Dense CO_2: An Innovative Method for Sterilization of Venous Catheters at Low Temperature

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Venous catheters are important medical devices which are more and more employed in medical treatment. After use, they are currently not re-used, a practice which is very expensive due to their cost. For this reason it is very interesting to be able to set up a sterilizing technology that permit to re-use them without changing their physical properties and the microbial adhesion. The methods usually employed for sterilization are steam (autoclave) and pure ethylene oxide (at low temperature).
The aim of this work is to study the effects of different sterilization methods on catheters artificially contaminated with bacteria and yeast. Four processes were investigated: steam, ethylene oxide (Et.Ox), supercritical carbon dioxide (sc-CO_2) and supercritical nitrogen protoxide (sc-N_2O).
The results obtained demonstrate that the supercritical fluid sterilization method provides an effective inactivation of all the microorganism tested, and that no significant modification was found in the microbial adhesion capacity to the catheter surface when 10 subsequent treatments were performed on the same sample.
Inactivation kinetic for sterilization by sc-CO_2 and sc-N_2O were carried out at 40°C and 100 bar. The best results were obtained with sc-CO_2, which allowed a faster treatment.

KEYWORDS: catheters, sterilization, supercritical fluids

INTRODUCTION

In recent years many works appeared on the possibility to reuse medical equipment, in particular cardiac interventional catheters for which the sterility is an important requirement. It would be very interesting to be able to set up a technology for catheter re-use without changing their physical properties.
Pure ethylene oxide (Et.Ox) is the most commonly used in low temperature sterilization technique. Due to ethylene oxide's toxicity [1], a long aeration process is required to remove the residual agent and its by-products from sterilized materials; despite this precaution, level of Et.Ox residues have been detected in reused catheters sterilized by it [2]. Autoclave involves exposure to high temperatures and steam, which may thermally degrade sensitive materials such as those of catheters shell.
At the moment, few data are available on the practice of electrophysiology catheter reuse [3]. It seems that no deleterious effects on patient outcome and the potential risk of transmitting infective organisms is very low.
For this reason, the aim of this work was to determine the feasibility of using high pressure (dense or supercritical) CO_2, or N_2O, for the sterilization of cardiological catheters, as an alternative method to conventional techniques such as steam autoclave and Et.Ox. Recent studies have shown that the treatment with supercritical fluids has been successful in the inactivation of a wide range of bacteria, yeasts and viruses at relatively low temperatures [4-6].
At this scope, the inactivation kinetics of three kinds of bacteria and a yeast by dense gas treated was studied and the microbial adhesiveness on catheter was analyzed up to 10 sterilization cycles.

MATERIALS AND METHODS

Microorganisms and growth conditions

All experiments were carried out using a Gram-positive (*Staphylococcus aureus*) and two Gram-negative *(Escherichia coli, Pseudomonas aeruginosa*) bacteria and a yeast *Candida albicans*.
C. albicans and bacteria were grown in Sabouraud dextrose broth and brain heart infusion broth (Difco), respectively, and incubated at 37°C under aerobic conditions. Cells from cultures in the exponential phase of growth were harvested by centrifugation at 2,000g for 10 min and the pellets were washed twice in sterile distilled water before blood or medium contamination.

Catheters

The experiments were carried out using the following catheter kinds, indicated in the text as A, and B respectively:

A. SPLINKER™ (Medtronic, Inc., Minneapolis, USA);
B. CA Seldiflex™ (Plastimed Laboratoire, Fr.).

Catheter contamination and sterilization methods

Each segment was contaminated by immersion for 60 min at 37°C in 10 mL of property medium or human blood artificially contaminated with one of four microorganism considered, collected in sterility, reprocessed, washed in sterile water, and allowed to air dry before being sterilized
To study the inactivation kinetics by supercritical CO_2 and N_2O on bacterial and yeast, contaminated catheter segments (2±0.1 cm length) were sterilized with dense-CO_2 or dense-N_2O at 40°C, 100 bar for 1, 5, 10, 20, 30 and 50 min.
To study the effects of repeated dense-CO_2 and Et.Ox sterilization treatments on microbial adhesiveness, catheter segments (2±0.1 cm length) were sterilized (0, 1, 5, 10 times), contaminated, and dehydrated as above described.
The inactivation and the adhesiveness evaluation was analyzed by introducing each catheter segment in a tube containing 5 mL of sterile distilled water and vortexing for 1 min. Then the amount of microbial cell was counted by plating undiluted and 10-fold diluted samples on brain heart infusion agar (for bacteria) or Sabouraud dextrose agar (for *C.albicans*). After 24 h incubation at 37°C, the cfu/mL was evaluated.

Experimental apparatus

In Figure 1 the set-up of the high pressure pasteurization apparatus is outlined. The plant is provided with a high pressure storage reservoir to reduce the pressurization time of the reactors system. Details of this apparatus are reported elsewhere [7,8].
The plant is equipped with two thermostatic water baths: one for the reservoir and the other for the reactors. In the case of the experiments with CO_2, the reservoir was loaded using a HPLC pump, so the temperature of the two baths was the same throughout the experimental time, while in the case of N_2O the fluid was cooled in a heating exchanger at 5°C and fed at the same temperature into the reservoir. Then the valves V1-V4 were closed and N_2O was heated until the pressure in the reservoir reached the desired value.
The contaminated catheters were loaded, under sterile conditions, into the reactors, which were then immersed in the thermostatic bath. After temperature equilibration, the six samples were subjected to the identical pressure of CO_2 or N_2O for different treatment times, opening the valves V3-V10: in this way, a fast pressurization rate by the supercritical fluid was achieved.

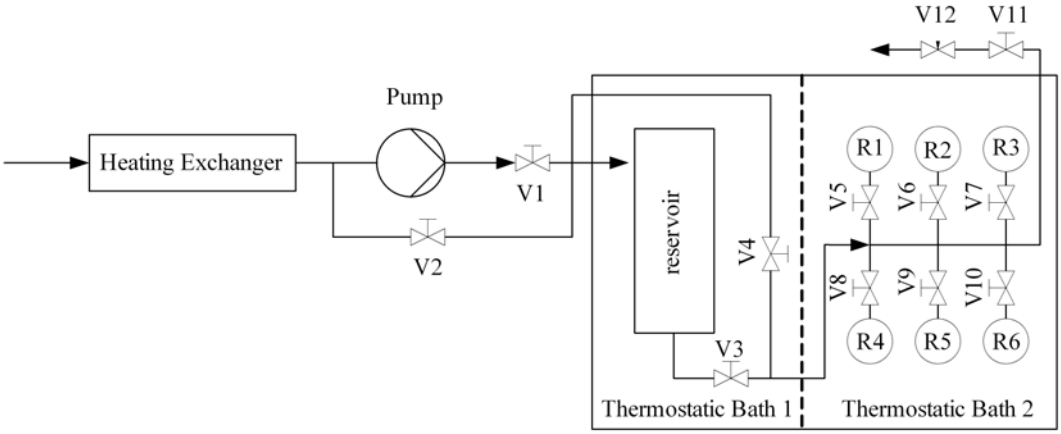

Figure 1. Block Diagram of plant for high pressure treatment: R1-R6: high pressure reactors; V1-V11: on-off valves, V-12: regulating valve.

Each catheter undergoes the dense-CO_2 or dense-N_2O treatment for the suitable time, then the reactor was depressurized and opened, under sterile conditions, for further analysis of the sample.

Reprocessing protocol

The catheter segments reprocessing protocol was as follows:

a) decontamination by 10 min immersion in 0.1% NaDCC (sodium dichloroisocyanurate dehydrate, Bionil®; Eurospital, Trieste, Italy);
b) cleaning by 10 min immersion in proteolytic enzymatic detergent (Septozim CE®; Farmec, Verona, Italy);
c) washing in sterile water followed by air drying.

RESULTS AND DISCUSSION

In the first part of the work, the sterilization of catheter by dense-CO_2 was studied in the following way: segments of catheter A were kept immersed, for 60 min at 37 °C, in medium artificially contaminated with one of the four microbial strains (about 10^6 cfu/mL), dried under sterile air flux, reprocessed and sterilized. After the sterilization step each sample was analyzed by the methodology described above and the inactivation expressed as $\log(N_0/N)$ is reported in Figure 2 for each microbial strain considered.

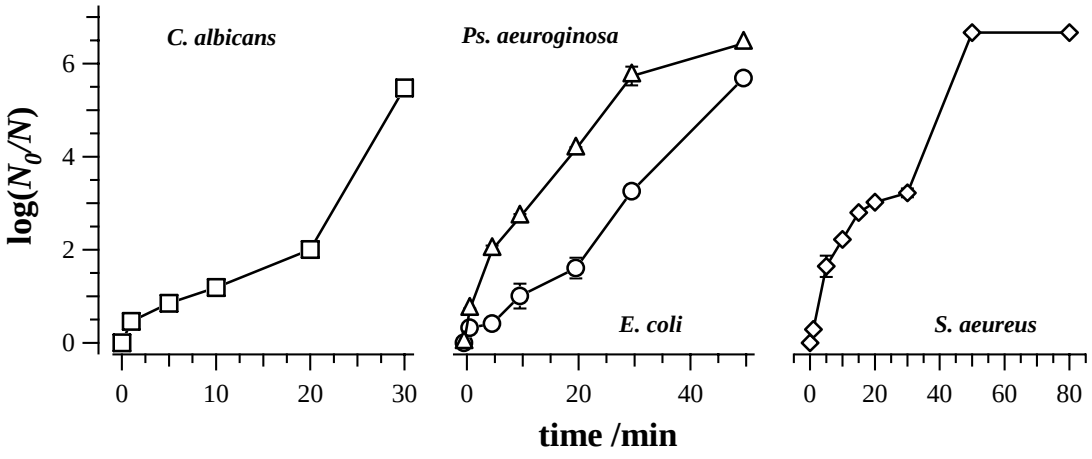

Figure 2. Inactivation kinetic of catheter artificially contaminated with microbial strain suspended in medium by supercritical CO_2 process.

In Figure 2, it is possible to observe the different effect of supercritical CO_2 on microbial strain inactivation. Sterility was obtained after 30 min of treatment for the yeast and 50 min for the all the bacteria.
The inactivation was due to effect of dense-CO_2 and not to the thermal treatment which caused a reduction at maximum of 0.4 log (Table 1).

Table 1. Microbial load (cfu/cm) before (N_0) and after (N) the thermal treatment at 40°C and ambient pressure.

		Initial microbial load	**Thermal treatment**	
		***N*₀ (cfu/cm)**	***N* (cfu/cm)**	***Log(N₀/N)***
S. aureus	Gram-positive	6 187 500	5 414 584	0.058
E. coli	Gram-negative	483 750	581 250	-0.080
Ps. aeruginosa	Gram-negative	2 725 000	2 490 625	0.039
C. albicans	yeast	300 000	122 500	0.389

Them, we have analyzed the sterilization by sc-CO_2 and sc-N_2O of catheters contaminated with blood and a microbial strain. The results are reported in Figure 3 for each microorganism considered and the two fluids employed.

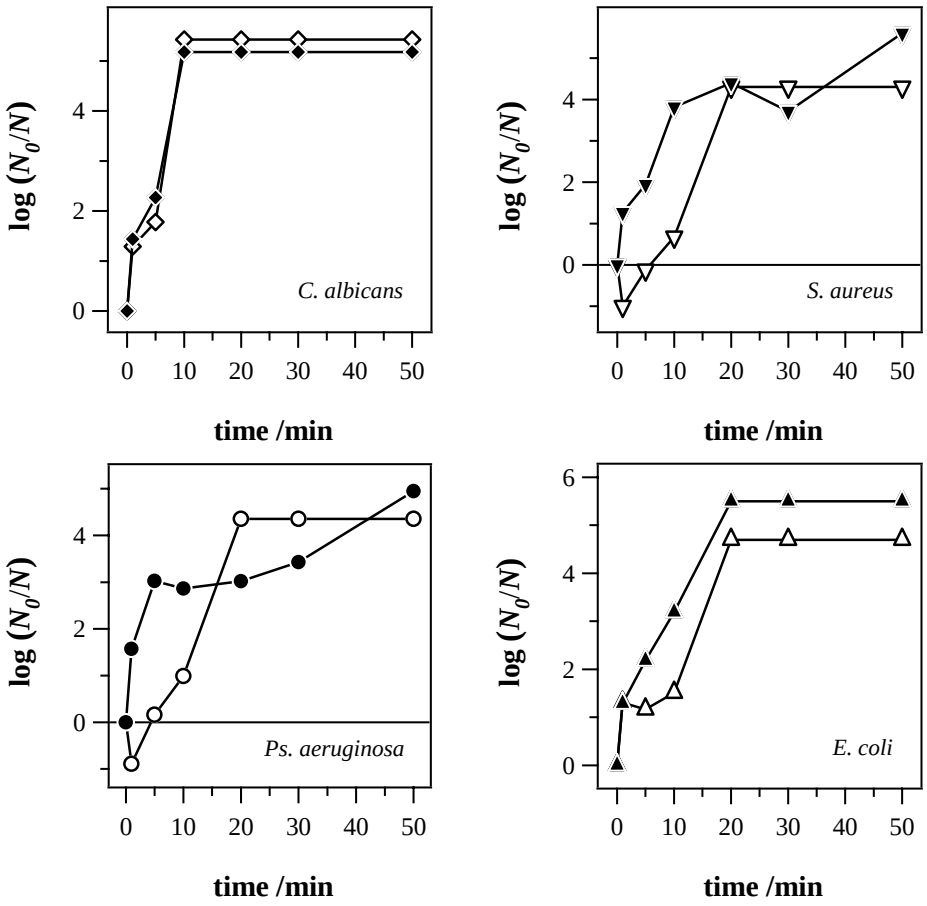

Figure 3. Inactivation kinetics of catheter artificially contaminated with microbial strain suspended in blood by sc-CO_2 (empty symbols) and sc-N_2O (filled symbols) process.

Figure 3 shows the inactivation kinetics of three bacteria and a yeast (*C. albicans*) by supercritical fluid sterilization process. The inactivation rate was different for the four strain considered: it was faster for *C. albicans*. For the yeast and *E. coli*, the two supercritical fluids had similar effect on microbial inactivation, in the other two case (*S. aureus and Ps. aeruginosa*), the treatment with sc-CO_2 lead to a complete sterilization in less time: about 20 min instead of 50 min for sc-N_2O. Also in this experiment, the inactivation was due to the supercritical fluids treatment and not to the thermal one (about 1 log of inactivation after 50 min at 40°C and ambient pressure).
It is reported that the sterilization process may produce modifications on the external surface of the polymeric catheter shaft such as roughness [9,10], favouring blood proteins and bacterial adhesion [11]. Therefore, as the catheter reuse requires the repetition of the process, we have examined the effect of repeated sterilization treatments with dense-CO_2 on cells adhesiveness on catheter B, for all the micro-organisms considered.
As shown in Figure 4, the treatment with dense-CO_2 of catheters B enhances the binding capacity of *E. coli* cells. For all the other bacteria and mould species no significant modifications in the cell-catheter binding were observed.

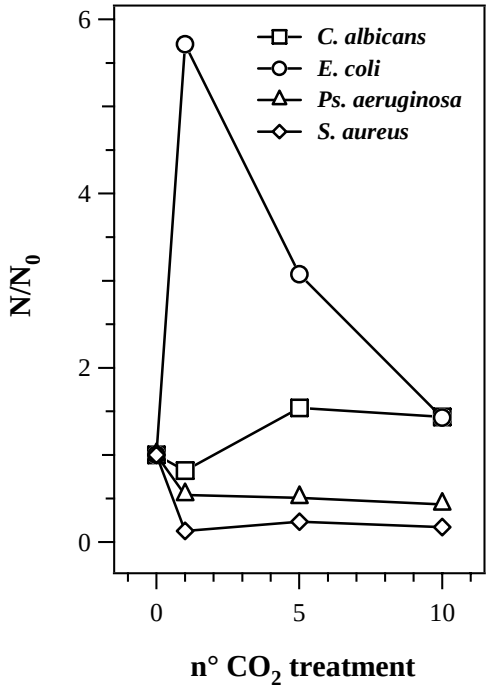

Figure 4. Effect of repeated sterilization treatments (up to 10 times) on microbial adhesion capacity on catheter B.

These results underline that the sterilization with dense-CO_2 does not induce modification on the catheter shell, which could be responsible of enhancing the binding capacity of microorganisms. This demonstrates the feasibility of the sterilization treatment in view of the reuse of this medical device.
The work on comparison among the sterilization capacity of two standard methods (Et. Ox., autoclave) and the innovative supercritical one (dense-CO_2 and N_2O) is currently in progress.

CONCLUSION

The effectiveness of dense-CO_2 and dense-N_2O treatment for the sterilization of medical devices was explored. The results obtained demonstrate the possibility of achieving complete

inactivation of microbial cells (Gram-positive and Gram-negative bacteria, and yeast) from artificially contaminated catheters, despite the large inocula used.
The treatment with dense-CO_2 was faster than that with dense-N_2O.
The advantage of the presently proposed method can be summarized as follows: i) the process requires low temperature; ii) no toxic compounds are produced during the process; iii) no significant modification in the microbial adhesiveness to the catheter shaft is detected also after 10 subsequent treatments; iv) dense-CO_2 treatment is quite fast, due to the great penetration capacity of CO_2 into microbial cells.
The effectiveness of the dense-CO_2 sterilization method at low temperature (40 °C) may open an avenue for processing many thermally labile medical devices, provided that changes of the mechanical properties of catheters are excluded and that also the CO_2 antiviral activity is properly evaluated.

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