Is using an open-reservoir cardiopulmonary bypass circuit after 6 days on standby safe?


*Department of Medical Engineering, National Hospital Organization Kure Medical Centre and Chugoku Cancer Centre, Hiroshima, Japan

**Department of Internal Medicine, National Hospital Organization Kure Medical Centre and Chugoku Cancer Centre, Hiroshima, Japan

Department of Cardiovascular Surgery, National Hospital Organization Kure Medical Centre and Chugoku Cancer Centre, Hiroshima, Japan

Department of Anesthesiology, Critical Care and Pain Medicine, National Hospital Organization Kure Medical Centre and Chugoku Cancer Centre, Hiroshima, Japan

*Corresponding author. Department of Medical Engineering, National Hospital Organization Kure Medical Centre and Chugoku Cancer Centre, 3-1, Aoyamacho, Kure, Hiroshima 737-0023, Japan. Tel: +81-823-223111; fax: +81-823-210478; e-mail: tagayam@kure-nh.go.jp (M. Tagaya).

Received 7 July 2015; received in revised form 16 September 2015; accepted 21 September 2015

Abstract

OBJECTIVES: To investigate the sterility and biocompatibility of a stored open-reservoir cardiopulmonary bypass circuit maintained on standby.

METHODS: A total of four cardiopulmonary bypass circuits were assembled, primed and left to recirculate. One unit was placed in a positive-pressure operating room and the other three were placed in the intensive care unit. The primed solutions, which employed Ringer’s acetate, hydroxyethylated starch and hydrate steroid, were sampled after 0, 24, 48, 72, 96, 120 and 144 h in all cardiopulmonary bypass circuits to measure the bacteria count, endotoxin count and chemical substances within the primed solution. Chemical substances were detected by assessing the following: the total organic carbon by the combustion oxidation infrared spectrometry, and molecular weight spread by gel permeation chromatography. The environments were left unattended and were uncovered during the storage period to mimic the clinical scenario.

RESULTS: There were no bacteria in any of the primed solutions, and only very minute concentrations of endotoxins were detected, both in the operating room and in the intensive care unit. The total organic carbon concentration was slightly more concentrated in the 144-h samples when compared with that in the 0-h samples. However, the molecular weight spread of the 0-h sample was identical to that in the 144-h sample.

DISCUSSION: With regard to the presence of bacteria and endotoxins, we noted that the hardshell reservoirs in the cardiopulmonary bypass circuit were effectively sealed and not invaded by bacteria. With regard to the presence of chemical substances, we noted that an increase in total organic carbon concentration was caused by bedewing, and that there was no release of chemical substances such as a polymer-coating agent, or other molecular materials in the primed solution.

CONCLUSIONS: There was no contamination or release of chemical substances in 6-day old cardiopulmonary bypass circuits maintained on standby, confirming that they are safe to use in terms of sterility and biocompatibility.

Keywords: Cardiopulmonary bypass • Extracorporeal membrane oxygenator • Standby • Polymer • Coating • Contamination

INTRODUCTION

Open-reservoir cardiopulmonary bypass (CPB) circuits are commonly used in cardiovascular and thoracic surgeries. In the emergency setting, a CPB circuit is commonly prepared before starting the surgery. However, if cardiovascular and thoracic surgeries are aborted, the CPB circuit is wasted. If the wet-primed oxygenator is used for another surgery on the following day, it is essential that there is no bacterial contamination, malfunctioning of the membrane oxygenator or release of chemical substances. Some investigators have reported that there was no bacterial contamination or released plasticizer in wet-primed oxygenators maintained in recirculation in crystalloid solution for 7–14 days [1–3], and others have reported that recirculated wet-primed oxygenators maintain the function of the membrane oxygenator [3–5]. Given the aforementioned information, it can be considered that a recirculated wet-primed oxygenator, which has been assembled several days before, can be used. However, although standby CPB circuits are available for use, many physicians still do not use them, largely owing to the fact that reports on bacterial contamination in the...
clinical setting (i.e. not only in a laboratory) and release of chemicals other than plasticizer have not yet been published.

Hence, in this report, preprimed CPB circuits recirculated for several days were tested to assess bacterial contamination and released chemical substances in the operating room (OR) or intensive care unit (ICU). We aimed to determine whether CPB circuits primed for several days can indeed be used in the clinical setting.

MATERIALS AND METHODS

Oxygenator and circuit

We used Ringer’s acetate, hydroxyethylated starch and hydrate steroid, in the study setting, as a default in all the experimental priming solutions because the experiment mimicked the clinical scenario. Additionally, none of the study experiments involved the use of heparin. The experimental oxygenator and circuit (Fig. 1) consisted of a hardshell reservoir (MERA, Tokyo, Japan), membrane oxygenator (MERA), arterial filter (MERA), polyvinyl chloride tube (MERA), haemoconcentrator (JMS, Tokyo, Japan) and two roller pumps (Sarns, Ann Arbor, MI, USA). The wall inside the circuit and the contact face with primed solution were coated with a polymer-coating agent. The polymer macromolecular materials in the circuit included the following: polypropylene, polycarbonate and silicone in a membrane oxygenator; polyethylene terephthalate, polycarbonate and urethane in a heat exchanger; polyester, polypropylene, polyurethane and silicone in a hardshell reservoir; polyester in an arterial filter; polyethylene in a sample port; polyvinyl chloride in the circuit tube; silicone, alkyl group carbon and polyethylene glycol in a coating agent (Table 1). The hollow fibre of the oxygenator is made from polypropylene and is coated with silicone.

The experimental circuit was opened in a sterile fashion and primed with 1000 ml of Ringer’s acetate, 1000 ml of hydroxyethylated starch and 24 ml of a hydrate steroid. The primed solution

Figure 1: Diagram of the experimental circuit. (a) Reservoir, (b) membrane oxygenator with heat exchanger, (c) arterial filter, (d) CDI 100 cube (Terumo, Tokyo, Japan) and (e) the haemoconcentrator, which are connected with a 3/8-inch polyvinyl chloride tube. (f) The diaphragm for pressure measurement, (g) CDI 500 cube (Terumo) and (h) sampling port are connected with a 1/8-inch polyvinyl chloride tube. The suction circuits are connected to the reservoir with a 1/4-inch polyvinyl chloride tube, and are fixed on (i) roller pumps, which are filled with air, without fluid. The distal suction circuits are capped but not sealed. (j) The waste liquid bottle is connected to the outlet of a filtration port on the haemoconcentrator with a 3/8-inch polyvinyl chloride tube, and is occluded (i.e. it is filled with air, without fluid). The primed solution is circulated in the main circuit, which includes the membrane oxygenator and arterial filter, and is recirculated in the haemoconcentration circuit, which includes the haemoconcentrator and roller pumps. The haemoconcentration circuit is bifurcated from the main circuit. The primed line remains untouched after priming is initiated, and the connection between the tube and (k) solution bag is exposed to the atmosphere. (l) The sterile package is sealed to maintain sterility of the surgical field tube. (m) The vent cock on the reservoir is capped but not sealed.
was recirculated at 3 l/min in the main circuit and at 0.2 l/min in the haemoconcentration circuit during the test phase.

Sterility studies

One test circuit was set up using sterile technique and primed in a positive-pressure OR. The primed solution was continuously recirculated in the test circuit. Twenty-four hours later, 60-ml samples were collected at the sample port (Fig. 1) to test for bacteria and endotoxins. Thereafter, test samples were collected to test for bacteria and endotoxins every 24 h until 144 h. These 60-ml samples were collected with a sterile syringe and were divided as follows: 50 ml for bacterial detection, 4 ml for endotoxin detection and 6 ml for rejection. Bacteria were detected using the membrane filter method (37-mm quality monitor; PALL, Covina, CA, USA). Endotoxins were detected using an endospecy test (LSI Medience Corp., Tokyo, Japan). The test circuit was stored 3 m from the surgical bed in the OR, was left unattended and was uncovered during the storage period. The priming solution bag was kept in connection with the priming tube during the test phase. In the OR, other surgeries (e.g. lung and peripheral vascular surgeries) were performed during the study period. The temperature in the OR was controlled at 24°C by an air conditioner.

In the ICU, where patients are repeatedly hospitalized and released, three test circuits were set up using sterile technique, and primed. The primed solution was continuously recirculated in the test circuit. Twenty-four hours later, 60-ml samples were collected to test for bacteria and endotoxins. Thereafter, the test samples were collected to test for bacteria and endotoxins every 24 h until 144 h. One of the three test circuits was used to detect both endotoxins and bacteria at each time point; for endotoxin and bacterial detection, the 60-ml samples were collected with a sterile syringe and were divided as follows: 50 ml for bacterial detection, 4 ml for endotoxin detection and 6 ml for rejection. In contrast, the other two test circuits were used only for bacterial detection; for bacterial detection alone, the 60-ml samples were collected with a sterile syringe and were divided as follows: 50 ml for bacterial detection and 10 ml for rejection. The methods of detection of bacteria and endotoxins in the ICU were the same as those in the OR. The test circuit was stored 3 m away from each bed in the ICU, was left unattended and was uncovered during the storage period. The priming solution bag was kept in connection with the priming tube during the test phase. The temperature in the ICU was controlled at 26°C by an air conditioner.

Table 1: Material of the cardiopulmonary bypass circuit

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane oxygenator</td>
<td>Polypropylene</td>
</tr>
<tr>
<td></td>
<td>Polycarbonate</td>
</tr>
<tr>
<td></td>
<td>Silicone</td>
</tr>
<tr>
<td>Heat exchanger</td>
<td>Polyethylene terephthalate</td>
</tr>
<tr>
<td></td>
<td>Polycarbonate</td>
</tr>
<tr>
<td></td>
<td>Urethan*a</td>
</tr>
<tr>
<td>Hardshell reservoir</td>
<td>Polyester</td>
</tr>
<tr>
<td></td>
<td>Polypropylene</td>
</tr>
<tr>
<td></td>
<td>Polyurethane</td>
</tr>
<tr>
<td></td>
<td>Silicone</td>
</tr>
<tr>
<td>Arterial filter</td>
<td>Polyester</td>
</tr>
<tr>
<td>Sample port</td>
<td>Polyethylene</td>
</tr>
<tr>
<td>Tube</td>
<td>Chloroethene</td>
</tr>
<tr>
<td>Coating agent</td>
<td>Alkyl group carbon</td>
</tr>
<tr>
<td></td>
<td>Silicone</td>
</tr>
<tr>
<td></td>
<td>Polyethyleneglycol</td>
</tr>
</tbody>
</table>

*a: Adhesive material.

Table 2: Bacterial and endotoxin counts

<table>
<thead>
<tr>
<th>Storage</th>
<th>Detection</th>
<th>Duration (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>OR</td>
<td>Bacterial counts (CFU/ml, n = 1)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Endotoxin counts (EU/ml, n = 1)</td>
<td>0.002</td>
</tr>
<tr>
<td>ICU</td>
<td>Bacterial counts (CFU/ml, n = 3)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Endotoxin counts (EU/ml, n = 1)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*n is the number of test circuits used for assessment.

OR: operating room; ICU: intensive care unit; CFU: colony-forming unit; EU: endotoxin unit.

Release of chemical substances

The CPB circuit comprises various substances composed of macromolecular materials (Table 1). Therefore, we measured the total organic carbon (TOC), because carbon is included in all of these macromolecular materials. Combustion oxidation infrared spectrometry was employed to measure the TOC. Furthermore, we compared the molecular weight spread in the primed solution between the 0-h sample (sampled immediately after starting recirculation) and 144-h sample by gel permeation chromatography (GPC). The test circuit, recirculated duration, sampled time and storage were the same for the single sterility test circuit used in the OR as in the three sterility test circuits used in the ICU. The primed solution was recirculated in the test circuit, and 100-ml samples were immediately collected with a sterile syringe for the TOC and GPC analyses. TOC samples were collected every 24 h until 144 h. At this time (144 h), a GPC sample was also collected. All samples were stored in glass bottles at 0°C until the TOC and GPC were measured.
molecular weight spreads of the 0-h sample, 144-h sample, blank and dibutyl phthalate (as the standard substance) are shown in Fig. 3. The peaks in the 0-h chart were almost identical to the peaks in the 144-h chart, suggesting that no chemical substances were released. However, as seen in Fig. 3, the third peak in the 144-h chart showed an insubstantial difference compared with the third peak in the 0-h chart (the 144-h chart had a slightly peaked shoulder). Hence, it is possible that a peak caused by the release of molecular material may potentially be hidden under the third peak.

RESULTS

Sterility studies

In the OR, the temperature and humidity were 24.3 ± 0.6°C, and 25 or 26%, respectively, during the test phase. In the OR, the bacterial counts were 0 colony-forming units (CFUs)/ml for all samples, whereas the endotoxins ranged from 0.001 to 0.003 endotoxin units (EU)/ml in each sample. The temperature and humidity of the ICU were 26.9 ± 0.3°C and 27 ± 2%, respectively, during the test phase. In the ICU, the bacterial counts were 0 CFU/ml for all tests, and the endotoxins ranged from 0.002 to 0.004 EU/ml for each sample (Table 2). In all test circuits, bedewing was observed on the inside wall of the reservoirs.

Release of chemical substances

The shifts in the TOC concentration over time are shown in Fig. 2. The TOC concentration in the 144-h samples was slightly more concentrated compared with that in the 0-h samples.

The molecular weight spreads of the 0-h sample, 144-h sample, blank and dibutyl phthalate (as the standard substance) are shown in Fig. 3. The peaks in the 0-h chart were almost identical to the peaks in the 144-h chart, suggesting that no chemical substances were released. However, as seen in Fig. 3, the third peak in the 144-h chart showed an insubstantial difference compared with the third peak in the 0-h chart (the 144-h chart had a slightly peaked shoulder). Hence, it is possible that a peak caused by the release of molecular material may potentially be hidden under the third peak.

DISCUSSION

Sterility and the release of chemical substances

The results of the sterility studies suggested that there was no bacterial contamination in the primed and recirculated circuit, even though the test condition was similar to the condition in clinical practice. Thus, our results indicated that neither a bacterial inroad nor breeding was present in the circuits, regardless of whether the circuits were stored in a positive-pressure environment, suggesting that the surrounding environment did not affect the bacterial growth. Dalstrom et al. reported that, in a positive-pressure OR, an uncovered sterile tray was contaminated after 4 h of exposure [6]. Based on this information, the sterility study in this report indicated that the reservoir was sealed effectively, as it was highly likely that environmental contamination was present in the ICU. With regard to the primed solution, it has been reported that proteins, lipids and phospholipids are necessary for the growth and survival of bacteria [7, 8]. However, the growth and survival of bacteria in hydroxethylated starch have not been previously reported. Furthermore, with regard to the study duration, previous studies reporting circuit sterility for 7 days have been published [1, 2], however, these studies were only laboratory-based. Moreover, Karimova et al. reported that a primed, recirculated circuit can be maintained in a sterile state for up to 14 days in the clinical setting, but they recommended maintaining the circuit at 8°C [3]. In our study, we confirmed that the circuit can be left unattended in a sterile state for up to 6 days in the clinical setting, because no bacteria contamination was noted over 6 days in normal clinical conditions (i.e. normal storage, normal temperature and normal primed solution with a plasma substitute).

Endotoxins are known to be produced when gram-negative bacteria die [9]. The allowed threshold for endotoxins is 5 EU/kg/h for most intravenous applications [10], with the endotoxin content of distilled water being ~20 EU/ml [11]. Thus, in our study, endotoxins were detected in only very limited concentrations, confirming the safety of using a 6-day old circuit in terms of the endotoxic threat.

The COC results indicated that the carbon concentration in the primed solution increased with time, and there are two hypotheses to explain this finding: (i) an increasing amount of carbon due to the release of any chemical substances, or (ii) a decrement of the primed solution due to vaporescence. Hypothetically, if chemical substances were released in the CPB circuit, the coating agent from the polymer-coated polyvinyl chloride tube may have been released first. When the coating agent on the polyvinyl chloride tube is denuded, three problems may occur: plasticizers may be released by the exposed polyvinyl chloride, the coating agent may be harmful to humans and platelet preservation may be impaired [12–15]. However, the result of the GPC analysis suggested that no macromolecular materials were released in the primed solution. This finding, along with the observation of bedewing, suggests that the reason for the carbon concentration increment in this case was vaporescence, indicating that there was no release of chemical substances.

With regard to plasticizers in CPB circuits, Riley et al. reported that normal saline priming does not lead to the release of plasticizer for up to 4 weeks [16]. Han et al. also reported that no accumulation of plasticizer in the circulating plasmalyte was detected in their study [17]; furthermore, Karimova et al. suggested that plasticizer was not released, despite detecting plasticizer in the primed recirculated solution, because the plasticizer levels measured for 2 weeks were lower than the plasticizer levels detected in the priming solution bag [3]. Based on these reports, our finding that no substances were released after 6 days in the recirculation circuit seems adequate. Furthermore, if there is a tentative risk of plasticizer release (based on the presence of a slightly peaked shoulder in Fig. 3), the primed...
solution only has to be filtrated and diluted before use. Filtration and dilution can remove any substances with a molecular weight of <1000. Accordingly, Tagaya et al. reported that small molecular materials can be removed by ultrafiltration or dialysis [18, 19]. With regard to the issues with biocompatibility and platelet preservation mentioned above, a heparin-coated or polymer-coated circuit has been reported to significantly prevent inflammation and to significantly preserve platelets compared with a non-coated circuit [14]. Therefore, denuding the coating agent may have a significant negative impact on CPB surgeries. However, our results (Fig. 3) indicated that the polymer-coating agent can remain intact for (at least) up to 144 h. To our knowledge, this is the first report on the longevity of a polymer-coating agent of a preprimed CPB circuit. Finally, with regard to the release of chemical substances, we confirmed that the CPB circuit can be safely used for up to 6 days in a normal clinical situation.

Clinical use

If a primed circuit is used after 6 days on standby, it must be ensured that there is no bacterial contamination, malfunctioning of the membrane oxygenator or release of chemical substances. In this study, two of these matters were addressed: the risk of infection and the release of chemical substances. However, we did not evaluate the remaining concern, i.e. the risk of membrane oxygenator malfunctioning, because there is already a variety of information available on this topic. Previous studies have suggested that adequate oxygenator function can be preserved for >14 days in crystalloid prime [3, 5]. However, it should be noted that these previous studies were performed in a laboratory, although these findings are presumed to mimic clinical practice well. On the other hand, Gao et al. reported a decline in gas transfer after only 72 h with priming with 4.5% albumin but not after priming with normal saline [4]. Their report indicated that maintaining gas transfer differs between priming with crystalloid and albumin. In this study, there was a risk of a decline in gas transfer, because we employed a plasma substitute in the primed solution. However, we predict that this decline in gas transfer is non-significant with regard to performing CPB surgery, because the membrane oxygenator uses only a small part of its gas transfer capacity throughout the surgery (i.e. the capacity of gas transfer is significantly larger than the aforementioned decrease in gas transfer) [20, 21]. Therefore, a 6-day old circuit can be considered acceptable from a comprehensive perspective.

Limitations

This study used only one circuit, which consisted of a single reservoir, a single membrane oxygenator and a single arterial filter. However, different coating agents are employed by different manufacturers, and the fact that this study only tested one coating agent is its major limitation. Therefore, further studies performed with circuits comprising several kinds of coating agents should be conducted in the future to confirm our findings.
CONCLUSIONS

Our results revealed that a preprimed recirculated CPB circuit can be safely used (in terms of sterility and biocompatibility) for at least 6 days after priming. This information may contribute to the flexible use of preprimed recirculated CPB circuits in the clinical setting.

ACKNOWLEDGEMENTS

We thank Hironori Izutani, Cardiovascular and Thoracic Surgery, Ehime University Graduate School of Medicine, Ehime, Japan, for providing valuable advice; Hideki Kanoh, A-kit Corp., Gifu, Japan, for providing analytical assistance; the medical staff of the ICU and OR in Kure Medical Centre and Chugoku Cancer Centre, for their cooperation; and Editage (www.editage.jp), for English language editing.

Conflict of interest: none declared.

REFERENCES