

# OVER TWO MONTHS HEPARINLESS VENOARTERIAL BYPASS IN GOAT WITH A NEWLY DEVELOPED CARDIOPULMONARY SUPPORT SYSTEM TREATED WITH A NOVEL ANTITHROMBOGENIC MATERIAL

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## INTRODUCTION

Prolonged cardiopulmonary support (CPS) using membrane oxygenator such as extracorporeal membrane oxygenation (ECMO) or percutaneous cardiopulmonary support (PCPS) is a potent therapeutic option in treating the patients with life threatening respiratory and/or circulatory failure. The current CPS equipment, however, has several problems as limiting barriers to its popular use. Problems to be solved for applying the CPS system to prolonged support include: 1) thromboembolism originating from the system due to its poor antithrombogenic property, 2) hemorrhagic complication in association with necessitated systemic heparinization, 3) plasma leakage from microporous membrane oxygenator, 4) hematological reaction caused by direct blood-gas contact in such microporous membrane, and 5) poor durability of the centrifugal blood pump that contains shaft seal mechanism. If these problems are overcome, more liberal application of CPS to patient population with high risk of bleeding or with need of long-term support will come to be realistic. It is, therefore, of great clinical significance to develop such CPS system that can be used for over a month without systemic anticoagulation.

We have recently developed a revolutionary CPS system by accumulating novel technologies including a potent antithrombogenic surface treatment and an extremely durable oxygenator, and successfully conducted over-two-months heparinless venoarterial bypass in a series of animal experiments. This paper represents the details of our CPS system and the summary of animal experiments.

## MATERIALS AND METHODS

### Description of CPS System

The CPS system is composed of a compact oxygenator, a centrifugal pump, uptake and return canulae, and connecting tubings. The newly developed oxygenator (Platinum Cube NCVC<sup>®</sup>, Dainippon Ink and Chemicals, Tokyo, Japan), with 0.8 or 1.3 m<sup>2</sup> of membrane surface area with 95 or 130 ml of priming volume, consists of a special polyolefin-made hollow fiber membrane in which micropores are blind-ended to create an ultra thin dense layer of less than 0.2  $\mu$ m thickness, thereby preventing plasma leakage with this unique microstructure [1].

The fibers are arranged at even intervals with woven vertical polyester threads to optimize the inter-fiber flow distribution. For blood pump system, we employed a commercially available progressive centrifugal pump (RotaFlow<sup>®</sup>, Jostra, Hirrlingen, Germany) that is driven by an outer motor via magnetic coupling and eliminating shaft seal mechanism to obtain long-term durability.

The entire blood-contacting surface of the CPS system is treated with a novel powerful heparin bonding material (T-NCVC<sup>®</sup> coating) to impart antithrombogenicity. T-NCVC<sup>®</sup> coating is characterized as an ionic complex of bonded heparin and aliphatic coupling reagents. It contains several kinds of long-chain dialkyl groups that enhance its hydrophobic properties, and constrain immobilized heparin from leaching into the circulating blood. By optimizing the combination of dialkyl groups, heparin molecules are hardly released when contacting to the blood but are retained on the material surface. As a consequence, differently from the other heparin bonding materials, T-NCVC<sup>®</sup> is endowed with high longevity and strong antithrombogenicity at the same time. Furthermore, T-NCVC<sup>®</sup> can be coated on any type of base material by simple coating procedure with low treatment cost.

### Heparinless Long-Term CPS Animal Testing

Animal testing for heparinless long-term CPS was undertaken in eight adult goats weighing from 42 to 60 kg. The experimental protocol was as follows. Under general anesthesia, the uptake and return canulae were inserted into the right cervical vein and the right carotid artery, and the tip of multiple holed uptake canula was anterogradely advanced into the inferior vena cava at the diaphragm position. The CPS system was connected and positioned on the harness installed on the animals' back, and the venoarterial bypass was instituted. Systemic anticoagulation was not conducted during the course of perfusion, except for a small dose of heparin at the time of canulae insertion (100 IU/kg) and minute amounts of heparin in the pressure monitoring lines (less than 2 IU/kg/h in total).

After the surgery, the animal was extubated, transferred to the cage, and allowed to drink or feed, stand up or sit down, ad libitum. Bypass flow was maintained between 1.5 and 3.0 L/min, and an oxygen gas flow to the oxygenator was kept at the same rate as the bypass flow rate.

**Table 1** Summary of Heparinless Venoarterial Bypass Animal Study with T-NCVC<sup>®</sup> Treated Cardiopulmonary Support System

No.	Body weight (kg)	Bypass flow (L/min)	Anticoagulants	Device failure	Plasma leakage	Condition	Bypass duration (days)
1	42	2.0 – 2.9	no	no	no	good	41
2	45	1.7 – 3.2	no	no	no	good	65
3	50	1.8 – 2.2	no	no	no	good	35
4	58	2.0 – 2.2	no	no	no	good	36
5	60	2.0 – 2.5	no	no	no	good	60
6	60	2.6 – 2.8	no	no	no	good	28
7	58	2.0 – 2.8	no	no	no	good	34
8	60	2.0 – 2.5	no	no	no	good	39

Inflow and outflow blood gases were periodically analyzed to assess the gas-exchange performance. Clotting status was evaluated by measuring activated clotting time (ACT), partial thromboplastin time (APTT), blood heparin concentration, platelet count, fibrinogen, antithrombin III, and clotting factor XIIIa. Hematological and blood chemical studies including plasma free hemoglobin were also performed periodically. At the end of experiment, each system was disassembled for the inspection of thrombus formation, and each animal was subjected to postmortem examination.

## RESULTS

Overall results of the experiments are summarized in Table 1. All animals underwent the installation surgery uneventfully, and demonstrated good general condition without any untoward incident till they were electively sacrificed. The CPS system could be continuously perfused without systemic anticoagulation during the scheduled periods of as long as 65 days. Bypass flow rates ranged between 1.8 and 2.9 L/min on average. Gas-exchange function was kept stable at around 90-150 ml/min in O<sub>2</sub> transfer and 60-140 ml/min in CO<sub>2</sub> removal, which were sufficient level for provided blood flow throughout the experiments. Plasma leakage was not observed in any case.

The ACT and APTT levels were almost constant at 100-130 sec and 40-60 sec, respectively, and the blood heparin concentration was less than detectable level at any time. There were no substantial changes in platelet count ( $35\text{--}52 \times 10^4 /\mu\text{l}$ ), fibrinogen (200-300 mg/dl), antithrombin III (120-140%), and clotting factor XIIIa (140-175%) during the entire course of experiments. Plasma free hemoglobin (always less than 20 mg/dl) and the other hematological and blood chemical data also remained normal.

No perceptible thromboembolism was found at necropsy in any animal. In macroscopic and microscopic views, the hollow fibers of the oxygenator were mostly free of thrombus formation against prolonged perfusion without systemic anticoagulation, although a few clots were found mainly in the marginal area of the inlet and outlet ports. The other parts of the CPS circuit including the centrifugal pump and canulae were completely free of thrombus.

## DISCUSSION

Characteristic features of our CPS system are recapitulated as excellent long-term durability, and capability of heparinless perfusion.

Excellent long-term durability is attributable to the employment of the progressive centrifugal blood pump containing no shaft seal mechanism and, moreover, the newly developed oxygenator made of special hollow fiber membrane. Microporous membrane, most commonly used in the current membrane oxygenator, has disadvantages including plasma leakage accompanying the loss of hydrophobicity and other deleterious effects caused by direct blood-gas contact. In the present study, the longevity and reliability of our newly developed oxygenator were satisfactorily demonstrated by continuous perfusion for up to 65 days without any manifestation of plasma leakage. Furthermore, this distinctive feature provides the likelihood of setup and storage in preprimed condition [2]. Preprimed setup, which is so far as

is known attempted for the first time by our group, not only facilitates quick implementation of CPS in emergency situation but probably expedites usual prophylactic or elective application.

Bleeding accompanying systemic heparinization remains the most common problem in ECMO or PCPS [3]. Clotting formation inside the device and consequently necessitated systemic heparinization have been the major barriers to the prolonged use of CPS system, rendering the risk of bleeding and thromboembolism. Bleeding in sites of canulae insertion, surgery, or injury makes the management of patients extremely difficult. In the present study, our CPS system coated with T-NCVC<sup>®</sup> demonstrated excellent antithrombogenic property. Within a perusal of the relevant literatures, our experience of 65 days use of the CPS system is the exceptionally longest record of continuous single system use without systemic anticoagulation. This system will provide a privilege to avoid bleeding by minimizing or even eliminating the necessity of systemic anticoagulation, and will open the door to candidates for emergency CPS particularly those with trauma and cerebrovascular bleeding in whom systemic heparinization has been contraindicated. It may also be used for long-term CPS in various clinical settings such as bridge to lung or heart-lung transplantation.

While our heparin treatment technique was thus proved to be of great efficacy, still necessary for extermination of thrombus formation is refinement of the blood flow condition within the AL from hydrodynamic point of view. Another research project in our institute, therefore, focuses on incorporating a centrifugal pump with an oxygenator to be a single device in an effort to eliminate channeling or stagnation of the blood flow, as well as to reduce blood contacting surface area and priming volume [4]. The prototype device was confirmed to provide ideal inside flow conditions, and the surface modification technique used in the present study is being applied as the next step in the development of this device.

In summary, a durable and thrombo-resistant CPS system has been developed and evaluated in venoarterial bypass chronic animal experiments. The system could be perfused for over two months without systemic anticoagulation. The favorable results of this study strongly indicate that the newly developed CPS system has an ability to be used for prolonged heparinless ECMO or PCPS.

## REFERENCES

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