Bioburden Reduction of Four Decontamination and Cleaning Protocols for Reprocessing Single-Use Cardiac Electrophysiology Catheters

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Abstract

Decontamination and cleaning protocols are critical in reprocessing medical device. Protocols should be designed to remove biological debris, reduce bioburden and minimize the risk for health professionals. These issues are of utmost importance when reprocessing of single-use-only labeled devices is considered. This study evaluates the efficiency in reducing bioburden of four protocols for decontaminating and cleaning electrophysiology single-use catheters.

The proximal portion of sixty cardiac catheters was soiled in vitro by human blood spiked with Bacillus subtilis or Pseudomonas aeruginosa. Contaminated devices were treated according to one of the following experimental protocols: i) chlorine solution followed by enzymatic solution; ii) enzymatic solution followed by chlorine solution; iii) polyphenolic emulsion; iv) polyphenolic emulsion followed by enzymatic solution. Untreated device were considered as positive controls. Bacterial bioburden was quantified by enumerating colony forming units. Device sterility was checked by broth-enrichment and prolonged incubation time.

Chlorine based protocols showed higher biocide properties (>2.6-3.8 log reduction) than polyphenolic based protocols (1.9-3.2 log reduction), irrespectively from bacterial strain and amount. A further reduction was related to the enzymatic treatment.

To maximize the bactericidal efficiency and also guarantee an effective cleaning, the protocol should provide at first the cleaning phase by the enzymatic detergent, followed by treatment with the chlorine releasing solution. Since this protocol does not realize an immediate decontamination of the device, adequate safety measures for health professionals should be introduced. Wherever these risks cannot be reduced, polyphenols based protocols with immediate device decontamination after use should be considered.

ABBREVIATIONS

CFU: Colony Forming Unit; NADCC: Sodium-Dichloroisocyanurate; SUDS: Single-Use-Only Labeled Devices; C+: Positive Control; EM: Electron Microscopy

INTRODUCTION

Pre-sterilization protocols are fundamental procedures to achieve an acceptable sterility assurance level in the re-processed device after clinical use. Many guidelines and recommendations reported about the influence of the pre-sterilization microbial and organic load for successful devices reprocessing [1-3]. This issue has been specifically recognized for low temperature sterilization processes. Penna and co-workers reported that the effectiveness of gas-plasma sterilization is critically influenced by the presence of a high bioburden level [4]. Pre-cleaning, including decontamination procedures and cleaning protocols are therefore of special significance to the entire sequence of medical device processing and should be carefully considered when designing the reprocessing protocol for a medical device. Moreover, the need for protection of health professionals during handling contaminated devices has to be taken into account. International standards demands for a careful evaluation and minimization of the risk for workers. According to the Italian Government regulations [5], a decontamination procedure of recognized efficacy against HIV has to be conducted immediately after use on every medical device that has been in contact with human blood. Finally the sensitivity and compatibility of the device materials to the used chemicals has to be considered to avoid compromising device safety and efficiency. The optimal
decontamination/cleaning process have to comply with all these aspects.

This issue is of utmost importance when reprocessing of single-use-only labeled devices (SUDs) is considered. To reduce procedural costs, many cardiologic centers consider reprocessing and reusing electrophysiology and ablation percutaneous cardiac catheters [6,7]. Anyway, uniform and clear guidelines to properly guarantee safety and efficacy of the reprocessed devices have not been defined yet. Health agencies and national governments often adopt a cautionary approach discouraging SUDs reprocessing, advocating a general shortage of scientific investigation able to document possible additional risks both of transmitting infectious disease and adverse reaction to disinfectants when catheter are reused after careful cleansing [8] and sterilization by either ethylene oxide [9,10] or hydrogen peroxide [11].

Considering the crucial role of decontamination and cleaning procedure for SUDs reprocessing success, we aimed to evaluate four different protocols for conditioning electrophysiology single use catheters before sterilization process in order to optimize bactericidal efficacy of the pre-sterilization treatment. Integration of the results with previous finding from microscopic investigation [12] could help in identifying the best protocol able to maximize both disinfection and cleaning properties.

MATERIALS AND METHODS

Carrier devices

Sixty non-irrigated cardiac electrophysiology and ablation catheters of different manufacturers (Bard Inc. New Jersey USA; Medtronic Inc. Minnesota USA; Biosense-Webster Inc. California USA) were collected from the Cardiology Department of the S. Chiara Hospital in Trento after first clinical use (Table 1). Devices were disinfected cleaned and sterilized by hydrogen peroxide gas plasma technique (Sterrad100S®, ASP, Johnson & Johnson Inc.).

According to previous studies [13,14], the proximal shaft portion was considered as the most complex and critical part of these devices because it is inserted in the circulatory stream, presents many interfaces between the polymeric shaft and platinum electrodes, and is subjected to the major mechanical and thermal stresses during clinical use. The 5 cm long proximal portion of 60 catheter shafts was excised by using aseptic pliers and the sectioned end of the shaft was sealed by silicone rubber to avoid contamination of the internal lumen that is not exposed to blood during clinical use.

Three lots (I, II, and III) of 20 shaft portions (here considered as representative for the whole device) each were created and separately processed and tested for different inoculum conditions.

In vitro contaminated blood soiling (simulated use)

Each lot of devices was contaminated in-vitro by 1 hour immersion in 10 mL whole human blood (anti-coagulated with sodium citrate) spiked by different bacteria species at different bacterial loads, simulating the worst case scenario for clinical use [15,16]. I lot was contaminated by Bacillus subtilis (ATCC 6633) at a nominal inoculum of 10^6 CFU/mL. II lot and III lot were contaminated by Pseudomonas aeruginosa (ATCC 27853) at nominal inocula of 10^5 and 10^6 CFU/mL respectively. Bacteria strains were previously cultured in trypticase soy broth and subcultured on sheep-blood agar. B. subtilis was selected as the most resistant sporulating species in respect to low temperature sterilization techniques [11,17], while P. aeruginosa is usually recommended for testing disinfectants resistance.

After soiling procedure, each samples group was extracted from blood and left drying in a biohazard cabinet for two hours simulating the time before devices processing after clinical use [14].

Decontamination and cleaning protocols

As reported in Table 2, four different experimental protocols were designed and tested for decontamination/cleaning: i): chlorine solution followed by enzymatic solution; ii): enzymatic solution followed by chlorine solution; iii): polyphenolic emulsion; iv): polyphenolic emulsion followed by enzymatic solution. Chemicals composition, active principle, and modality of use are detailed in Table 3.

Sixteen samples per lot were decontaminated and cleaned according to one of the four experimental protocols. Each quartet included four catheter types as presented in Table 1. One additional representative quartet of samples per lot underwent no treatment and was considered as a positive control (C+) for the quantification of the bioburden on the device surface.

Each device was processed singularly according to the scheduled experimental protocol by providing the required contact time in 200 mL of the treatment solution/s. After completing the protocol, each sample was rinsed by 30 s vortex mixing in 200 mL of sterile water three times.

Cultural techniques

Colonies forming units per device (CFUs/sample) were quantified by applying a Cleri-modified quantitative technique [18,19]. Briefly, each catheter segment was transferred into a screw cup sterile container with 5 mL of trypticase soy broth and vortexed for 1 min. Three aliquots (1, 10, 100 μL) of the vortexed medium per container were withdrawn and inoculated onto sheep-blood agar plates. Inoculated plates were incubated aerobically at 37°C. CFUs were enumerated at 24 and 48 hour.

A qualitative culture by broth-enrichment was also realized to check for device sterility adapted from a previously presented protocol for cardiac catheters [14]. Briefly, 40 mL of trypticase soy broth was added in each container to guarantee the complete submersion of the device segment and a large availability of nutrients. Containers with device and liquid medium were incubated aerobically at 37°C, checking daily for broth turbidity. Turbid media were subcultured onto sheep-blood agar plates and all the recovered organisms were identified. Broths showing no turbidity after 30 day of incubation were subcultured in sheep-agar plates and checked for supporting bacteria growth as described previously [14]. Colony counting was performed up to 10^5 CFUs per plate. According to this limit, the most suitable dilution was selected per each device type and protocol. Survival counts per group were averaged over the four sample tested per each protocol and reported as CFUs per device.
Table 1: Electrophysiology and ablation cardiac catheter tested in the study.

<table>
<thead>
<tr>
<th>Device Type/s (manufacturer)</th>
<th>Catheters amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protocol i</td>
</tr>
<tr>
<td>RF Conductor™ 7Fr (Medtronic Inc.)</td>
<td>3</td>
</tr>
<tr>
<td>Torkr™ Josephson type (Medtronic Inc.)</td>
<td>3</td>
</tr>
<tr>
<td>Compli™ Cournand type (Biosense-Webster Inc.)</td>
<td>3</td>
</tr>
<tr>
<td>Woven™ 5Fr Cournand type (Bard Inc.)</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
</tr>
</tbody>
</table>

Abbreviations: C+: positive control

Table 2: Decontamination and cleaning protocols.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>i)</td>
<td>1, Chlorine solution; 2, Enzymatic solution</td>
</tr>
<tr>
<td>ii)</td>
<td>1, Enzymatic solution; 2, Chlorine solution</td>
</tr>
<tr>
<td>iii)</td>
<td>Polyphenolic emulsion</td>
</tr>
<tr>
<td>iv)</td>
<td>1, Polyphenolic emulsion; 2, Enzymatic solution</td>
</tr>
</tbody>
</table>

Table 3: Solutions, chemicals, and methods used in decontamination and cleaning protocols.

<table>
<thead>
<tr>
<th>Solution/emulsion (Trade name)</th>
<th>Active principle</th>
<th>Main components</th>
<th>Modality of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine (Bionil®; Eurospital, Trieste, Italy)</td>
<td>Hypochlorous acid</td>
<td>Sodium dichloroisocyanurate Boric acid</td>
<td>9.2 g/L of water (5000 ppm free chlorine), 10 min</td>
</tr>
<tr>
<td>Enzymatic (Septozim CE®; Farmec, Verona, Italy)</td>
<td>Alkaline protease</td>
<td>Enzymatic mixture</td>
<td>1% vol in water (40-50°C), 10 min</td>
</tr>
<tr>
<td>Polyphenolic (Colloidale Gamma®; Farmec, Verona, Italy)</td>
<td>Carbolic acid</td>
<td>o-Phenylphenol</td>
<td>3% vol in water</td>
</tr>
<tr>
<td>o-Benzyl-p-chlorophenol Sodium alcan-sulphonate Sodium sulphoricinate</td>
<td></td>
<td>15 min</td>
<td></td>
</tr>
</tbody>
</table>

Samples were defined as “sterile” if no growth of bacteria was present on both solid and liquid media.

Bacterial logarithmic reduction due to the applied experimental protocol was also computed by subtracting the mean log (CFUs) of processed devices from the mean log (CFUs) of positive controls.

RESULTS

The mean number of CFUs per device after the application of the four different protocols is reported in Figure 1. Each graph presents data pertaining to one of the three tested lots. CFUs amount is presented at 24h and 48h of incubation to elicit any possible bacteriostatic effect as revealed in Figures 1b (protocol iii)) and 1c (protocol iv)). Positive controls (C+) are also reported for easy of comparison. Table 4 summarizes the microbial logarithmic reduction of the four protocols in all the three tested condition (i.e. lot). Results indicate a higher biocide properties (>2.6-3.8 log reduction) of the hypochlorous acid based protocols (i) and (ii)) irrespectively from the test strain and the inoculum concentration. Differently, a lower bactericidal activity was associated to the use of polyphenol based protocols (iii) and iv)) (1.9-3.2 log reduction). Moreover, a difference in the number of surviving microorganisms between protocol iii) and protocol iv) was present, showing a reduction in the CFU/device due to the addition of the enzymatic treatment in the protocol.

Sterile samples were obtained only from samples at low nominal inoculum (I lot and II lot). Two sterile samples were found in I lot after protocol ii). In II lot, two samples treated with protocol ii) and one sample with protocol i) were sterilized.

DISCUSSION

This study complements previous electron microscopy (EM) data by quantifying CFUs at catheter surface before and after the application of different experimental protocols. Investigations performed by scanning and transmission EM on cardiac catheter subjected to the same protocols here presented, allowed to identify and localize the amount of biologic residuals at the catheter surface [12]. The EM investigation elicited the presence of bacteria within the residual soil at the electrode-shaft interface. However no information of microorganism’s viability was available from that investigation. It was shown than the use of a chlorine-based solution as the first step in decontamination-cleaning protocols resulted in the fixation of the blood proteins, blocking red cells and bacteria in a compact organic soil [12]. Proteins denaturation compromised also the efficacy of subsequent treatments with enzymatic solution showing an
Figure 1 Retrieved Colony forming units per device (CFUs/sample) after the application of the four different experimental protocols for decontamination and cleaning (i, ii, iii, iv). a) I lot contaminated with blood spiked with *B. subtilis* (10^8 CFU/mL). b) II lot contaminated with blood spiked with *P. aeruginosa* (10^6 CFU/mL). c) III lot, contaminated with blood spiked with *P. aeruginosa* (10^8 CFU/mL). Bar charts presents mean and SD values of four measurements.

**Abbreviations:** C+: positive control; N.G: no CFU on agar medium

interaction during disinfection process. Moreover, the intrinsic chemical biocide strength of the biocide is of basic relevance. Standard tests for bactericidal disinfectants activity are usually conducted by assessing viability of planktonic bacteria [20,21]. These testing methodologies could address for the right choice of the chemical to use but the relative activity of a disinfectant agent against a surface adhered bacterium or even a coagulum protected spore, could be markedly lower and can follow different inactivation kinetics. The methodological approach used in this study was able to quantify the reduction of the bacterial bioburden adhering to the device surface.

All protocols assured a bioburden reduction of 1.9 log or higher thus confirming a disinfection effect, but chlorine based protocols (protocols i and ii)) were more effective in respect to polyphenol based treatments (protocols iii and iv)).

By considering the two tested bacteria species it is also possible to evidence that the microbial reduction in *B. subtilis* spiked samples is higher than in *P. aeruginosa* treated with the same treatment. This confirms the higher resistance to disinfectants of gram-negative bacteria.

The broad spectrum of bactericidal and virucidal activity of chlorine solutions is well documented [20,22,23] but concentration of the disinfectants must refer to available chlorine after the chlorine demand of the biologic soil has been satisfied [22,24]. Concentration of 5000 up to 100000 ppm of free chlorine could be required for decontamination of organic fluids [1]. Therefore the absence of any pre-treatment on soiled devices decreases the biocide activity [23] as well as the cleaning efficiency. Protocol [1] was drafted by considering the presence of organic matter on the devices. NaDCC was selected as the favorite chlorine-releasing compound for assuring a suitable level of hypochlorous acid (the prime and more effective source of free chlorine) even in such disadvantageous condition. However, as previously evidenced by Kampf and collaborators [25], an effective cleaning should precede the chemical disinfection especially for highly soiled devices. The use of the proteolytic detergent might favor the subsequent decontamination treatment by NaDCC, enhancing its biocide strength by limiting the free chlorine organic sequestration and giving an effective contribution to the pre-sterilization treatment. This effect, though not clearly documented in this study, could occur in case of low availability of free chlorine. In that situation, the reduction of the whole organic load could bring to a relevant reduction of the bacterial bioburden even though no biocide action is related to the detergent [16]. So that, the use of a chemical with good cleaning properties is a key step also for its decontamination effect and should be recommended in reprocessing medical devices.

Differently, the higher cleaning efficiency of the polyphenolic treatments, previously documented by EM [12], was not reflected into a higher bactericidal activity. Although the efficiency of polyphenolic emulsions against gram-positive and gram-negative bacteria has been reported [26] and phenolic derivatives are considered effective as fungicidal and tuberculocidal, their use in this specific application was not as effective in reducing the bacterial bioburden as the application of chlorine compounds based protocols. Limitations in the use of polyphenols were insufficient cleaning of the NaDCC pre-treated device. These evidences should be considered in defining the optimal protocol.

Decontamination efficacy strongly depends on the bioburden amount by factors as the depth of action and the chemicals
Table 4: Bacteria reduction after different decontamination and cleaning protocols.

<table>
<thead>
<tr>
<th>Device lot</th>
<th>Initial Bioburden (C+)</th>
<th>Mean log reduction ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protocol i</td>
</tr>
<tr>
<td>(blood inoculum)</td>
<td>CFU/device</td>
<td></td>
</tr>
<tr>
<td>I lot</td>
<td>B. Subtilis (10^8 CFU/mL)</td>
<td>2.1x10^4</td>
</tr>
<tr>
<td>II lot</td>
<td>P. Aeruginosa (10^6 CFU/mL)</td>
<td>9.1x10^3</td>
</tr>
<tr>
<td>III lot</td>
<td>P. Aeruginosa (10^6 CFU/mL)</td>
<td>2.1x10^4</td>
</tr>
<tr>
<td>IV lot</td>
<td>P. Aeruginosa (10^6 CFU/mL)</td>
<td>1.6x10^4</td>
</tr>
</tbody>
</table>

Abbreviations: SD: standard deviation; C+: positive control; CFUs: colony forming units

Notes: *Mean Log bacteria reduction was calculated as the difference between the log of the number of CFUs retrieved from sample undergoing no treatment and the log of the number of CFU retrieved after each treatment protocol. Only 48h colony count was considered in this computation.

Previously reported, showing a limited sporicidal effect [1] and disinfection of HIV, HBV and HCV is reliable only by providing a low blood amount, the correct agents concentration and the presence of a cationic surfactant for avoiding organic inhibition [1,21]. To overcome this limitation, polyphenolic detergents formulations make use of ionic and non-ionic surfactants, enhancing both the dispersion properties in water of these non-soluble compounds and their cleaning efficacy. Anyhow our experimental data suggest a limited bactericidal activity on highly soiled devices. Some concern about toxic residual is moreover related to the use of polyphenolic compounds for polymeric device disinfection. Chemical affinity of the compounds to some polymeric segments may favor polyphenol absorption and potential release during subsequent reuse. Moreover the interaction with medical grade polymers could modify mechanical and thermodynamic properties or induce discoloration. Some authors discouraged their use also for semicritical devices [1] but recent formulations should be further tested.

Since the reprocessing of critical medical devices is nowadays a common practice in US and also in some European countries (e.g. Germany), the paper add some new information on the importance of decontamination and cleaning when a SUDs reprocessing and reuse policy is considered. The study was conceived as an in-vitro experiment and adds some value to the large body of evidences that should be collected to properly assess feasibility of reprocessing and reusing SUDs in a safe and effective way. Further investigations have to be provided about other critical points like sterilization efficacy, performance tests, correct repackaging, etc. that are mandatory for a complete and reliable reprocessing protocol.

Moreover, SUDs could be considered as a good model for many reprocessable and reusable medical devices that are often lacking in detailed indications about reprocessing. Results of this study could be partly extended on other reusable medical device with design and materials similar to electrophysiology catheters in order to optimize the decontamination and cleaning steps during reprocessing.

CONCLUSION

Testing efficacy of different decontamination protocols for reducing microbial load on critical medical device is of utmost importance for identifying the most effective treatment in reprocessing single-use catheters. The experimental approach presented here, simulated clinical use soiling with human blood and bacteria. Four possible cleaning and decontamination protocols were tested to better elucidate bactericidal properties of the chemicals in the decontamination of single-use electrophysiology catheters.

Differences between polyphenol-based and chlorine-based treatments were found, with a lower microorganism’s retrieval after using NaDCC solution. Although polyphenolic emulsion effectively remove blood and organic soil, this treatment realized a lower level of disinfection and concern about materials compatibility should be considered.

These evidences suggest that the use of protocols including chlorine releasing compounds and proteolytic detergent are the most bactericidal. However, to properly clean the soiled device, a two step protocol is required, providing at first the cleaning phase by the enzymatic detergent, followed by the immersion in the chlorinated solution. This approach maximizes the bactericidal efficiency and also guarantees an effective cleaning, as previously reported by EM investigations [12]. Taking into account that this optimized protocol does not provide for an immediate decontamination of the blood contacted device, adequate safety measures (e.g. the introduction of suitable personal protective equipment) should be introduced to minimize the risk of exposing health care workers to biological agents. Wherever these risks cannot be reduced, polyphenol-based protocols with immediate device decontamination after use should be considered.

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