing the hemodialyser more than once in order to reduce costs.

In view of the danger of parenteral as well a non-parenteral virus transmission between patients that might result from the reusing hemodialysers, reusable hemodialysers are restricted for use for a single patient. Equipment designed to carry out dialysis using reusable hemodialysers are commercially available and gradually, the reprocessing and reusing of new dialysers has become more widespread in clinical practice. The cost benefits achieved by reprocessing dialysers are significant, and the costs of dialysis can be reduced by approximately 75% by reprocessing and reusing dialysers 10 to 20 times.

Many procedures are available for preparing reusable dialysers (and reusable disposable hemodialyser blood line connections), including manual and automatic procedures. For reasons of efficiency, hemodialysis centers use machines which process the regeneration of many hemodialyser devices simultaneously. All of these machines generally operate using the following sequence of steps:

1. washing and flushing the blood compartment of the dialyzer with water;
2. ultrafiltration of water from the dialysate compartment to the blood compartment;
3. removing residual blood in the blood compartment;
4. washing and filling the dialyzer with a chemical sterilant to sterilize and prevent the growth of germs during storage;
5. washing the sterilant before the next use.

After the regeneration procedures, the dialysers should be stored until the next hemodialysis. The disinfection step employs chemicals which are effective for killing of microorganisms (bacteria and fungi) which may contaminate the blood compartment of the dialyser during the reprocessing. The disinfectant chemicals should also prevent the growth of microorganisms during the storage of the regenerated hemodialyser.

The quality control of these methods involves the determination that: 1) the regenerated hemodialyser is equivalent to a new dialyzer in terms of performance and in terms of biocompatibility of the dialysis membrane, and 2) sterilization of the reprocessed hemodialyser. These controls are performed by measuring the volume of the blood compartment, clearance data and blood compatibility parameters.

Generally, it is important to note that all of the chemical disinfectants used in reusing machines are oxidising agents (reactive oxygen substances) which are classified as toxic substances. The washing out of these substances before reusing the regenerated dialysers remains a crucial point, especially to ensure that long term clinical adverse clinical effects are to be avoided.

In the prior art various attempts have been made to develop suitable methods of reprocessing hemodialysers in combination with machines which could

be used to automatically clean and sterilize the disposable hemodialyser and the blood line.

In the prior art relating to sterilants generally, the conventional methods of controlling microbial growth involve the use of high concentrations of organic biocides or germicides (many of these are well known in the food industry). The substances are in general oxidative chemicals and act on the microorganisms in many ways by attacking the cell wall, or the cytoplasmic membrane, or vital cellular constituents. In medical application, aqueous or aqueous-ethanol peracetic acid solutions have been used typically to sterilize many surfaces of instruments (see, e.g. Machelwsky, P.S., *Artificial Organs* 1993, 17, pages 147-152). The approach of using these disinfecting solutions in reprocessing hemodialysers has been documented in "AAMI Recommended Practice for Reprocessing Hemodialyzers" (*Association for the Advancement of Medical Instrumentation, Va, 1993*). The methods which have been proposed involve the application of high concentrations of formaldehyde, glutaraldehyde, peracetic acid and hydrogen peroxide.

Another approach for disinfecting hemodialysers that involves heating (instead of using germicidal sterilants) has also been proposed in the prior art by Kaufman *et al.* (Clinical Experience with Heat Sterilization for Reprocessing; *ASAIO* 1992; Vol 38; No 3; pages M338-M340). In this procedure, the hemodialyser is heated above 100°C for approximately 20 hours. Although this procedure eliminates the toxicity problems, it suffers from the fact that many hemodialyser membranes are damaged during the heating process.

In the prior art by Levin (US Patent No. 5,609,100), an improved method is described which involves using an aqueous solution containing a less toxic chemical such as citric acid and then subjecting the dialyzer to a reduced elevated temperature below 100 °C (95°C).

The prior art relating to reuse systems focused on the technology principles of reuse apparatus which could be utilized in conjunction with hemodialysis machines and/or on apparatus which could automatically clean, flush and disinfect the hemodialyser, the blood lines and the dialysis machine. The prior art did not focus on the nature of the sterilants used, but instead concentrated on designing apparatus which could be utilized as a close system while using toxic chemicals in high concentration. For instance, Mellot (US Patent No. 3,753,493) discloses a automatic cleaning apparatus based on a arrangement of valves, timer, and solenoids. Shaldon (US Patent No. 3,871,913) discloses a reuse device which is connected in parallel with the dialysis machine and can be used after the therapy session in such a way that both dialyzer compartments can be flushed, cleaned and sterilized. Hardy (US Patent No. 4,166,031) also disclosed a device with a parallel flow arrangement for cleaning, flushing, and sterilizing. Mason (US Patent No. 3,920,030) discloses a device using tubular pumps for moving solutions through the dialyzer and solenoids for closing the flow. Boag (US Patent No. 4,695,385) discloses an electronic control system for a dialysis reuse device involving the working steps of the cycles and conductivity measurements as quality control. Twardowsky (US Patent No. 5,484,397) discloses a hemodialysis machine for a reusable dialyzer and blood lines and which automatically prepares a cleaning and rinsing solution from dry chemicals.

Numerous articles have been published on the multiple use of hemodialysers and on the effects of effects of different reprocessing chemicals and techniques on the dialyzer biocompatibility and performance. In this respect, it has been documented that dialysis in facilities that reprocess hemodialysers using oxidative sterilants may be associated with worse survival than dialysis in facilities that do not reprocess hemodialysers (Feldmann HI et al; JAMA; 276(8); pages 620-625). By Ng Y.Y. *et al* (Artif. Organs 1996; 20(1)). Within the growing practice of dialyzer reuse in the last few years, it has become apparent that clinical findings raise important concerns about potentially avoidable mortality among US hemodialysis patients treated in dialysis facilities reprocessing hemodialysers. Problems related to the reusing of hemodialysers has been documented to involve

chronicle adverse reactions due to use of high concentrations of biocides. For instance, it seems also that with the increased application of reprocessing hemodialysers the risk of allergic and pyrogenic reactions appears to be increased (Klinkman H et al; Artif Organs 1996; 20(5); pages 426-432). Biocides are in general highly toxic in the quantities known to be required for effective control of microbial growing. In addition environmental regulations restrict the amount of chemicals that can safely be discarded into the environment. A number of clinical studies have focused on the oxidative stress in hemodialyzed patients and the long-term effects of dialyzer reuse practice. In this respect Sies H (Am J Med 1991, 91 (suppl 3C) pages 31-37) demonstrated a shift in the oxidant/antioxidant balance. Other studies include (Trznadel *et al.*, Free Radi. Biol. Med. 1990, 8, pages 429-432; Köse K et al, Clinical Biochemistry 1997, 30(8), pages 601-606). An additional problem involved with the practice of oxidative sterilant is their ability to cross-link proteins (Kato *et al.*, Journal Biol. Jt. Surgery 1991,73: pages 561).

In summary, problems are associated with known sterilants as regards the quantities that need to be employed, as well as with the high concentrations that are needed. As regards the concentrations needed, the known chemical sterilization methods react chemically with the used dialyser and produce chemical by-products within the reused dialyzer. These by-products are believed to be associated with adsorbed proteins that are present on the surfaces of the dialyser.

In view of the foregoing state of the art it is one objective of this invention to overcome problems associated with the oxidative stress due to the use of oxidative chemicals. This seem to be the key factor involved in adverse reactions observed by patients dialyzed with reused devices.

Thus we have determined that a key problem is that the oxidative chemicals used for disinfection oxidize adsorbed protein in the dialysers membrane during sterilization and storage. The oxidized adsorbed proteins may enter the patients and may react extracorporally with the biological system, thus increasing the risk of adverse reactions (including the contact phase activation due to the interaction

between the blood and oxidized protein at the membrane level). The effects of oxidative chemicals are poorly tolerated in uremic disease (Richard, M.S. *et al.*, Nephron 1991, 57, pages 10-15; Trnadel, K. *et al.*, Free Radic Biol Med 18989, 6, pages 393-397; Paul, J.L., Nephron 1993, 64, pages 106-109; Saint-Georges *et al.*, Kidney Int. 1989, 27, pages 274-277). This is because the uremic condition, is exacerbated by any oxidation/antioxidation imbalance.

Therefore, a need exists in hemodialysis for controlling the growth of microorganism in reused dialysers but also to improve the safety of the regeneration process in terms of acute and chronicle adverse reactions in patients. The ideal sterilant must be able to sterilize without adversely affecting the device and must be able to sterilize the dialyzer membrane (which may have been coated through many uses with human proteins) without altering the essential physical and biological nature of these proteins, especially the reaction of protein oxidation.

SUMMARY OF THE INVENTION

According to one aspect of the present invention, there is provided a method for decontaminating a surface of device that has been in contact with a biological fluid which comprises contacting the surface with one or more aqueous disinfectant solutions characterised, in that at least one of the solutions comprises a germicidal sterilant and at least one of the solutions comprises urea. Typically, in carrying out the method of the invention, the surface is contacted with an aqueous disinfectant solution comprising both a germicidal sterilant and urea in a single solution. Thus, in this embodiment, the invention comprises contacting the surface with one or more aqueous disinfectant solutions characterised, in that at least one of the solutions comprises both a germicidal sterilant and urea.

The germicidal sterilant is preferably selected so as to result in a synergism with urea to prevent the growth of bacteria and to prevent the oxidation of proteins. Thus, for example, the germicidal sterilant may be selected from peracetic

acid formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bithiocyanate, carbamate, DBNPA, quaternary ammonium compounds, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one, and 4,5-dichloro-1,2-dithio-3-one.

The concentration of urea (weight per volume) is preferably in the range between 50-200 mmol/l and the concentrations of the germicidal sterilant is less than 300 and preferably less than 200 ppm.

The method described above is of particular utility in preparing hemodialysis apparatus for reuse, i.e. the "device" referred to is a part of a dialysis apparatus.

Thus according to a further aspect, the invention provides a method for performing a biocompatible reprocessing operation on a hemodialysis device to allow its reuse and of controlling the growth of bacteria therein, comprising the step of introducing urea and a sterilant into the hemodialyser.

The reprocessing operation preferably includes the step of filling the blood and dialysate compartments of the hemodialysis device with a aqueous solution containing a mixture comprising urea and a germicidal sterilant. The concentration of urea (weight per volume) is preferably in the range between 50-200 mmol/l.

It has been found the combined use of a germicidal sterilant and urea results in a synergism which prevents the growth of bacteria and prevents the oxidation of proteins. This synergistic effect allows the concentration of germicidal disinfectant to be reduced to less than 300 ppm, whereby achieving inhibition of the growth of microorganisms but with no measurable oxidative effects on adsorbed proteins during sterilization and storage.

The germicidal sterilant is preferably peracetic acid, but other sterilants may be used, such as formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bithiocyanate, carbamate, DBNPA, a quaternary ammonium compound, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one, or 4,5-dichloro-1,2-dithio-3-one.

A typical regeneration procedure according to the invention includes the step of filling the blood and dialysate compartments of the hemodialyser device with a aqueous solution containing a mixture comprising as a first component urea, as second compound a germicidal sterilant and as a third compound another germicidal sterilant. The "second compound" may be peracetic acid and the "third compound" may be selected from formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bithiocyanate, carbamate, DBNPA, quaternary ammonium compounds, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one, and 4,5-dichloro-1,2-dithio-3-one. Preferably, the total of the concentrations of peracetic acid and the third germicidal sterilant is less than 200 ppm. As a final step ascorbic acid (w/v) may be circulated through the dialyser before reuse to neutralize residues of the germicidal sterilant.

Aqueous disinfectant solutions for decontaminating hemodialysers prior to reuse, comprising a germicidal sterilant and urea form a further aspect of the invention.

In its more preferred embodiments, the present invention provides an improved method for the reprocessing of hemodialysers without the use of high levels of germicidal sterilants. According to the forgoing prior art, it is a particular objective of the invention to provide a method for reprocessing dialysers in terms of the biocompatibility of the dialyzer membrane in cleaning the dialyzer, in reducing the protein oxidation and in minimizing the risks of adverse reactions in patients. It is a further objective of the invention to provide a method for sterilizing, cleaning and reprocessing dialysers which optimizes the storage and the conservation of the biocompatibility and efficiency properties of the semi-permeable membrane.

In its more preferred aspects, the present invention further provides compositions for use in hemodialyser reprocessing machines. The composition include very low concentrations of peracetic acid or other germicide sterilants and

an effective amount of urea. The formulation of the composition of the present invention consists of a mixture of urea and a low concentration of germicidal sterilant which provides a synergistic activity against microorganisms and fungi and prevents the oxidation of adsorbed proteins. In addition to the formulation of the composition, the invention also provides a method of perfusing the dialyzer before use with a composition comprising one or more anti-oxidants.

As indicated, the present invention in its preferred embodiments provides a method for inhibiting the growth of microorganisms in reusing hemodialysers. The method includes the step of adding manually or through an automated machine to the water a very low amount of germicidal sterilant and an effective amount of urea. Combining the germicidal sterilant with urea has been found to enhance the effectiveness of the sterilization by reducing the risk of protein oxidation. In addition perfusion of the reprocessed hemodialyser (manual or through an automated machine) with an antioxidative compound such as ascorbic acid will further clean the dialyzer and neutralize possible oxidized adsorbed protein. The biocide may be chosen from the group consisting of: peracetic acid, formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bithiocyanate, carbamate, DBNPA, quaternary ammonium compounds, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one and 4,5-dichloro-1,2-dithio-3-one.

Preferentially, peracetic acid is the germicidal sterilant used. Naturally, a mixture of the germicidal sterilants can also be used.

In one embodiment, preferably peracetic acid or an other germicidal sterilant is added to the water system. Alternative germicidal sterilant to peracetic acid may be chosen from the group consisting of formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bithiocyanate, carbamate, DBNPA, isothiazolin, quaternary ammonium compounds, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one, or 4,5-dichloro-1,2-dithio-3-one. The concentration of peracetic acid or other germicidal sterilants is in the range between 10 and 500 ppm ("ppm" = 1 part per million per weight).

Preferentially, a concentration of at least 200 ppm is optimal to inhibit bacteria growth and also to reduce the risk of protein oxidation. An alternative is a mixture of two or more germicidal sterilants comprising peracetic acid and second chemicals from the group consisting of formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bithiocyanate, carbamate, DBNPA, isothiazolin, quaternary ammonium compounds, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one, and 4,5-dichloro-1,2-dithio-3-one. The total concentration of peracetic acid and the second germicidal sterilant is in the range between 100 and 500 ppm. Preferentially, a concentration of 100 ppm of peracetic acid and 100 ppm for the second sterilant is optimal to reduce the risk of protein oxidation.

In another embodiment, urea is added prior to the germicidal sterilant in the water system.

The main advantage of this invention is that by using a mixture of germicidal sterilant and urea there is provided an improved composition on the one hand for inhibiting the growth of organism in the hemodialyser and on the other hand, for desorbing protein from the dialyzer membrane. The concentration of urea used is preferably between 20 and 50 mmol/l.

In one embodiment of the invention, after reprocessing and storing, the hemodialyser is washed with a ascorbic acid solution. The main advantage of this procedure, is to neutralize the possible residual germicidal sterilants which were not removed by washing. To achieve these objectives, the invention as a result of the reduced need for germicidal sterilants, provides an improved anti-oxidative method for reprocessing, storage and preparation of dialyzers. The improved procedure comprises the steps of: flushing and washing, sterilizing with the composition of the invention, storing with the composition of the invention, washing with pure water, and flushing with an anti-oxidative solution.

Moreover, an advantage of the present invention is that it provides a more cost effective and environmentally friendly method for reprocessing hemodialysers.

Additional features and advantages of the present inventions are incorporated in the following preferred embodiments.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention provides, for reprocessing of a dialyser, improved methods and chemical compositions to be administrated in a fluid system. The invention relates to the application of a mixture of a sufficient amount of urea and low amount of peracetic acid or other biocides that exhibits a synergistic effect when added to a fluid. After reprocessing and storing the used dialyser, the invention also proposes the flushing of the dialyser prior clinical use with an antioxidative solution, preferably an acid ascorbic solution

The most suitable germicidal sterilant to be mixed with urea is peracetic acid. However, other germicidal sterilants which can be used in this invention include: peracetic acid, formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bithiocyanate, carbamate, DBNPA, quaternary ammonium compounds, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one, and 4,5-dichloro-1,2-dithio-3-one. The germicidal sterilants can be obtained from a number of chemical suppliers. The composition also may include a mixture of sufficient amount of urea, peracetic acid and second germicidal sterilant. The invention also provides a method of preparing the reprocessed dialyser with an ascorbic acid solution prior clinical use.

The invention can be used for a variety of dialysers in which the semi-permeable membrane is in the form of hollow fibers or in form of sheet or plates. In particular, the invention can be used for reprocessing dialysers for blood purification. But, it also can be used for reprocessing filters for plasmapheresis and for washing used peritoneal dialysis fluids from dialysers after use.

Urea, ascorbic acid, peracetic acid or other germicidal sterilants solutions used in the practice of the invention are preferably drug grade and dissolved in ultrapure water, i.e. water which was been treated by reverse osmosis and which has been filtered to remove bacteria and pyrogens.

The method of reprocessing the dialyser is preferably based on the use of a composition of a concentration of 20-40 mmol/l of urea with peracetic acid or other germicidal sterilant at the concentration of 200 ppm. Such use provides an unexpected synergistic effect for inhibiting the growth of microorganisms but has no measurable oxidative effects on the adsorbed proteins during sterilization and storage. The beneficial effect in the inhibition of the protein oxidations is primarily due to the fact that the oxidative germicidal sterilants is considerably reduced to a lower concentration. The synergistic relationship is present in that the cooperative action of the combined urea with peracetic acid yields a total effect which is greater than peracetic acid at 200 ppm (in this context, "ppm" means 1 part per million by weight per volume), compared to use of peracetic acid at higher concentration. The amounts of peracetic acid and urea suggested for effectiveness in this invention depend on the quality of the water to be used to make the solution, the microbiological count as determined by the colony forming units, the solution temperature, and the pH.

By way of example, a composition comprising 20-40 mmol/l urea with a mixture of peracetic acid with one or more germicidal sterilants with the end concentration of 200 ppm can be used in this invention. The synergistic relationship of urea and peracetic acid is increased by the presence of other germicidal sterilant.

The overall process of the invention can follow the following reprocessing steps. The used dialyser will undergo a flushing and washing procedure through the blood compartment of the dialyzer using reverse osmosis (RO) water. For the first step, the cleansing of the used dialyser is preferably performed using ultrapure RO water to avoid bacterial endotoxin contamination and the introduction of water impurities in the dialyser.

In the second step, the procedure applied involves filling the blood and dialysate compartments of the dialyzer with chemical composition. In this step, the

solution of 200 ppm of peracetic acid (alternatively with another germicidal material as discussed above, or a mixture) and 10-50 mmol/l urea is primed in the dialyser.

In the third step, the blood and the dialysate compartment will be closed, and the dialyser can be stored closed at room temperature, or preferably at + 4 °C. Prior to reusing the dialyser, it is necessary to remove all the chemicals from both the blood and dialysate compartments. This is done in the fourth step by priming and washing the chemicals from the blood and dialysate compartment with sufficient amount of reverse osmosis pure water. In this step it is necessary to use endotoxin and bacterial free water easily obtained by ultrafiltrating the RO water. Finally, prior to clinical use, possible residues of oxidative chemicals are neutralize by filling of the blood compartment with a 0.9% saline solution containing 5mg/ml ascorbic acid (w/v). It may be preferable to circulate the solution for a period at least 30 min to neutralize residues of the germicidal sterilant. All the steps may performed manually or automatically.

After the described reprocessing treatment, the dialysers are ready for use in a dialysis treatment. Prior to use, the mixture containing the germicidal sterilant and urea are removed from the dialysate and blood compartment using by washing with a sufficient amount RO water to remove the chemicals. The dialyser is then primed with sterile saline containing 5mg/ml ascorbic acid until the blood compartment is free of air. Thus the ascorbic acid saline solution is recirculated through the blood compartment in a close loop fashion while the dialysate is passed though the dialysate compartment. This last procedure can be performed in an automated or in an manual fashion . This procedure has been found to remove and neutralize even the smallest traces of peracetic acid and other germicidal sterilants. In this respect, an analysis of the residual germicidal sterilants is not necessary while using very low amount on one hand and the use of ascorbic acid on the other hand.

For the conventional hemodialysis treatment typical with three-time per week dialysis), the reprocessing of the dialyser is performed three times per week with

a storage time of 48 hours. The process is thereafter repeated multiple times (normally a maximum of twenty times, preferably a maximum of sixteen times) until the dialyser fails the conventional transmembrane pressure test. The reprocessed dialysers should not be transferred from one patient to another (as in conventional reprocessing methods).

DESCRIPTION OF DRAWINGS

- Figure 1 shows the results of experiments to assess the disinfectant ability of compositions according to the invention.
- Figure 2 shows the results of experiments to assess the extent to which compositions according to the invention oxidise proteins.
- Figure 3 shows the results of assessing Bradykinin release following reprocessing of a dialyser by various methods.
- Figure 4 shows the results of assessing clearance following reprocessing of a dialyser by various methods.
- Figure 5 shows the results of assessing biocompatibility following reprocessing of a dialyser by various methods.

The present invention will be described by the following examples.

Example 1

This example demonstrates the synergistic effects of the compositions of the present invention to sterilize hemodialysers. Hemodialysers employed were made from a polysulfone membrane manufactured by Fresenius Medical Care (Bad Homburg, Germany), designated as F60, which had been clinically used for the first time.

In vitro testing: in this experiment, the hemodialysers were contaminated with *Pseudomonas aeruginosa* strain PA103. Bacteria were first cultured in trypticated soy agar plates (TSA) for 8 days at 32 °C. Spores were harvested by flooding the surface with bicarbonate buffered sterile water. The suspension was used to inoculate a bicarbonate dialysate solution, which was incubated by shaking at 37 °C. When the growing phase of 10^9 /ml colony forming unit was reached, the used dialysers were flushed by recirculation for 60 minutes with the contaminating solutions. Immediately after contamination, the dialysers were flushed and washed with sterile water to remove the contaminating solutions according to Step 1 of the invention, filled with various compositions of the invention, and stored for different incubation times. Experiments were performed for several time periods, i.e, 0 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, 72 hours. The compositions were : composition A: 50 mmol/l urea and 200 ppm peracetic acid; B: 50 mmol/li urea in 200 ppm hydrogen peroxide; C: 50 mmol urea in 200 ppm methylene bithiocynate; D: 50 mmol/l urea in 200 ppm carbamate; E: 50 mmol/l in 200 ppm 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one; F: 50 mmol/l in 200 ppm 4,5-dichloro-1,2-dithio-3-one.

At different times during incubation 1 to 48 hours, samples were taken from the blood compartment of the dialysers and analyzed for colony forming units. Control experiments used conventional peracetic acid solutions (negative control) and a sterile saline solution (negative control). The hemodialysers were incubated at room temperature. For testing the colony forming units, the entire content of each dialyser was removed and 9-fold serial dilutions were performed in sterile water (from 10^{-1} up to 10^{-9}). Colony forming units were measured by incubating 100 μ l of the sample in agar using standard methods (Ref: 4354424; Becton Dickinson UK Ltd, Oxford) for 5 days at 28 °C temperature.

The results of the experiments are shown in Figure 1a. As shown in the figure, after approximately 8 hours incubation the different compositions of the invention containing urea were the same as the standard peracetic acid

composition and 10 times more efficient in killing bacteria than water alone. In addition long term incubation up to 72 hours did not result in a loss of efficiency with respect to the capacity to kill bacteria. In an additional set of experiments, the preferred composition of the invention (composition A) was tested for its ability to kill several microorganism at room temperature: *Pseudomonas maltophilia*, *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium chelonae*, *Aspergillus niger*. All these organisms may accidentally contaminate hemodialysers. Experiments were also performed for several time periods, i.e, 0 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, 72 hours. Control experiments were conventional peracetic acid solutions (negative control) and a sterile saline solution (negative control). The hemodialysers were incubated at room temperature. For testing the colony forming units, the entire content of each dialyser was removed and 9 -fold serial dilutions were performed in sterile water (from 10^{-1} up to 10^{-9}). As shown in Figure 1b, all the organisms were killed after at least 60 minutes incubation and were not different from the standard peracetic acid solution.

These results show that bacteria killing for all germs investigated was achieved within one hour storage of the treated dialyser

Example 2

This example demonstrates that dialysers reprocessed with a composition in accordance with the invention are free of adsorbed oxidized proteins as compared with conventional hemodialysers. This leads to a better biocompatibility of the dialysis membrane in terms of not activating the contact phase of the circulating blood during hemodialysis.

New dialysers (made with polysulfone membrane manufactured by Fresenius Medical Care Bad Homburg, Germany) were perfused with human plasma for four hours using a dialysis machine. The dialysers were treated according to the

reprocessing procedure of the invention. The reprocessing steps were repeated 50 times for each dialyser. The dialyzers were rinsed with 2 l saline to eliminate residual blood, thus the dialysate and the blood compartment were primed and incubated at room temperature for 120 min with a detergent containing: 50 mmol diethanolamine/HCl (pH=11.5) + 100 mmol NaCl + 1% Triton X. After incubation, the dialyzers were rinsed with a 1% Triton and a total volume a 100 ml eluate has been collected. The collected eluates were washed with a PBS solution (pH = 7.4) containing 0.05% Thimerasol and concentrated by a centrifugal filter (MILLIPORE; Ultrafree-15; Biomax-5) with a NMWL of 5,000. The detection of oxidized protein was performed by competitive enzyme linked immunoassay using a polyclonal antibody DNP-derivatized proteins. The method applied is based on the fact that oxidative modification of proteins involves the introduction of carbonyl groups into protein side chains by a site-specific mechanism. The carbonyl groups of oxidized proteins in the samples (obtained by the eluates) were derivatized to 2,4-dinitrophenylhydrazone (DNP-hydrazone).

In vitro testing: Figure 1a shows the extent of the oxidation of the dialyser adsorbed proteins treated with the different compositions of the invention compared with treatment with a conventional peracetic acid composition. The tested compositions in accordance with the invention were : composition A:, B, C, D, E:, as described in Example 1.

Clinical testing: the Figure 2b shows similar results during clinical trials using a the preferred composition containing urea and low concentrations of peracetic acid in comparison with the standard reprocessing procedure. The tested composition in accordance with the invention was composition A as described in Example 1.

Example 3

This example demonstrates that dialysers reprocessed with the composition containing urea and low concentration of peracetic acid in accordance with the

invention are effective in reducing the risk of bradykinin in patients receiving ACE-inhibitors such as Captopril. This performance is related to the fact that the dialyser membrane protein-layer of the dialyser reprocessed according to the invention is not negatively charged through oxidation.

Clinical testing: The tested composition in accordance with the invention was composition A as described in Example 1. Following the experiments of Example 1 for clinical testing, covalent coupling of the desorbed proteins onto polystyrene microwells (CovaLink NH ; Nunk) for the use in an assay was applied. The coupling of proteins has been achieved using N-hydroxysuccinimide (NHS esterified molecules). The final protein concentration was 10 µg/ml; 2) 100 µl of the protein solution were then pipetted into well of the CovaLink plate and covered at the top. The plate was then incubated in the refrigerator at + 4°C for 30 min.; 3) After incubation, the plate was washed x6 times with 0.05 M phosphate buffered saline (pH = 7,2), dried and stored at + 4 °C until use. Thus wells were washed and incubated again with autologous patient plasma of patients treated with the reprocessing procedure of the invention versus the standard procedure containing 0.1 µg captopril. Bradykinin generation was measured at different incubation times. Figure 3 shows the bradykin release in the described testing system. As shown, the activation of the contact was demonstrated using proteins exposed with the conventional reprocessing system. In contrast, there were no contact phase activation though proteins exposed with the reprocessing system of the invention.

Example 4

This example demonstrates that dialysers reprocessed with urea and low concentration of peracetic acid in accordance with the invention are effective in performing hemodialysis in terms of clearance of low and middle molecular weight toxins.

In vitro testing: hemodialysis was performed on a series of patients using Fresenius Medical Care Hemodialysers F60 reprocessed in accordance with the

invention using the preferred chemical composition. The tested compositions in accordance with the invention was composition A as described in Example 1. After different numbers of cycles of clinical use and reprocessing, dialyser performance capacity was tested *in vitro*. In this respect, the extracorporeal circuit, blood pump and plastic vessel used in simulating an *in vitro* hemodialysis were according to ISO 8638. Clearances studies of small, middle and large molecules were performed according to the International standard (ISO 8637). An open loop dialysis circuit was mounted in a dialysis machine (4008, Fresenius Medical Care, Bad Homburg Germany) and comprised a bag reservoir containing 2 liters, sterile dialysis tubes (Fresenius Medical Care) and the dialyzer. The clearance rates of urea, creatinine, phosphate and Vitamin B12 were stated for the range and dialyzing fluid flow rates and include of 200ml/min, the ultrafiltration was 10ml/min. The blood flow rate was always measured by weighing the medium pumped. The dialysate fluid flow rate was 500 ml/min. The blood compartment was perfused with the test substances dissolved in dialyzing fluid. For the middle molecule β 2-microglobulin, we used uraemic plasma obtained during the treatment by plasmapheresis of uremic patients.

Figure 4a shows the clearance data. Figure 4b shows the total membrane protein adsorption due to the generation of a secondary layer. As demonstrated, clearance data for small and middle molecules after different reprocessing procedures using the preferred composition in accordance with the invention were found to be substantially the same as the clearances achieved by the same dialyser before the first use.

Examples 5

This example demonstrates that dialysers reprocessed with urea and low concentrations of peracetic acid in accordance with the invention are effective in performing hemodialysis biocompatibility in terms of platelet and complement activations.

In vitro testing: the blood-compatibility testing circuit used was a close system according to ISO 8636 in which the test medium was heparinized fresh human blood. Thrombin antithrombin III (TAT) measurements were performed using ELISA (Enzygnost TAT micro) purchased by Behring (Germany, Marburg). Complement C3a measurements were performed by commercially available ELISA test provided by Amersham.

Figure 5 shows the blood-compatibility data. As shown, TAT and C3a after different reprocessing series using the preferred composition in accordance with the invention, were found to be substantially better as the same dialyser before the first use. These data demonstrated the blood compatibility performance of a dialyser reprocessed with the composition in accordance with the invention.

**CLAIMS**

1. A method for decontaminating a surface of device that has been in contact with a biological fluid which comprises contacting the surface with one or more aqueous disinfectant solutions characterised, in that at least one of the solutions comprises a germicidal sterilant and at least one of the solutions comprises urea.
2. A method according to Claim 1, which comprises contacting the surface with an aqueous disinfectant solution comprising both a germicidal sterilant and urea.
3. A method for decontaminating a surface of device that has been in contact with a biological fluid which comprises contacting the surface with one or more aqueous disinfectant solutions characterised, in that at least one of the solutions comprises a germicidal sterilant and urea.
4. A method according to claim 2 wherein the germicidal sterilant is selected so as to result in a synergism with urea to prevent the growth of bacteria and to prevent the oxidation of proteins.
5. A method according to any preceding claim wherein the germicidal sterilant is an oxidising agent.
6. A method according to Claim 5 wherein the germicidal sterilant is peracetic acid.

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7. A method according to any of Claims 1 to 4 wherein the germicidal sterilant is selected from peracetic acid formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bithiocyanate, carbamate, DBNPA, quaternary ammonium compounds, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one, and 4,5-dichloro-1,2-dithio-3-one.
8. A method according to any preceding claim wherein the concentration of urea (weight per volume) is the range between 50-200 mmol/l.
9. A method according to any preceding claim wherein the concentration of the germicidal sterilant is less than 200 ppm.
10. A method according to any preceding claim wherein the device is a part of a dialysis apparatus.
11. A method for performing a biocompatible reprocessing of a hemodialysis device to allow its reuse and of controlling the growth of bacteria therein comprising the step of introducing urea and a sterilant into the hemodialyser.
12. A method according to Claim 11, including the step of filling the blood and dialysate compartments of the hemodialysis device with a aqueous solution containing a mixture comprising urea and a germicidal sterilant.
13. A method according to claim 12 wherein the concentration of urea (weight per volume) is the range between 50-200 mmol/l.
14. A method according to any of Claims 11 to 13 wherein the germicidal sterilant is selected so as to result in a synergism with urea to prevent the growth of bacteria and to prevent the oxidation of proteins.

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15. A method according to any of Claims 11 to 14 wherein the concentration of the germicidal sterilant is at a concentration of less than 300 ppm, whereby providing a synergistic effect with urea for inhibiting the growth of microorganisms, but with no measurable oxidative effect on adsorbed proteins during sterilization and storage.
16. A method according to claim 15 wherein the germicidal sterilant is peracetic acid.
17. A method according to claim 14 wherein the germicidal sterilant is formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bithiocyanate, carbamate, DBNPA, a quaternary ammonium compound, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one, or 4,5-dichloro-1,2-dithio-3-one.
18. A method according to any of Claims 11 to 17, including the process step of filling the blood and dialysate compartments of the hemodialyser device with a aqueous solution containing a mixture comprising as a first component urea, as second compound a germicidal sterilant and as a third compound another germicidal sterilant.
19. A method according to claim 18 wherein the second compound is peracetic acid and the third compound is selected from formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bithiocyanate, carbamate, DBNPA, quaternary ammonium compounds, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one, and 4,5-dichloro-1,2-dithio-3-one.
20. A method according to claim 19 wherein the total of the concentrations of peracetic acid and the third germicidal sterilant is less than 200 ppm.
21. A method according to any of Claims 11 to 20 wherein ascorbic acid (w/v) is circulated through the dialyser before reuse to neutralize residues of the germicidal sterilant.

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22. An aqueous disinfectant solution for decontaminating hemodialysers prior to reuse, comprising a germicidal sterilant and urea.
23. A aqueous disinfectant solution according to Claim 22 wherein the germicidal sterilant is an oxidising agent.
24. A aqueous disinfectant solution according to Claim 23 wherein the germicidal sterilant is peracetic acid.
25. A aqueous disinfectant solution according to any of Claims 22 to 24 wherein the is germicidal sterilant is selected from peracetic acid formaldehyde, glutaraldehyde, hydrogen peroxide, methylene bistiocyanate, carbamate, DBNPA, quaternary ammonium compounds, 4,5-dichloro-2-N-octyl-4-isothiazolin-3-one, and 4,5-dichloro-1,2-dithio-3-one.
26. A aqueous disinfectant solution according to any of Claims 22 to 24 wherein the concentration of urea (weight per volume) is the range between 50-200 mmol/l
27. A aqueous disinfectant solution according to any of Claims 22 to 26 wherein the concentrations of the germicidal sterilant is less than 200 ppm.

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