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BRIEF COMMUNICATION

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Up to 151 days of continuous animal perfusion with trivial heparin infusion by the application of a long-term durable antithrombogenic coating to a combination of a seal-less centrifugal pump and a diffusion membrane oxygenator

Abstract We developed a new coating material (Toyobo-National Cardiovascular Center coating) for medical devices that delivers high antithrombogenicity and long-term durability. We applied this coating to an extracorporeal membrane oxygenation (ECMO) system, including the circuit tube, cannulae, a seal-less centrifugal pump, and a diffusion membrane oxygenator, to realize prolonged cardiopulmonary support with trivial anticoagulant infusion. The oxygenator consisted of a hollow-fiber membrane made of polymethylpentene, which allows the transfer of gas by diffusion through the membrane. The centrifugal pump was free of seals and had a pivot bearing. We performed a venoarterial bypass in a goat using this ECMO system, and the system was driven for 151 days with trivial anticoagulant infusion. Plasma leakage from the oxygenator did not occur and sufficient gas-exchange performance was well maintained. In the oxygenator, thrombus formation was present around the top and the distributor of the inlet portion and was very slight in the outlet portion. In the centrifugal blood pump, there was some wear in the female pivot region and quite small amounts of thrombus formation on the edge of the shroud; the pivot wear seemed to be the cause of the hemolysis observed after 20 weeks of perfusion and which resulted in the termination of the perfusion. However, no significant amounts of thrombus were observed in other parts of the system. This ECMO system showed potential for long-term cardiopulmonary support with minimal use of systemic anticoagulants.

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Introduction

The extracorporeal membrane oxygenation (ECMO) system has been widely used for life support of patients with severe respiratory failure, acute circulatory failure, or both. The ECMO system has been shown to provide excellent oxygenation and hemodynamic support; however, the ECMO systems presently available have major problems with regard to antithrombogenicity and long-term durability. Poor blood compatibility, including the destruction or consumption of blood components and thromboembolism, is a common problem with ECMO systems, resulting in the need for systemic heparinization, which entails a risk of hemorrhagic complications. Some of these devices also have durability problems, such as serum leakage from the membrane oxygenators and seal rupture around the shafts of centrifugal pumps, making it necessary to replace the membrane oxygenators and pumps every few days. These drawbacks lead to unsatisfactory clinical results.

We have attempted to develop an ECMO system with high antithrombogenicity and high durability to facilitate continuous ECMO support. We have previously reported the development of a new ECMO system that could be continuously maintained for 34 days with trivial anticoagulant infusion.[1] This system has shown the potential to provide long-term cardiopulmonary support with minimal anticoagulants. The system employs a heparin-bonding technique [Toyobo-National Cardiovascular Center (T-NCVC) coating; National Cardiovascular Center, Osaka, Japan, and Toyobo, Osaka, Japan] and a microporous hollow-fiber membrane oxygenator with a dense layer at blood- and gas-contacting surface made of polymethylpentene (Platinum Cube NCVC; Dainippon Ink and Chemicals, Tokyo, Japan), both of which were newly developed for this project, along with a long-term durable centrifugal pump (Rotaflow; Jostra Hirringen, Germany). We newly

applied this heparin-bonding technique, which was applied to the Platinum Cube NCVC, to another long-term durable oxygenator, the Quadrox D (Jostra), which employs a hollow-fiber diffusion membrane made of polymethylpentene. The purpose of this study was to evaluate the long-term durability under chronic animal perfusion using a system consisting of a Quadrox D oxygenator, a Rotaflow centrifugal blood pump, circuit tubes, and cannulae, all of which were coated with T-NCVC. The results of the present study indicate that this system can be perfused for at least 151 days without device exchange under trivial levels of heparin infusion.

Materials and methods

ECMO system

The oxygenator of this ECMO system was the Quadrox D (Jostra), which consists of a tight hollow-fiber diffusion membrane made of polymethylpentene with a heat exchanger made of polyethylene. Its priming volume is 250 ml and the surface area is estimated to be 1.8 m^2. Due to the merits of the diffusion membrane, it was hoped that plasma leakage from the membrane would be prevented, even during prolonged use. A centrifugal pump with a priming volume of 40 ml, RotaFlow (Jostra), was employed as the pump system. It contains no seals, thereby improving the system's durability; it has a pivot bearing system with sapphire ball (male pivot) and polyethylene (female pivot).[2] The entire blood-contacting surface of the system was coated with the newly developed T-NCVC coating.

Antithrombogenic coating

The newly developed heparin coating material, the T-NCVC coating, is a complex of bonded heparin and aliphatic coupling reagents. It contains several long-chain dialkyl groups, which enhance its hydrophobic properties and prevent immobilized heparin from leaching into the circulating blood. When this material comes into contact with blood, most of the heparin molecules are not released but are retained on the material surface, ensuring high levels of antithrombogenicity.[1,3]

Chronic animal evaluation

Under general anesthesia with isoflurane and nitrous oxide, a goat weighing 60 kg underwent a venoarterial bypass between the right cervical vein and the right atrium using the above-described ECMO system. Systemic anticoagulants were not administered during the entire course of the experiment, except for a heparin injection at the time of initiation of perfusion with a dose of 100 I.U./kg and a trivial fluid infusion containing heparin to keep the pressure-monitoring line open (less than 2 I.U./kg/h). We attempted to maintain the bypass flow at between 25 and 50 ml/min/kg (between 1.5 and 3.0 l/min) with a constant rotation speed of the rotary blood pump, and with a 100% oxygen flow maintained at the same rate as the bypass flow. Throughout the experimental period, the goat could sit and stand up with free access to water and food. We attempted to maintain this perfusion as long as possible. The in vivo perfusion would be terminated when harmful complications occurred in the animal, such as disorders of organ function, thromboembolic complications, severe infection, or bleeding, or when there was a need for device exchange due to continuous hemolysis, the impossibility of meeting the bypass blood flow, or deterioration in the gas-exchange performance of the oxygenator.

Data collection regarding the gas-exchange performance of the oxygenator and changes in blood components and blood chemistry

Gas-exchange performance was evaluated with regard to the oxygen transfer rate and the carbon dioxide removal rate at 1, 2, 4, 8, 12, 16, 20, and 21 weeks after the initiation of ECMO perfusion at oxygen/blood flow ratios of 1.0 and 3.0 at a pump flow of 2.5 l/min using a blood gas analyzer (ABL 500, Radiometer, Copenhagen, Denmark). Bypass flow was measured with a Doppler flowmeter (Transonic, Ithaca, NY, USA). Changes in blood components, including hemoglobin, platelet count, plasma free hemoglobin, activated coagulation time (ACT), activated partial thromboplastin time (APTT), and plasma levels of fibrinogen degradation products (FDP), antithrombin III (ATIII), and heparin were measured and evaluated.

Pathological examination of the animal after the termination of perfusion

At the end of the experiment, the animal was dissected and evaluated pathologically.

Evaluation of devices

During animal perfusion, the condition of the ECMO system was examined at least once a day, focusing on the existence of thrombus, serum leakage from the oxygenator, and the rotation condition of the centrifugal blood pump. At the end of the experiment, the devices perfused, including the inflow and outflow cannulae, the circuit tube, the centrifugal blood pump, and the oxygenator, were evaluated, focusing on changes in the surface.

Animal care

The animal was cared for by well-trained staff including veterinarians, in accordance with the *Principles of Laboratory Animal Care and Use of Laboratory Animals* by the National Academy of Sciences, as published by the US National Institutes of Health (NIH).

Results

General conditions

The condition of the animal was continually stable without marked complications, except for the hemolysis observed from the 20 week after the initiation of ECMO perfusion. The animal was electively killed on the 151st perfusion day because of decreasing levels of hemoglobin due to hemolysis. Bypass flow was maintained at between 33 and 42 ml/min/kg (between 2.0 and 2.5 l/min) at a constant rotation speed of the centrifugal blood pump.

Gas-exchange performance of the oxygenator and changes in blood components and blood chemistry

Gas-exchange performance

Serum leakage from the membrane oxygenator was completely prevented. There was no marked deterioration in either the oxygen transfer rate or the carbon dioxide transfer rate. Sufficient gas-exchange performance was maintained during the entire experimental course at both an oxygen/blood flow ratio of 1.0 (oxygen transfer rate: 119, 146, 143, 135, 116, 114, 143, and 124 ml/min; carbon dioxide transfer rate: 51, 57, 52, 100, 51, 90, 67, and 83 ml/min at 1, 2, 4, 8, 12, 16, 20, and 21 weeks after the initiation of ECMO perfusion, respectively) and a ratio of 3.0 (oxygen transfer rate: 167, 153, 155, 137, 122, 109, 152, and 180 ml/min; carbon dioxide transfer rate: 124, 116, 100, 119, 89, 91, 78, and 87 ml/min at 1, 2, 4, 8, 12, 16, 20, and 21 weeks after the initiation of ECMO perfusion, respectively).

Changes in blood components and blood chemistry

ACT and APTT were maintained in the physiological range (ACT: 118, 125, 136, 131, 116, 123, 112, 129, and 133 s; APTT: 54.8, 57.8, 60.7, 86.6, 80.5, 63.5, 50.6, 45, and 60.8 s before perfusion and at 1, 2, 4, 8, 12, 16, 20, and 21 weeks after the initiation of ECMO perfusion, respectively). Plasma heparin was not detected at all (0, 0, 0, 0, 0, 0, 0, 0, and 0 mg/dl before perfusion and at 1, 2, 4, 8, 12, 16, 20, and 21 weeks after the initiation of ECMO perfusion, respectively). Platelet counts were maintained at an almost constant level after gradually decreasing up to 4 weeks (51.9, 48.5, 41.1, 24.5, 23.5, 16.7, 23.5, 17.5, and 15.9 $\times$ 10^4/mm^3 before perfusion and at 1, 2, 4, 8, 12, 16, 20, and 21 weeks after the initiation of ECMO perfusion, respectively). FDP and ATIII were maintained in the physiological range (FDP: 1, 5, 7, 6, 8, 5, 5, 6, and 7 mg/ml; ATIII: 127%, 124%, 122%, 134%, 132%, 121%, 132%, 124%, and 138% before perfusion and at 1, 2, 4, 8, 12, 16, 20, and 21 weeks after the initiation of ECMO perfusion, respectively). No obvious elevation of plasma free hemoglobin was observed, and hemoglobin levels were maintained, until the 20th week after the initiation of ECMO perfusion. However, hemolysis was observed from the 20th week, and hemoglobin levels continued to decrease until termination of the perfusion (hemoglobin: 11.9, 9.7, 10.5, 11.3, 11.2, 9.2, 8.9, 10.8, and 8.3 g/dl; plasma free hemoglobin: 4.3, 3.8, 0.4, 0.4, 2.1, 4.3, 1.2, 5.8, and 31.7 mg/dl before perfusion and at 1, 2, 4, 8, 12, 16, 20, and 21 weeks after the initiation of ECMO perfusion, respectively).

Pathological examination of the animal after the termination of perfusion

In terms of macroscopic and microscopic observations, no marked thromboembolism or other pathological changes

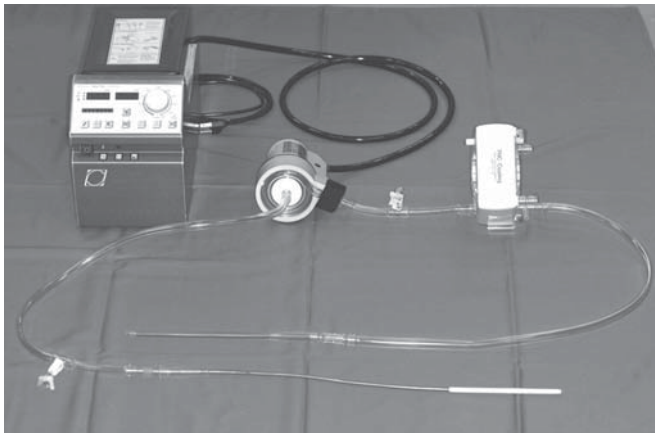

Fig. 1. Application of the Toyobo-National Cardiovascular Center (T-NCVC) long-term durable antithrombogenic coating to the combination of a Rotaflow seal-less centrifugal pump and a Quadrox D diffusion membrane oxygenator

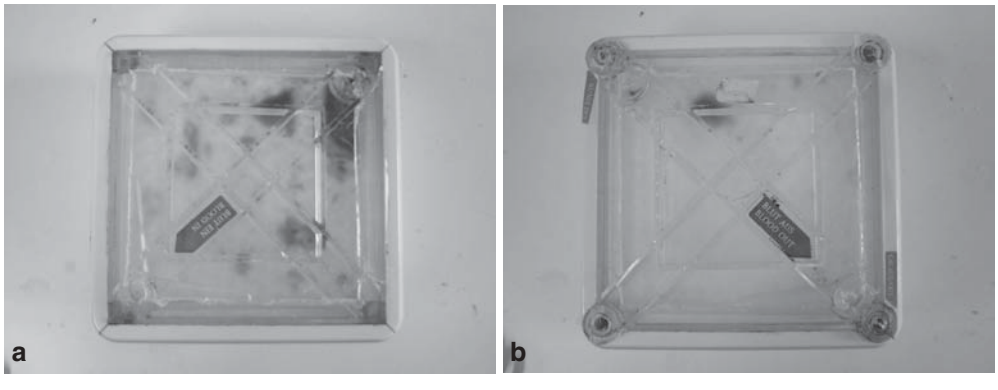

Fig. 2a,b. The oxygenator after 151 days of perfusion. Thrombus formation was present around the top and the distributors of the inlet portion **a**, while it was very slight in the outlet portion **b**

were doserved, except for those found in the lungs. In the lungs, severe alveolar fibrosis with topical athelectasis was found, which is thought to be the result of ischemia of the lung accompanying reduced pulmonary flow due to veno-arterial ECMO perfusion.[4]

Evaluation of devices

Oxygenator

Thrombus formation was almost nonexistent on the hollow fibers of the oxygenator. However, some thrombus formation was observed at the top of the inflow region, at which the blood flow was supposed to be slowest or stagnant, as well as around the flow distributors in the inflow portion; the thrombus formation was clearly very slight in the outflow portion.

Pump

No marked thrombus formation was observed, even around the pivot of the pump. Nevertheless, there was some wear in the female pivot region and quite small amounts of thrombus formation on the edge of the shroud; the pivot wear seemed to be the cause of the hemolysis observed after 20 weeks of perfusion. This erosion was thought to be due to the deterioration of the polyethylene female pivot owing to extremely long-term continuous perfusion with a device not designed to be driven for such a long period.

Cannulae and circuit tubes

The circuit tubes as well as the inflow and outflow cannulae were clear. In the region where the inflow and outflow cannulae were inserted, thrombosis was observed around the cannulae adjacent to the suturing used to stabilize the cannulae.

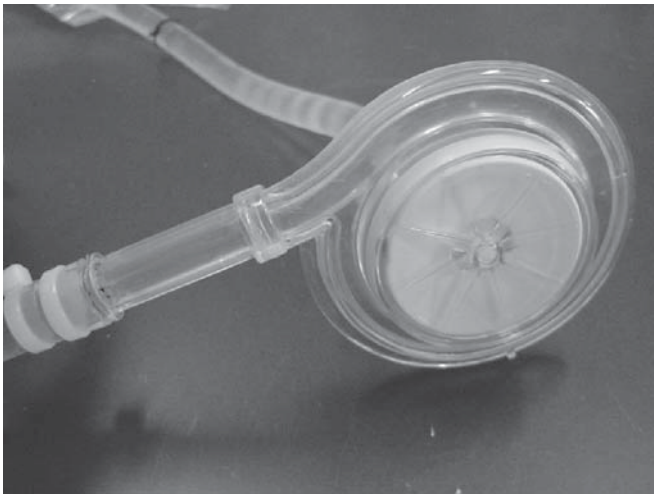

Fig. 3. The centrifugal pump after 151 days of perfusion. No thrombus formation was observed in the centrifugal pump except for minor amounts of thrombus formation on the edge of the shroud

Discussion

In this study, a T-NCVC-coated ECMO system achieved secure perfusion for 151 days without device exchange by employing a newly developed long-term durable heparin-bonding technique in the diffusion membrane-type oxygenator and the seal-less centrifugal pump.

We have described herein how the combination of the Quadrox D oxygenator and the T-NCVC coating presents a feasible option for continuous long-term perfusion. It is speculated that the diffusion membrane-type oxygenator Quadrox D achieved long-term durability because of the avoidance of serum leakage observed in conventional porous-type membrane oxygenators. In a previous experiment, we also succeeded in continuous long-term perfusion using the Platinum Cube NCVC, which is a microporous membrane oxygenator with a dense layer at the blood- and gas-contacting surface. There is no evidence regarding the durability of the Platinum Cube NCVC up to 151 days, since the previous experiment was scheduled to be terminated after 1 month.[1] To determine the difference between the diffusion membrane type and the microporous membrane type with a dense layer in terms of long-term durability is one of our next challenges.

In the oxygenator, thrombus formation was present around the top and the distributor of the inlet portion and was very slight in the outlet portion. However, no marked

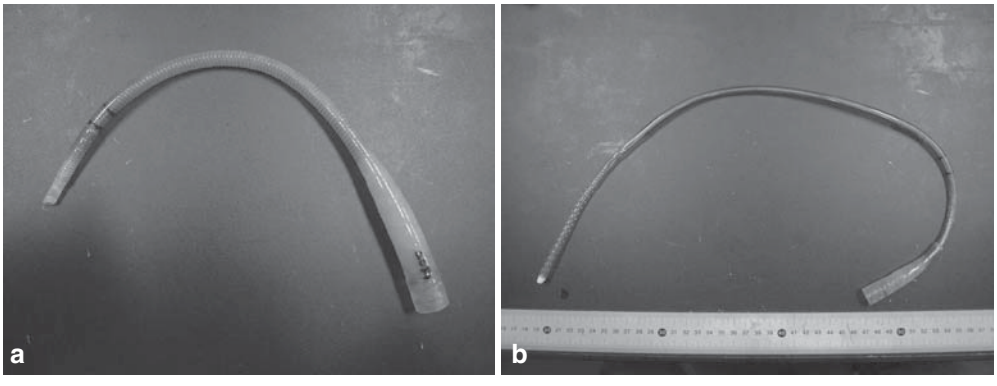

Fig. 4. The inflow **a** and outflow **b** cannulae after 151 days of perfusion. No thrombus formation was observed in the inflow or outflow cannulae

thrombus was observed in other parts of this ECMO system. The areas where thrombus was noted were exclusively stagnant or turbulent areas in terms of blood flow. The improvement of antithrombogenicity in such areas with the realization of a smooth flow pattern remains a challenge.

The mechanism of the centrifugal blood pump in which the sealing system around the bearing was eliminated enabled the pump to run for 151 days, whereas it was originally designed only for use over several days of perfusion as well as for cardiopulmonary bypass during cardiovascular surgery. Nevertheless, perfusion in the present study had to be terminated due to an increase in hemolysis caused by problems with the centrifugal blood pump, as some erosion to the pivot region was observed to cause hemolysis starting at 20 weeks of perfusion. The pivot bearing system with a sapphire ball (male pivot) and polyethylene (female pivot) has a definite limitation in terms of durability due to mechanical wear. However, the combination of the Rota-Flow and T-NCVC coating is expected to be sufficiently beneficial because the current duration of ECMO perfusion in the clinical setting is generally of the order of several weeks. In the future clinical application of this ECMO system to severely ill patents, there might be some unexpected findings that have not been clarified in this brief communication using a healthy animal.

In conclusion, this newly developed ECMO system could be appropriate for the application of long-term perfusion with minimum anticoagulants with a reduced need for device exchange. This system is expected to provide improved clinical results and to increase the number of ECMO applications, including in patients with hemorrhagic complications. However, this report demonstrates use of the system in only one case. A detailed study is needed to better show the reliability and consistency of this system.

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