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Are Dialysis Devices Usable as Ozone Gas Exchangers?

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Abstract: A study aimed to compare the efficiency of the ozone transfer of four hydrophilic dialysis filters, and one hydrophobic gas-exchange device (GED) has been performed. Obviously, the former should be specifically used only for dialysis. Unfortunately, some clinicians incautiously use them as GEDs. It has been shown that: (i) dialysis filters present a wide range of gas-exchange yield (from 0 up to 70%), often related to variability according to the treatment time; (ii) by scanning microscopy, it has been noticed that hollow fibers are somewhat altered by ozone; and (iii) because their constitutive materials may be not ozone-resistant, they may release toxic compounds harmful for the patients. On the contrary, the appropriate GED is ozone-transfer efficient, is ozone-resistant, and is suitable for blood autotransfusion and ozonation. **Key Words:** Ozone—Extracorporeal circulation—Hollow fibers—Gas-exchange device.

INTRODUCTION

To the best of our knowledge, the first approach aiming to simultaneously oxygenate and ozonate human blood was proposed by Wainwright (1). In such a case, blood withdrawn from a patient runs inside a tilted glass tube against a gas mixture composed of oxygen-ozone flowing in a countercurrent direction. The procedure appeared to be effective, but it presented a risk of viral infections even though the system had to be sterilized for each treatment. A similar stainless steel system recently proposed by Latino and Keyser (2) has the same drawbacks. Since 1992, in cooperation with the Nephrology Unit of the Polyclinic of Siena (Italy), a dialysis-like system, where the dialysate was substituted with O_2–O_3 gaseous mixture similarly directed in a countercurrent fashion for ozonating blood, was investigated. The dialysis filter is referred to as an artificial kidney. Blood withdrawn from the patient runs inside hollow fibers of different hydrophilic composition while the dialysate runs outside in countercurrent fashion. Dialysis membranes not only need to be efficient at clearing wastes, but must also be biocompatible with human blood. Filter permeability is influenced by pore size, number of pores, and thickness of the membrane. The surface area of the membrane determines the available area for diffusion and ultrafiltration (3). After a few years of research, it was realized that the system was unsuitable because the dialysis filters: (i) were poorly effective for gas exchange; (ii) it may have reacted with ozone, possibly releasing toxic materials into the blood; and (iii) caused a loss of solutes and water. Moreover, they allowed some platelet adhesion, leukocyte activation, and produced some immune responses (4,5). The conclusion that dialysis filters were unsuitable for blood ozonation, chiefly for the hydrophilic nature of the hollow fibers and the possible release of toxic compounds has been [3] reached. For this reason, the examination of single-use and ozone-resistant hydrophobic membranes, made of polypropylene, hence impermeable to water but freely permeable to O_2–O_3, was started. At first, the gas-exchange devices (GEDs), even using heparin, were minimally biocompatible (6) until two years ago, when a suitable phosphorylcholine-coated

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one was used (7). In the latest GED, denominated D100, in comparison to dialysis filters, blood runs outside the fibers against the gas mixture inside. This GED has proved to be quite effective and atoxic when used for blood ozonation (8,9). Common dialysis filters are usually sterilized with gamma radiation, even if both ethylene oxide and steam could be used (10). However, ozone is also occasionally used for the same purpose (11–16), as well as for polymer grafting (17–19) or, unfortunately, for performing recirculatory hemoperfusion against O_2–O_3, implying a risk for the patients (20).

The aim of this report is to show that, during these unorthodox practices using dialysis filters, their in vitro gas-exchange efficiency is often minimal and, even if such is not the case, they might potentially be hazardous for the patient because they are not specifically tested and/or approved for such purposes.

MATERIALS AND METHODS

Hollow fiber modules

The following four dialysis filters have been used and codified as follows:

1 heparinized cuprophan, Cobe Centrysystem 550, Secon GmbH, Germany;
2 cellulose triacetate high efficiency hollow fiber, CT150G, Baxter S.A., France;
3 polysulfone Rexbrane, Rexeed-18L, Asahi, Japan; and
4 polymethylmethacrylate, Filtryzer B3-2.0A, Toray, Japan.

In contraposition, a new and suitable GED, namely D100, made of phosphorylcholine-coated polypropylene (Sorin, Mirandola, Italy) was used as reference standard. All the devices and ancillary materials were sterile and used only once.

Schematic diagram of the circuit and operative conditions

Figure 1 describes in detail the various operative conditions and embodiments.

The dialysis filters, the GED, and lines were routinely rinsed with 2-L saline before starting the perfusion simulated by saline phosphate buffer (pH 7.4), in the presence of KI (0.12 M). The various ozone concentrations used throughout the experiments (between 0.5 and 2.5 µg/mL) were generated by Ozonline ECO_3 (Torino, Italy) using only pure, medical-grade oxygen representing at least the 95% of the gas mixture at a feed flow of 15 L/h. Ozone concentration was continuously monitored by a photometer, periodically checked by iodometric titration. The overall time of ozone treatment was 60 min.

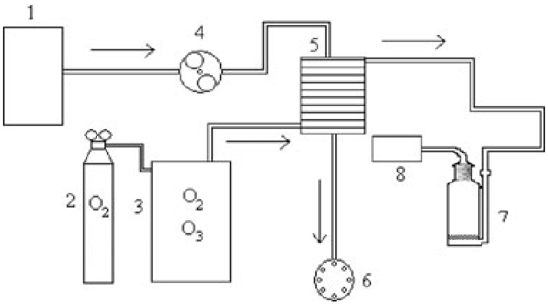

FIG. 1. Schematic diagram of the experimental device: 1 saline solution or buffer; 2 oxygen supply; 3 ozone generator with photometer; 4 peristaltic pump; 5 dialysis filter or GED; 6 sample collector; 7 silica gel trap; 8 ozone destructor. The peristaltic pump was always kept in "ON" position throughout the experiments.

Transfer and total yield of ozone were evaluated both during the early phase of treatment and after 60 min of ozone flowing, simulating the time of a therapeutic session.

When ozone, flowing in a countercurrent fashion outside the hollow fibers, permeated these same, it immediately reacted with the KI solution, generating I_2, which was titrated with $Na_2S_2O_3$ (0.0001–0.1 N) (21) (see Scheme 1).

Scheme 1. Iodine titration

$$O_3 + 2I^- + H_2O \rightarrow I_2 + O_2 + 2OH^-$$
$$I_2 + 2S_2O_3^{2} \rightarrow 2I^- + S_2O_4^{2-}$$

It indicates the reaction elicited by ozone and the classical reducing reaction for measuring the ozone. Furthermore, blank tests under the same conditions using only oxygen for the same time have been carried out.

Scanning Electron Microscopy (SEM) characterization

The morphology of hollow fibers before and after ozone treatment, or after saline phosphate buffer (pH 7.4) with and without I_2 (0.1 N) perfusion, was investigated by SEM studies. Both the external and internal hollow fiber surfaces were examined. The material was mounted on aluminum stubs by carbon conductive glue and coated with 20 nm gold in a Balzer's MED 010 sputtering device. The samples were finally observed with a Philips XL20 scanning electron microscope operating at an accelerating voltage of 5–20 kV, according to the material sensitivity.

Statistical analysis

Results were expressed as the mean of at least three independent measurements (CV% < 2). One-way analyses of variance performing the Bonferroni posttest (Instat software, version 3.0 GraphPAD Software Inc., San Diego, CA) were used for the statistical analysis of the results. Significance was defined as a P value less than 0.05 (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

RESULTS AND DISCUSSION

Efficiency evaluation

Owing to the fact that ozone is a strong oxidant to be used with great concern and with proper devices, a precise knowledge of the ozone effects on the devices during the overall treatment is essential. When ozone, flowing in a countercurrent fashion outside the hollow fibers of the dialysis filters, interacts with the KI solution flowing inside the fibers, it immediately generates iodine, and in such a way, the device acquires an amber color. Transfer and total yield of ozone were evaluated during the early phase of treatment. The same evaluation was also repeated after 60 min of a continuous ozone flowing, simulating the time of a therapeutic session. Diagrams of Fig. 2 show the amount of ozone transferred of the four dialysis filters at the beginning (top) and at the end (bottom) of the experiment, respectively.

5 Apart from filter *4*, the ozone transfer increased in a linear fashion with respect to the ozone dose. Device *1* appears to be rather efficient and reproducible with time, followed by an almost analogous behavior of both devices *2* and *3*, at least for what the initial treatment is concerned. In fact, the presence of ozone inside the system *3* for about 60 min led to a significant variation in the ozone efficiency, especially for the doses up to 0.4 mg/min. On the contrary, device *4* seemed gas-transfer inefficient, even if a diffuse browning of the hollow fibers in proximity of the gas inlet and outlet was observed. Table 1 shows the ozone yield % at the beginning and at the end of the perfusion in relation to the ozone concentrations. In details it can be noted that while filter *4* is inefficient, filter *1* has yielded more constant and higher values. Moreover, filters *2* and *3* show a variable yield during the ozone treatment.

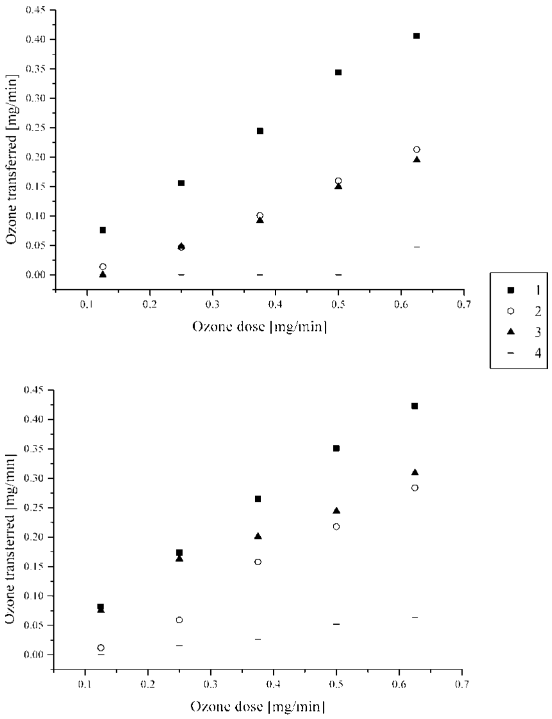

FIG. 2. Ozone transfer (mg/min) at the start (top) and at the end (bottom) of the ozone treatment. Numbers 1–4 refer to dialysis filters as described in "Materials and Methods."

TABLE 1. *Ozone yield of the four dialysis filters both at the start and at the end of experiments in relation to different ozone concentrations*

	Ozone yield [%]									
	0.5 µg/mL		1.0 µg/mL		1.5 µg/mL		2.0 µg/mL		2.5 µg/mL	
Hollow Fiber Modules	Start	End	Start	End	Start	End	Start	End	Start	End
1	** 61	66	** 62	69	** 65	71	ns 69	70	* 65	68
2	ns 11	10	** 19	24	*** 27	42	*** 32	44	*** 34	45
3	*** 0	60	*** 19	65	*** 25	54	*** 30	49	*** 31	49
4	ns 0	0	*** 0	6	*** 0	7	*** 0	10	* 8	10

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

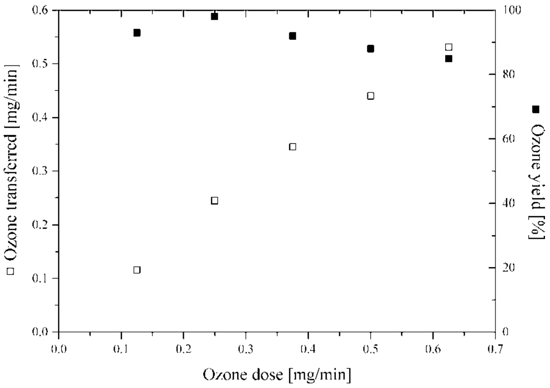

FIG. 3. Ozone transfer (□) and Ozone yield (■) for the GED.

The efficiency and ozone yield achieved using the GED D100, where the KI solution flows outside the fibers, are shown in Fig. 3, in relation to the data at the start of the evaluation. The ozone transfer is almost quantitative and significantly higher than in dialysis filters ($P < 0.001$). No differences of values at the end of the ozone treatment were observed, meaning a constant gas exchange (data not shown).

Owing to the fact that the gas mixture is composed of al least 95% medical oxygen, it must be pointed out that no appreciable oxidation of KI occurred during control experiments using only oxygen.

The morphology and structure of hollow fibers become the focus of the concern of the fiber integrity and how ozone significantly affects their stability during treatment.

SEM characterization

In order to examine whether ozone may damage the fiber membrane of the various filters, the hollow fiber surfaces by SEM were examined.

Hollow fiber specimens from filters *1, 2,* and *4* were very sensitive to high-energy electrons and they were preferentially observed at 5 kV. Both the outer and the inner surface of the fibers above these three filters are smooth and continuous. Fibers from filters *1* and *4* are instead morphologically unaffected by the ozone flow (data not shown). In fibers from filter *2*, the outer (Fig. 4A) and inner sides are unaltered by the ozone flow with respect to control (saline and KI solution), even though several little dips are always present in the inner side of the same fibers (Fig. 5A), and for this reason, no further ozone treatment evaluation is possible.

Specimens from filter *3* and from GED were observed at 10–20 kV. The outer surface of the filter *3* fibers (Fig. 4B) appears discontinuous as being made up of several stacked layers of a holey film. The inner side of this fiber, instead, has a barely wrinkled but continuous surface (Fig. 5B). In this side of the fibers and for the whole filter length, several structural alterations are observable as little dark spots, presumably due to the ozone flow (Fig. 6). This phenomenon suggests the reactivity of the fiber materials at the experimental conditions adopted. Both the outer

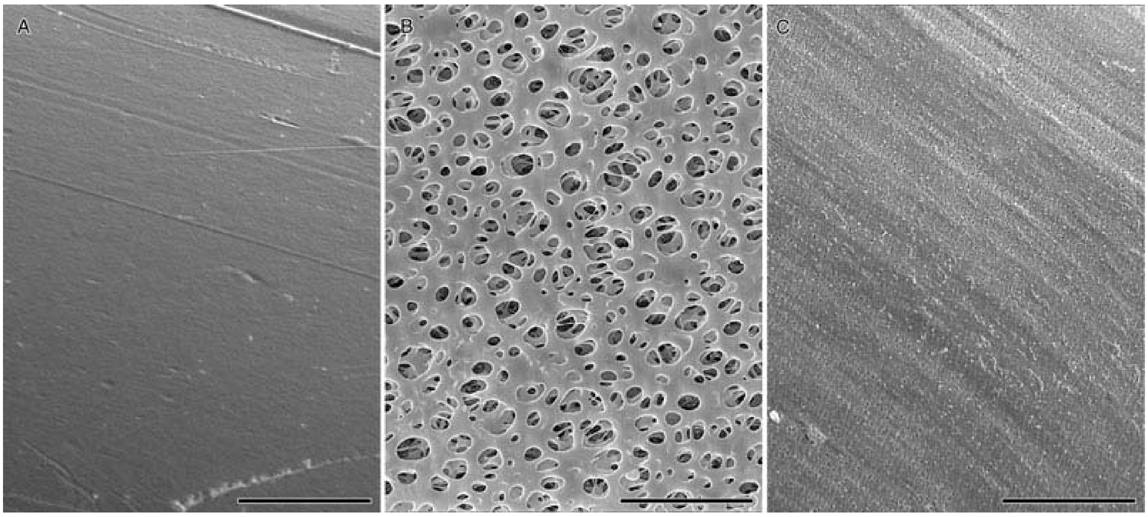

FIG. 4. Outer surface of the fibers from the devices after ozone treatment. (A) filter 2. (B) filter 3. (C) GED. Bar = 10 μm.

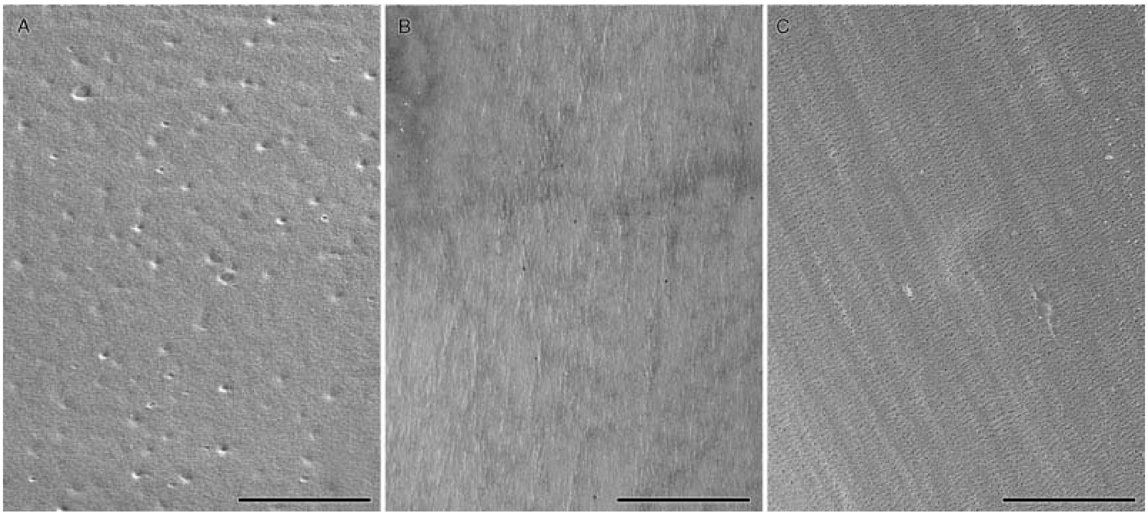

FIG. 5. Inner surface of the fibers from the devices after ozone treatment. (A) filter 2. (B) filter 3. (C) GED. Bar = 10 µm.

(Fig. 4C) and the inner (Fig. 5C) surface of the fibers from GED show the same morphological appearance, and they seem like to each other. SEM observation at higher magnification of GED fibers reveals a very close reticular structure (Fig. 7), with longitudinal thread crossed with irregular and circularly arranged matter. The fibers from this filter appear morphologically unaltered by ozone treatment.

CONCLUSIONS

Dialysis filters, to a different extent, allow a nonconstant transfer with time of the gas mixture O_2–O_3 into the saline–KI solution, variable in a proportional manner to the ozone amount for filters *2–4*. Filter *1* allows an ozone exchange ranging from 61 to 69% at the initial stage of the experiment, until a 71% ozone exchange at the end is reached. Clearly, these filters are suitable for dialysis but cannot be used as gas exchangers, even if a quantitative passage of ozone is reached. In fact, dialysis filters are made of hydrophilic materials that are not ozone-resistant and therefore they may react with ozone, and they may release unwanted chemicals potentially harmful for the patient. Moreover, the GED is made of hydrophobic, ozone-resistant polypropylene-based fibers. On the basis of the present findings, it is hoped that anyone incautiously using dialysis filter for blood

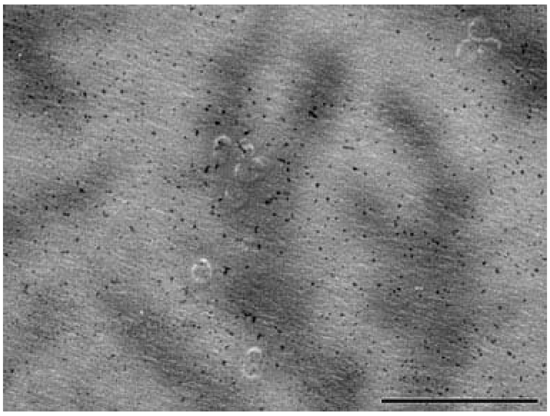

FIG. 6. Inner surface of fibers from filter 3. Many little dark spot are observable after ozone treatment. Bar = 5 µm.

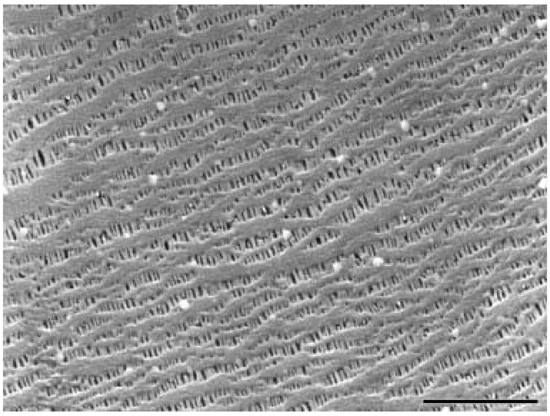

FIG. 7. The inner surface of the fibers from GED filter shows a very close reticular structure. Bar = 2 µm.

[8] extravascular ozonation should correctly adopt the appropriate device. Eventually, by considering the strong reactivity of ozone, its occasional use for sterilizing dialysis filters could be carefully evaluated because it may impair their function.

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