**In vitro** haemocompatibility of a novel bioprosthetic total artificial heart†

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**OBJECTIVES:** The CARMAT total artificial heart (TAH) is an implantable, electro-hydraulically driven, pulsatile flow device with four bioprosthetic valves. Its blood-pumping surfaces consist of processed bioprosthetic pericardial tissue and expanded polytetrafluoroethylene (ePTFE), potentially allowing for the reduction of anti-coagulation. This pre-clinical study assessed the in vitro haemocompatibility of these surfaces.

**METHODS:** Coupons of pericardial tissue and ePTFE were placed in closed tubular circuits filled with 12.5 ml of fresh human blood exposed to the pulsatile flow at 120 ml/min for 4 h (37°C). Silicone- and heparin-coated polyvinyl chloride (PVC) tubes served as positive and negative controls, respectively. Fresh blood from six donors was used to fill four sets of 12 circuits. Blood samples were taken at baseline and from each circuit after 4 h. Coupons of materials were examined with scanning electron microscopy.

**RESULTS:** The platelet count was 202 ± 45 × 10⁹ l⁻¹ at baseline. Four hours after circulation, the platelet counts were 161 ± 30 × 10⁹ l⁻¹ (compared with baseline, P = 0.0027) for pericardial tissue, 162 ± 35 × 10⁹ l⁻¹ (P = 0.0035) for ePTFE and 136 ± 42 × 10⁹ l⁻¹ for positive controls (P = 0.0021). Baseline plasma fibrinogen was 2.9 ± 0.5 mg/dl compared with 3.0 ± 0.5 mg/dl for pericardial tissue and 3.1 ± 0.7 mg/dl for ePTFE, indicating no marked fibrinogen consumption. Thromboxane B2 levels for positive controls were 33.3 ± 8.7 ng/ml compared with 16.2 ± 11.5 ng/ml for pericardial tissue (P = 0.0015) and 15.2 ± 4.7 ng/ml for ePTFE (P < 0.0001). Platelet adhesion was 2.87 ± 1.01 × 10⁹ cm⁻² for positive controls compared with 1.06 ± 0.73 × 10⁹ cm⁻² for pericardial tissue (P < 0.0001) and 0.79 ± 0.75 × 10⁹ cm⁻² for ePTFE (P < 0.0001). Thrombin–antithrombin III complex levels were 3.8 ± 0.5 μg/ml for positive controls compared with 1.9 ± 0.9 for pericardial tissue (P < 0.0001) and 2.1 ± 1.0 for ePTFE (P < 0.0001). With an electro-microscopic examination at ×600, only small depositions of platelets, erythrocytes and fibrin were noticed on the pericardial tissue samples and ePTFE samples. Silicone surfaces showed marked areas of thrombi, and PVC tubings a thin protein layer.

**CONCLUSIONS:** Haemocompatibility of the TAH blood-contacting surfaces was confirmed by in vitro studies showing a limited consumption of fibrin, limited thromboxane B2 release and platelet adhesion, and minor blood cell depositions on the surfaces. These results will be validated in clinical studies, with the aim of reducing anti-coagulation when using the CARMAT TAH.

**Keywords:** Artificial heart • Assisted circulation • Transplantation • Haemocompatibility

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**INTRODUCTION**

The prevalence of heart failure (HF) in Europe and the USA is ~12 million, of which 0.5–5% are in advanced HF refractory to medical management [1–4]. Cardiac transplantation remains the primary option for just a select group of end-stage HF patients due to the shortage of donor organs. The number of heart transplant procedures reported to the International Society for Heart and Lung Transplantation Registry has plateaued between 3200 and 3400 procedures per year since 2002 [5].

The adoption of left ventricular assist devices (LVADs) as a viable therapy for end-stage HF has resulted in increased referrals of patients on both sides of the spectrum, from New York Heart Association class I to III, to those in need of total cardiac replacement therapy [6–8]. For the latter group, pneumatically driven total artificial hearts (TAHs) and biventricular VADs have been used as a bridge to transplantation, at the cost of high morbidities related to the devices (infection, bleeding, stroke) and limited mobility for the patient [9–11]. The CARMAT TAH has been developed to minimize these drawbacks. Its blood-contacting surfaces consist of expanded polytetrafluoroethylene (ePTFE) and bovine pericardial tissue processed in glutaraldehyde [12]. These materials have demonstrated a high...
level of biocompatibility in applications such as cardiac valve replacement and vascular grafts [13, 14]. In particular, pericardial tissue has been used extensively in bioprosthetic valves [14].

In the preclinical testing phase of the CARMAT TAH, the in vitro haemocompatibility of these surfaces was assessed and the results are presented here.

MATERIALS AND METHODS

CARMAT total artificial heart

The CARMAT TAH contains two ventricles, each with a blood compartment and a liquid compartment, separated by a pulsatile hybrid membrane (Fig. 1). The hybrid membrane has a polyurethane layer at the liquid-contacting surface and bovine pericardial tissue on the blood-contacting surface. The fixed surface of the blood compartment is covered with ePTFE. Electro-hydraulic pumps create a systolic and a diastolic phase by moving the silicone fluid and deploying the hybrid membrane. The stroke volume (30–65 ml) and the beat rate (35–150 min⁻¹) of the prosthesis adapt automatically in response to changes in preload, detected by the pressure sensors located in the device. The resulting pulsatile blood flow ranges from 2 to 9 l/min with a flow adjustment on the right side to correct for the bronchial shunt. Bioprosthetic valves at the inlet and outlet of each blood compartment maintain the forward flow. The prosthesis is partially surrounded by a flexible compliance bag. A percutaneous driveline delivers power to the prosthesis and serves as a communication channel.

Test loops and test coupons

Test loops have been used as a simplified system to evaluate the haemocompatibility of the blood-contacting materials of the TAH. A dynamic blood flow model described previously was used for the tests (Fig. 2) [15].

The hybrid membrane coupons measure 50 × 30 mm² and consist of bioprosthetic pericardial tissue (Neovasc, Inc., Richmond, BC, Canada) attached to polycarbonate urethane (Advansource Biomaterials, Wilmington, MA, USA). The hybrid membrane is hermetically sealed and wrapped around the polyvinyl chloride (PVC) rings (5 mm in diameter) on both the ends with the pericardial tissue facing inwards, chemically sterilized and stored in 0.6% glutaraldehyde.

The ePTFE coupon (50 × 30-mm²; Bard Peripheral Vascular, Inc., Tempe, AZ, USA) is thermoformed and wrapped around the 5-mm diameter PVC rings. A 12-mm diameter heparinized PVC tube is placed around the ePTFE cylinder as reinforcement.
The hybrid membrane tubes (after rinsing three times in saline for 10 min) and the ePTFE tubes were connected to a 5-mm diameter heparin-coated PVC tube and subsequently to a 3-mm diameter heparin-coated PVC tube, which was part of a closed-loop circuit with a one-way ball valve as shown in Fig. 2. The negative control circuits were made of a similar heparin-coated PVC tube without the graft content, whereas the silicone rubber (polydimethylsiloxane) circuit contained a 12-mm silicone sheet and served as a positive control. Four batches of 12 circuits (with a volume of 12.5 ml) were built.

Fresh blood from six donors was drawn in syringes with heparin 1.5 U/ml and used within 30 min to fill the circuits. The pulsatile flow was maintained at 120 ml/min for 4 h (37°C).

Blood sampling and assays

Blood samples were taken at baseline (before filling the circuits) and 4 h after circulation in the test loop. Samples were analysed immediately for cell count and platelet count. A portion of the blood was anticoagulated with ethylenediaminetetraacetate acid (EDTA) to prepare the plasma. The plasma was used for analysis of thromboxane B2 (TxB2), thrombin–antithrombin III (TAT) complexes, fibrinogen, elastase and haemoglobin concentrations. Haematology measurements included erythrocytes, leucocytes, platelets, haemoglobin and fibrinogen.

The thrombin formation during the in vitro experiments was determined by means of TAT complexes in the EDTA plasma. An enzyme-linked immunosorbent assay was performed subsequently with capture and detection antibodies (Cederlane Laboratories, Hornby, Canada).

Activation of the arachidonic acid pathway in platelets results in the release of the potent platelet-aggregating agent thromboxane A2, which is rapidly converted into the inactive product thromboxane B2. Thromboxane B2 was measured by means of an enzyme immunoassay (Biotrak, Amersham, UK), based on labelled TxB2 competing with the TxB2 sample. In this test, the label is a peroxidase that converts the substrate tetramethylbenzidine, yielding a yellow colour which is measured at 450 nm by a spectrophotometer (Powerwave 200; Biotek Instruments, Winooski, VT, USA).

The platelet binding was measured based on the release of platelet acid phosphatase in the citrate buffer (pH 7.4), containing p-nitrophenyl phosphatase and Triton X-100. Substrate conversion is proportional to the amount of platelets, which was determined by a standard curve and the platelet count.

Fibrin-binding assays were performed by incubating washed biomaterials in the Tris buffer (pH 7.4), containing a labelled antibody to human fibrin, without cross-reacting with fibrinogen (American Diagnostica, Stamford, CT, USA). Label detection was based on the time-resolved fluorescence of europium (Victor, PerkinElmer, Waltham MA, USA).

Validation of heparin leaching from coated polyvinyl chloride tubing and haemolytic activity

Two PVC circuits were coated with polyethylenimine and subsequently with heparin by submersion in a 10 IU/ml heparin solution in the same manner as all the other circuits used for the test materials. After coating, washing and drying, the circuits were filled with 50 mM Tris buffer (pH 7.5). The Tris solution was circulated in the PVC circuits for 4 h at 37°C. Samples were collected at 0, 2 and 4 h and evaluated for thrombin inhibition in the presence of antithrombin III, to indicate heparin activity. The calculated heparin concentration was 0.02 (=0.2% of incubated heparin) at 0, 2 and 4 h, indicating that thrombin was virtually not inhibited by the circulated Tris solution and that heparin leaching did not occur.

Haemolytic activity of the test coupons is determined in a separate experiment, using 2-cm² test materials incubated with washed erythrocytes for 24 h under static conditions. The free haemoglobin and total haemoglobin concentrations were determined and their ratio was calculated as a measure of haemolysis.

Material surface characterization 4 h after circulation

The incubated coupons were washed with phosphate-buffered saline and each coupon was cut into three pieces. Two pieces were used in studying the binding of platelets and were labelled antibodies to fibrin. One piece was fixated immediately in glutaraldehyde to visualize the blood-contacting surface by means of scanning electron microscopy (SEM).

Data presentation and analysis

Results of the blood samples are presented as mean ± standard deviation. Paired Student’s t-tests were used to compare the test results of baseline, positive controls or negative controls (IBM SPSS Statistics, Chicago, IL, USA).

RESULTS

Haematology data are presented in Table 1. Erythrocyte counts and haemoglobin levels did not change significantly from baseline 4 h after flow in any of the circuits. Compared with the platelet counts at baseline (202 ± 45 × 10⁹ l⁻¹), the platelet counts were significantly lower in positive controls (126 ± 42 × 10⁹ l⁻¹, P = 0.002), ePTFE (162 ± 35 × 10⁹ l⁻¹, P = 0.031) and bioprosthetic pericardial tissue circuits (161 ± 30 × 10⁹ l⁻¹, P = 0.021), but remained within the normal ranges. Leukocyte counts increased significantly in circuits with bioprosthetic pericardial tissue (5.9 ± 1.6 × 10⁹ l⁻¹ versus 5.1 ± 1.0 × 10⁹ l⁻¹, P = 0.006), but remained within the normal ranges. There was no significant change in the levels of haemoglobin.

Plasma fibrinogen concentrations remained constant in all the circuits, indicating no significant consumption of fibrinogen by adhesion to the material surfaces or due to fibrin clot formation. Activation of the coagulation system was demonstrated by significantly higher levels of plasma TAT III complexes in all the circuits compared with baseline (P < 0.0001, Fig. 3). Negative controls, ePTFE and bioprosthetic pericardial tissue generated significantly less thrombin than the positive control circuit (P < 0.005).

Thromboxane B2 release from platelets increased significantly from baseline in all the circuits, with the highest levels in positive controls (P < 0.0001, Fig. 3). In the cases of ePTFE (15.21 ± 4.72 ng/ml) and bioprosthetic pericardial tissue (16.17 ± 11.46 ng/ml), thromboxane B2 release was significantly lower compared with the positive controls (33.34 ± 8.74, P < 0.005).
Elastase release from leukocytes increased significantly in all the circuits compared with baseline values (1.6 ± 0.9 μg/ml), especially in the pericardial tissue circuit (8.6 ± 3.2 μg/ml, Table 1).

Platelet adhesion on ePTFE (0.79 ± 0.75 × 10^9 cm^−2) and bioprosthetic pericardial tissue (1.06 ± 0.73 × 10^9 cm^−2) was significantly lower when compared with that on positive controls (2.87 ± 1.01 × 10^9 cm^−2, *P < 0.0001) and significantly higher when compared with that on negative controls (0.12 ± 0.03 × 10^9 cm^−2, *P < 0.01, Fig. 4). Fibrin binding on both the test materials (ePTFE: 4.80 ± 1.80 × 10^9 cm^−2 and bioprosthetic pericardial tissue: 5.27 ± 2.74 × 10^9 cm^−2) was similar to that on positive controls (5.18 ± 2.32 × 10^9 cm^−2, Fig. 4).

Haemolysis, determined during a 24-h direct exposure to the surfaces in a separate experiment, was calculated as the ratio of free-to-total haemoglobin release from erythrocytes. In the presence of all the materials, haemolysis was <0.4% on average and lowest in pericardial tissue (0.14 ± 0.10%) and ePTFE (0.10 ± 0.02%, Fig. 4).

### Scanning electron microscopy

Magnified SEM photographs (×12) were taken for all the four types of materials. The silicone surface (positive controls)
contained areas covered by a peelable film of fibrin, with erythrocytes and platelets trapped in the fibrin network (Fig. 5).

The heparin-coated PVC (negative controls) surface showed multiple, small spots on ×60 magnified images. At ×600 magnification, these spots appear to have a size of \(~10\ \mu m\) with pseudopodes and granules and appear to be leukocytes (Fig. 5).

The ePTFE surface contained areas of a thin fibrin network (nine of 12 samples) with platelets observed on two samples and appeared free from blood elements in three of the 12 samples (Fig. 6).

Bioprosthetic pericard material contained fibrin strands and erythrocytes in two of the 12 samples and platelets in two of 12 samples. The structure of pericardium has similarities to that of thick fibrin strands between which erythrocytes can be trapped. Six of the 12 samples contained almost no fibrin or platelets.

**DISCUSSION**

The blood-contacting surfaces of the CARMAT TAH ventricles demonstrated good haemocompatibility after exposure to circulating human blood *in vitro*. Platelet activation, measured by thromboxane B2 release and platelet binding to the surface, was significantly lower for ePTFE and pericardial tissue compared with the silicone surface, which served as a positive control. Activation of the clotting system was evident in all the circuits,
but significantly lower for ePTFE and pericardial tissue compared with the silicone surface. The findings were confirmed by SEM analysis of the surfaces. As anticipated, the silicone surface was covered with a loose fibrin layer containing organized thrombi, whereas the heparin-coated surface showed only the sporadic deposition of fibrin strands and blood cells. The ePTFE surface and the pericardial tissue showed similar surface activation with depositions of fibrin strands and blood cells.

Most aspects of haemocompatibility measured in this study showed that the material properties of ePTFE and pericardial tissue showed similarities to those measured in heparin-coated medical grade PVC, which is one of the most haemocompatible materials used routinely in the extracorporeal circuits [16]. The observed adhesion of platelets and fibrin to the ePTFE and pericardial tissue surfaces might be caused by the porous structure of ePTFE and the fibrous structure of pericardial tissue.

The materials of the blood-contacting cavities of the CARMAT TAH were selected based on their clinical record of durability and haemocompatibility. Bovine pericardial tissue have been used in bioprosthesis valves and their long-term clinical success has resulted in a shift towards accelerating greater use of tissue valves over the last decades [14, 17]. ePTFE has been used extensively in vascular grafts and demonstrated low thrombogenicity [13].

A potential concern regarding the closed-loop test circuits used in this study is the validity of the test results. Blood handling, storage and filling of the test loop may induce blood activation before it gets into contact with the test coupons. The absence of significant changes in haemoglobin, fibrinogen and erythrocyte count in all the circuits 4 h after pulsatile flow confirms that the circuits did not confound the test results. The 4-h test duration is considered representative of physiological conditions. If blood activation occurs, then it typically happens during the first hours of blood–tissue contact. Comparable in vitro haemocompatibility studies of vascular graft material confirmed blood activation after 60 min of blood contact [18]. Extension of test duration beyond 4-h circulation without gas exchange would result in blood cell degradation, and activation products would confound the results. Furthermore, haemolytic activity was low 24 h after direct blood–tissue contact.

The flaccid appearance (<1 mm thickness) of the ePTFE and pericardial tissue coupons posed a challenge during the preparation of the test tubes. To prevent collapsing of the coupons, they were mounted on the PVC rings that were kept at a distance by a larger-diameter tube covering the rings. Nevertheless, the macrostructure of the pericardial tissue and ePTFE coupons was not completely smooth. This could explain the moderate cell activation, resulting in thromboxane release from platelets and elastase release from leukocytes. Furthermore, the contact surface area of the pericardial tissue and ePTFE was larger than that of silicone rubber and PVC. As shown by the SEM photographs, pericardial tissue and ePTFE are fibrous structures. Despite the larger surface area, blood activation and platelet/fibrin binding were not much different from a heparin-coated surface and in some cases less than with silicone rubber.

Another potential issue is the relevance of these results. In the clinical prosthesis, the total surface area of the pericardial tissue is 240 cm² and that of ePTFE is 428 cm². Assuming a circulating blood volume of 5000 ml, this results in a surface/volume ratio of 0.048 cm²/ml for the pericardial tissue and 0.086 cm²/ml for ePTFE. For the current study, 5.56 cm² of the bioprosthetic pericardial tissue and of ePTFE was exposed to 12.5 ml of the blood, representing a surface/volume ratio of 0.463 cm²/ml for each material. This is a 9.7-fold exposure ratio for pericardial tissue and 5.4-fold for ePTFE compared with the in vivo contact ratio.

The in vitro tests were done in compliance with the recommendations for biological evaluation of medical devices of the International Organisation for Standardisation (ISO 10993:2009, www.iso.org) [19]. Although the standard comments that short-term tests are poor predictors of long-term blood device interactions, it further indicates that human blood should be used when possible and test durations of 4 h are acceptable to achieve a representative result.

Based on the low binding of platelets and fibrin, the absence of significant blood activation after circulation in addition to the low haemolysis level, it is concluded that the pericardial tissue and ePTFE in the experiments described herein have a good haemocompatibility. By using biocompatible materials, the requirement for anticoagulation drugs might be reduced, as well as the risk of coagulation-related events such as stroke and bleeding [20]. Validation of these findings will follow in the upcoming clinical studies of the CARMAT TAH.

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Conflict of interest: P.J. and A.C. are employees of Carmat SA. Carpenter holds equity in Carmat SA.

REFERENCES


APPENDIX. CONFERENCE DISCUSSION

Dr F. Wagner (Hamburg, Germany): You have come up with a very complex structure for a new artificial heart, and one thing that I noticed in your presentation is that you have an asynchronous pumping feature of the two chambers. You mentioned they are independent, load-dependent I assume. So the first question refers more to the technology of the heart. Is this system triggered by anything like an auto-fill mode or how does this work? Is it correct that you put together a circuit where you exposed the surface for only 4 h?

Dr Jansen: Yes.

Dr Wagner: What was your rationale to go with such a short exposure time? And the second question is you decided to put the pericardial membrane in the circuit without the underlying urethane-polymer combination that obviously exists in your heart because you mentioned that the pericardium is glued onto a urethane polymer in order to stabilize the membrane.

Dr Jansen: Yes.

Dr Wagner: But you decided in your circuit to use only pericardium instead of the combination of materials that you have in your artificial heart; what was the reason for that? I think it might alter your results because it changes the physical parameters of the pericardium pretty much if you glue it to a urethane.

Dr Jansen: Regarding the first question relating to how the device works, the animation indeed shows asynchronous pulsation. In fact, the device has possibilities to go synchronous and asynchronous because the membranes are activated independently by two motor pumps that can drive the system as you like and based on the inflow. As to your second question concerning the timing, the 4 h is basically what is recommended by the ISO standards. If you go less than 4 h, you do not see enough exposure and activation. If you go beyond 4 h, then the fact that there is no nutrition for the blood cells could be a confounding factor in your results. So 4 h is a well-established time frame in combination with the exposure ratio of the surface.

And then your last question about the tissue that we used. The pericardial tissue is the tissue that the blood faces. On the pumping side of the device we have the polyurethane tissue, so the hybrid membrane is what is used here. Also, in our test circuits, we used the hybrid membrane, and the polyurethane does not see the blood. So the polyurethane is a layer that supports the pericardium, because the pericardium only has a thickness of about 3 mm.

Dr G. Gerosa (Padova, Italy): The interface between blood and membrane does not cause only clot information, but the membrane that you presented is made from the xenograft material. So I guess you treat that membrane with glutaraldehyde, but glutaraldehyde is not completely masking alpha-gal epitopes. So do you have any data to see if it is still expressing alpha-gal antigens because this can play a role in terms of late calcification due to an inflammatory process.

Dr Jansen: We did do studies to look at glutaraldehyde remains in the peri-cardial tissue. The system is conserved in 0.6% glutaraldehyde solution, and there is, of course, an extensive rinsing procedure before you use the device. And we have done an in vitro test to look at those ratios in our laboratory just to make sure that we do not see the activation of calcification due to that. We also do calcification studies after the accelerated bench testing, so after we have exposed the membranes to 2 years of pulsatile pumping, we examine the calcification with histology and microscopy because, as you indicated, it is a potential issue if you use peri-cardial tissue.

Dr D. Loisance (Paris, France): May I ask you what your next step is, considering the limitation of the ISO standards, which are very clearly expressed in the description of the standards?

Dr Jansen: The next step is to complete the studies related to the device performance, the haemodynamic performance and the durability tests. Then we will go to an acute animal test situation where we will train the surgical teams. And parallel to that we have sessions where we do cadaver implant studies and fit studies, so it is a combination of activities on parallel parts where we involve the clinical investigators in the very early phase.

Dr C. Schmitz (Munich, Germany): Can you tell us where is the need for a system like that in place of totally implantable VADs?

Dr Jansen: It has been demonstrated by others that there could be a potential benefit of using pulsatile biventricular support versus nonpulsatile biventricular support, and I think this solution brings an opportunity to see whether total pulsatile support has an advantage for the physiology as opposed to continuous flow on both sides.