Published in final edited form as:

Artif Organs. 2013 August ; 37(8): 678–688. doi:10.1111/aor.12049.

Biocompatibility Assessment of a Long-Term Wearable Artificial Pump-Lung in Sheep

Kang Zhou, MD, PhD^{1,2}, Shuqiong Niu, MD¹, Giacomo Bianchi, MD^{1,3}, Xufeng Wei, MD, PhD¹, Narayana Garimella, PhD¹, Bartley P Griffith, MD¹, and Zhongjun J Wu, PhD¹

¹Artificial Organs Laboratory, Department of Surgery, University of Maryland School of Medicine, Baltimore, MD 21201, USA

²Division of Cardiothoracic Surgery, the 2nd Xiangya Hospital, Central South University, Changsha, Hunan 410011, P.R. China

³Ospedale del Cuore “G. Pasquinucci” - Fondazione Toscana Gabriele Monasterio CNR, 54100 Massa, Italy

Abstract

The purpose of this study was to assess the biocompatibility of a newly developed long-term wearable artificial pump-lung (APL) in a clinically relevant ovine animal model. The wearable APL device was implanted in five sheep through a left thoracotomy. The device was connected between the right atrium (RA) and pulmonary artery (PA) and evaluated for 30 days. Three sheep were used as the sham control. Platelet activation was assessed by measuring platelet surface P-selectin (CD62P) expression with flow cytometry and plasma soluble P-selectin with an enzyme-linked immunosorbent assay (ELISA). Thrombotic deposition on the device components and hollow fiber membranes (HFM) were analyzed with digital imaging and scanning electron microscopy (SEM). Surface P-selectin of the APL and sham groups changed significantly over the study period, but without significant differences between the two groups. Soluble P-selectin for the two groups peaked in the first 24 hours after the surgery. Soluble P-selectin of the APL group remained slightly elevated over the study period compared to the pre-surgical baseline value and was slightly higher compared to that of the sham group. Plasma free hemoglobin (PFH) remained in the normal ranges in all the animals. In spite of the surgery related alteration in laboratory tests and elevation of platelet activation status, the APL devices in all the animals functioned normally (oxygen transfer and blood pumping) during the 30 day study period. The device flow path and membrane surface were free of gross thrombus. Electron microscopy images showed only scattered thrombi on the fibers (membrane surface and weft). In summary, the APL exhibited excellent biocompatibility. Two forms of platelet activation, surgery related and device induced, in the animals implanted with the wearable APL were observed. The limited device-induced platelet activation did not cause gross thrombosis and impair the long-term device performance.

Address for Correspondence: Zhongjun J Wu, PhD, Department of Surgery, University of Maryland School of Medicine, 10 South Pine Street, MSTF-436, Baltimore, MD 21201, U.S.A., Phone: (410) 706-7715; Fax: (410) 706-0311, zwu@smail.umaryland.edu.

Author contributions:

Kang Zhou - Data collection, data analysis/interpretation, drafting manuscript, approval of article
 Shuqiong Niu - Performing experiments, data analysis/interpretation, approval of article
 Giacomo Bianchi - Performing animal experiment, data collection/analysis, approval of article
 Xufeng Wei - Performing animal experiment, data collection/analysis, approval of article
 Narayana Garimella - Performing SEM experiment, data collection/analysis, approval of article
 Bartley P Griffith - Concept/design, securing funding, approval of article
 Zhongjun J Wu - Concept/design, securing funding, critical revision of article, approval of article

Keywords

Biocompatibility; Platelet activation; Artificial lungs; Integrated Pump-Oxygenators; Extracorporeal membrane oxygenation

Introduction

Lung disease, behind cardiovascular illness and cancer, is the third largest killer in the United States. At present, irreversible and chronic lung disease can only be treated by lung transplantation. Unfortunately, lung transplantation is limited by the availability of donor lungs and the lack of bridge to transplantation options. Significant efforts have been devoted to the development of next-generation artificial lungs either as a long-term bridge to lung transplant for patients with lung failure or the short-term treatment for acute lung failure. At the present, artificial lungs are primarily made of or designed based on HFM. The key oxygenation element of a typical artificial lung consists of thousands of hollow fibers with a blood contacting surface area ranging from 0.32 m² to 2.5 m², depending on intended use indication (pediatric versus adult, partial versus full respiratory support). The large blood contacting surface of artificial lungs presents unique challenges to their long-term biocompatibility and use. Thrombus formation and cellular deposits in HFM have been the leading cause of the malfunction and failure of artificial lungs, resulting in poor long-term performance and frequent device exchange [1–3].

Platelets have long been regarded as the prominent cell involved in physiologic hemostasis and pathological thrombosis. Platelet hyper-reactivity and/or circulating activated platelets have been associated with many cardiovascular, infectious, metabolic and auto-immune disorders. It's well known that elevated shear stress and artificial surfaces in blood contacting medical devices can induce platelet activation. Activated platelets exhibit structural and functional changes, promoting aggregate formation and adhesion to artificial surfaces and eventually resulting in clot formation [4]. The release reaction of platelets is associated with the neo-expression of α -granule glycoproteins such as CD62P or CD63. CD62P (also known as P-selectin) will translocate from α -granules to the surface [5–7]. Structurally, platelet activation leads to an altered expression of already constitutively expressed surface glycoproteins. Then, it may be cleaved off and become a functional soluble form named as soluble P-selectin. Both the platelet surface expressed P-selectin and soluble P-selectin have been commonly used as indicators of platelet activation in many studies [8, 9]. Platelet activation has been linked to the increase of cellular deposition on the HFM and the decrease in the efficiency of gas transfer in artificial lungs during in-vivo evaluation [10] and clinical use [11].

To address the need of respiratory support devices for lung failure, a novel wearable APL has been developed for long-term ambulatory respiratory or cardiopulmonary support [12,13]. The APL combined a magnetically levitated impeller pump with a unique HFM bundle and capable of both blood pump and gas transfer functions in a compact unit. This study aimed to assess the biocompatibility of the novel APL in terms of platelet activation and device thrombosis. Both surface expressed and soluble forms of P-selectin were investigated for 30 days in a clinically relevant large animal model as well as the thrombosis on the flow path and HFM of the APL.

Materials and Methods

APL

The APL was designed as an ambulatory cardiopulmonary or respiratory support device. The APL design is suitable for both central and peripheral cannulation. The APL device can be paracorporeally placed. The controller, battery and oxygen source (tank or oxygen concentrator) for the APL device can be integrated into a mobile driver or a backpack, similar to those for circulatory assist devices, to allow the patient to be mobile. The geometry and flow path of the APL was optimized with computational fluid dynamics design and modeling process [12]. The configuration used in this series of experiments consists of an inlet cannula for venous drainage in the RA and an outlet cannula anastomosed to the main PA. The features and specifications of the APL have been described previously [13, 14]. Briefly, the APL is an integrated magnetically levitated pump-oxygenator with the following features: (1) blood flow of 3.5 liters/min; (2) oxygen delivery rate of 180 ml/min; (3) pressure head of minimum 110 mmHg; (4) minimized surface area for gas exchange; (5) minimal red blood cell (RBC) injury/hemolysis and platelet activation; and (6) support duration of up to 30 days and exchangeable. Figure 1 shows an ambulatory patient with the APL, the disposable pump-oxygenator head, and the motor drive and controller. As a lightweight paracorporeal device (0.54 kg), its dimension (117 mm in length and 89 mm in diameter) easily fit even small patients, without sacrificing the technical aspects. The priming volume of the device is about 115 ml. The oxygenation bundle is made of polymethylpentene HFM (Oxyplus, Membrana, Germany) with a surface area of 0.8 m².

Surgical Procedure

Eight Dorset hybrid sheep (45~65 kg) bred for laboratory research (Thomas Morris, Reisterstown, MD) were used in this study. All the surgical procedures and post-operative care were carried out according to the approved protocol by the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland School of Medicine. During the course of the animal experiments, all animals received humane care in accordance with the Guide for Care and Use of Laboratory Animals (NIH publication 86-23, revised 1996). The APL device was evaluated in five sheep while three sheep were used as the sham group.

The study sheep was anesthetized and vital signs were monitored as previously described [14, 15]. Under general anesthesia, the study sheep underwent left thoracotomy at the 5th intercostal space. After opening the pericardium, the RA and the great vessels i.e. aorta and pulmonary trunk, were exposed. After the sheep was heparinized to achieve an activated clotting time (ACT) of 250 seconds, a 10 mm Dacron graft at the tip of an arterial cannula (Abiomed, Inc., Danvers, MA) was anastomosed end-to-side to the main PA. A 32 Fr. venous drain cannula (DLP, Medtronic, Minneapolis, MN) was placed in the RA through the right atrial appendage using the double purse-string suture technique. After the inflow and outflow cannulae were de-aired and tunneled out of the chest to exit the skin on the upper left lateral chest wall, the primed APL device was connected and initiated for the in-vivo evaluation. A flow probe (Transonic System, Ithaca, NY) was placed around the outflow tubing to measure the device generated flow. The incision was closed and the animals were allowed to recover and survived for 30 days. At the end of study, each animal underwent necropsy and device explanation. The explanted device was examined grossly and microscopically for platelet deposition and thrombus formation. The sham surgery group underwent the left thoracotomy under general anesthesia. The thorax was then closed and an indwelling catheter was placed in the jugular vein for further blood sample collection.

Collection of Blood Samples

During the study period, heparin were continuously infused to maintain the ACT in the range of 150–180 sec. in the sheep with an implanted APL device. No heparin was infused in the sham sheep. Blood samples for analysis of platelet activation was carefully collected in tubes containing 1/10 volume of 3.8% sodium citrate through the inlet cannula of the APL device in the study animals or from an indwelling catheter in the left jugular vein in the sham animal. The baseline sample was collected immediately after anesthesia induction. Serial samples were taken at 0.5, 2, 6, 10 and 24 hours after the initiation of the device operation in the device animals or after completion of thoracotomy in the sham animals and twice a week thereafter. The baseline and weekly collected blood samples were also sent to an outside laboratory for measurements of lactate dehydrogenase (LDH), blood chemistry and complete blood count (CBC) (Antech Diagnostics, Lake Success, NY).

Platelet Activation Assays

A 100 μ l whole blood sample was fixed in 1.4 ml 1% paraformaldehyde for at least 30 min at 4°C immediately after collection. Then diluted blood containing approximately 2×10^6 platelets was centrifuged. The cell pellet was collected, re-suspended in 1ml BSA-PBS buffer (R&D, USA) and incubated with FITC conjugated anti-CD41/61 antibody for platelet identification and phycoerythrin-conjugated anti-CD62P antibody for detection of P-selectin expression on platelet surface. Both the antibodies were purchased from AbD Serotec (Raleigh, NC). Flow cytometry was performed on a FACSCalibur cytometer (BD Biosciences). The data were analyzed with CellQuest software (BD Biosciences). A total of 5,000 platelets were analyzed. Platelet activation status was determined by percentage of phycoerythrin-labeled CD62P positive platelets.

Plasma was collected from blood samples by centrifugation and stored at -80°C for measurement of soluble P-selectin. A custom ELISA for ovine blood analysis, modified from the original protocol described by Massaguer et al. [16], was used to quantify the concentration of soluble P-selectin in plasma. Briefly, the anti-CD62P monoclonal antibody (KO2.7, AbD Serotec) was used as the capture antibody and coated on MaxiSorp plates (Nalge Nunc International, Rochester, NY, USA) overnight at 4 °C. The plates were then with phosphate-buffered saline (Lonza, Walkersville, MD, USA) and blocked with 1% bovine serum albumin (BSA) (Sigma, USA) in PBS for 1 hour at room temperature. Plasma samples were 1:5 diluted in 1% BSA-PBS and incubated in the coated plates for 2 hours at room temperature. After washing with PBS containing 0.05% Tween 20 (Sigma-Aldrich, USA), the biotinylated anti-CD62P monoclonal antibody (K.O.1.12, AbD Serotec) was added as the detection antibody and followed by incubating with Avidin-peroxidase (Pierce). The concentration of soluble P-selectin was determined by incubating with TMB (Pierce) as the substrate, and the absorbance at 450nm was measured using a microplate spectrophotometer (Molecular Device). Serial dilutions of recombinant human P-selectin (R&D system) were made to generate a standard curve with a 4 parameter standard formula.

In-Vitro Platelet Stimulation

To understand the platelet activation in ovine and confirm the sensitivity of the platelet assays, fresh ovine blood collected from jugular vein of sheep was simulated mechanically by shearing the blood for 5 minutes using a high speed drill (22,000 rpm, 1/8" drill bit) and pharmacologically by collagen (4 μ g/ml) (Chrono-Log, Havertown, PA, USA), thrombin (0.2 U/ml), ADP adenosine diphosphate (ADP) (20 μ M) (Chrono-Log, Havertown, PA, USA), and phorbol 12-myristate 13-acetate (PMA) (100 nM) (Sigma-Aldrich, St. Louis, MO, USA). CD62p positive platelets and amount of soluble P-selectin in the stimulated ovine blood samples were quantified. To relate the measured plasma concentration of soluble P-selectin to the total P-selectin contained in platelets, a platelet lysis assay was

performed to examine the P-selectin contained in ovine platelets. Platelet rich plasma (PRP) was isolated from fresh citrate anticoagulated blood by centrifuging at $160\times g$ for 10 min. The number of platelets in the PRP was measured by the HEMAvet multispecies hematology systems (HV950FS, Drew Scientific, TX). The platelet pellet was then collected by centrifuging the PRP at 14,000rpm for 15 min and placed in lysis buffer (2 μ M EDTA 0.1% triton-X100 PBS) at an initial concentration of 400×10^3 platelets/ μ l for 60 min at 4°C. Platelet lysate was serially diluted with 1%PBS-BSA for quantification of P-selectin in different number of platelets with ELISA.

Examination of Thrombosis in the APL

Each of the explanted APL devices was immediately rinsed gently with saline until the non-adherent blood cells were washed away and the discharged saline became clear. The intact APL device was digitally photographed externally. The device was then cut and disassembled. The impeller and internal surface were digitally photographed. Thrombus deposition on the blood contacting surfaces of the device was recorded. The HFM bundle was cut to remove the potting material embedded in the fiber bundle. The HFM sheet was then unfolded and laid out for digital photograph. Any areas of significant gross thrombotic deposition were recorded and imaged following the method described in the reference [10]. Images of the HFM were analyzed with imageJ (NIH, USA). The color images were converted to the binary images using a color intensity threshold, and then the areas of thrombotic deposition would change to black (positive) while the remaining areas were white (negative). The percent of the whole mat positive for deposition was then calculated. This process was repeated three times and the data were averaged.

Pieces of the HFM (1 cm²) were cut for scanning electron microscopy analysis. Fixed samples were rinsed with saline and then place in 1% osmium tetroxide (EMS 0.1M P18ES) for 30 min. After rinsing, the samples were serially dehydrated by carbon dioxide critical point drying, followed by sputter coating (EMS 350) with gold-palladium. Samples were visualized by Quanta 200 scanning electron microscope (FEI, Hillsboro, OR, USA).

Data Analysis

All the data are presented as mean $\pm$ SE among the sheep over a 5-day period. A mixed model was used for statistical analysis over the time by using sheep number as the subject variable and using the sample collecting time point and the animal group as the fixed, repeated-measure variables. Paired T-test was used to compare the differences between the device and sham groups at the same time point. $p<0.05$ is considered as significant difference. Comparisons were performed with SPSS software (PASW Statistics 18, IBM, Armonk, NY, USA).

Results

General Condition of Study Animals

Both the animals implanted with the APL and the sham animals survived for 30 days without complications and any health related issue. The APL in the five device animals functioned normally (oxygen transfer and blood pumping) during the 30-day study period [17]. The animals were electively terminated at the study end-point. Both the device and sham groups had an acute and surgery-related alteration in laboratory tests for blood counts, kidney function, liver function, and cell and tissue injury. Figure 2 shows the change in hematocrit and platelet count in the two groups of the animals over the 30 day period. The hematocrit in the device group dropped significantly from the baseline value after the surgery, remained low in the first few days and then gradually increased to the baseline level thereafter. The hematocrit of the sham sheep also dropped after surgery, but the degree was

relatively not so severe compared with that of the device sheep. The platelet count in the device and sham sheep also dropped after the surgery too, but exhibited an uptick trend in the first week, peaked around the day 10 and then returned to the baseline level gradually. The changes in hematocrit and platelet count during the first week after the surgery could be attributed to blood loss and fluid infusion during the surgery and surgery-related consumption of blood elements. Other laboratory tests, including alanine transaminase (ALT), aspartate aminotransferase (AST), and creatine phosphokinase (CPK), were all significantly elevated in both the device and sham animals during the first week and gradually returned during the second week and remained stable throughout the study. The creatinine and blood urea nitrogen (BUN) values in the three device animals and all the sham animals remained in the normal range throughout the study while the creatinine and BUN values in the other two device animals had an acute elevation at days 1 to 6 and returned to the normal range at day 9 and remained stable thereafter.

Hemolysis

Figure 3 shows the concentrations of PFH and LDH from the device and sham sheep during the 30-day study period. The concentration of PFH from the device sheep was well below 20 mg/dL over the study period and comparable to that from the sham sheep, indicating no hemolysis. However the LDH level significantly elevated from the baseline value in both the device and sham sheep in the first two weeks after the surgery, but gradually returned to the normal range. This elevation was clearly associated with the tissue injury induced by the surgical procedure since this happened in both the sham and device sheep.

Platelet Activation

Figure 4 shows the P-selectin expression on the surface of circulating platelets in both the device and sham animals. The percentage of the CD62P positive platelets in the total circulating platelets in both the device and sham groups fluctuated over the 30 day study period. The platelet activation as indicated by CD62P expression on the platelet surface remained below 10%. There was no significant difference in platelet activation over the 30 day study period between the two groups ($p=0.16$) although there was significant difference in the platelet activation at some individual time points between the two groups when analyzed with the t-test. Figure 4(b) shows the percentage of the platelet activation in the expanded time scale during the first 24 hours after the surgery. Since the overall level of platelet activation was below 10%, the difference noticed at some time points might not be clinically significant.

Figure 5 shows the soluble P-selectin concentration in the plasma from the two groups of animals. The plasma soluble p-selectin concentration was normalized with the platelet number since all activated platelets might release P-selectin into the plasma. Both the groups exhibited a significant elevation ($p<0.05$) in the plasma P-selectin during the first 24 hours after the surgery. The plasma P-selectin decreased after the first 24 hours for both the device and sham animals. The soluble P-selectin of the device group remained slightly elevated over the course of the study while that of the sham group decreased after the first two weeks, but increased to the same level as that of the device group at the end-point of the study. In the expanded time scale during the first 24 hours, the peak value of the device group was higher than that of the sham group. This difference might be caused by the combined effects of the surgical trauma and blood contact with the foreign surface of the APL. Although the soluble P-selectin in both the device and sham animals was slightly elevated, the clinical significance remained to be explored.

Figure 6 shows the percentage of CD62P positive platelets and the normalized soluble P-selectin concentration in the blood samples stimulated by mechanical shearing and the four

chemical agonists. There was an insignificant increase in the percentage of CD62P positive platelets from the baseline level (8.1% vs. 8.6%, $p=0.44$) after the blood was subjected to mechanical shearing with a high speed drill bit for 5 minutes. However, the soluble P-selectin concentration increased more than 3 times from the baseline level (2.5 vs 7.8 ng/10³ platelets, $p=0.0016$) after being sheared with the high speed drill bit. The four agonists all caused ovine platelet activation at various levels as indicated by both the CD62P expression on the platelet surface and soluble P-selectin. PMA was the strongest agonist by the two platelet activation markers. The addition of PMA to ovine blood resulted in a 6.5 fold increase in the CD62P positive platelets and an 8.8 fold increase in the P-selectin concentration from the baseline levels. The percentage of the CD62P positive platelets increased 4.9, 2.5 and 1.5 times from the baseline value, respectively, after being simulated by ADP, thrombin and collagen. In parallel, the soluble P-selectin concentration increased 7.7, 3.5, and 1.1 times from the baseline value, respectively. These results indicated that the platelet activation process or status induced by mechanical forces might be different from the chemical agonists.

Figure 7 shows the total amount of P-selectin in lysed platelets versus platelet numbers. There is an almost linear relationship between the concentration of soluble P-selectin and the number of lysed platelets. The results indicate that the amount of P-selectin present in ovine platelets is 8.2 $\mu\text{g}/10^3$ platelets. The secreted P-selectin was only a small portion of the stored P-selectin in platelets even when more 50% platelets expressed P-selectin on their surface in the PMA-activated ovine blood.

Platelet and Thrombotic Deposition

Figure 8 shows the images of the five explanted APL devices, the steps between connectors and tubing, and the impeller/diffuser region of one explanted device. The explanted devices were generally clean and no massive occlusive clots or deposited materials in the fiber bundle were noticed as commonly observed in the explanted oxygenators used in cardiopulmonary bypass or respiratory support. Three of the five explanted devices were free of gross thrombotic formation in the fiber bundle and flow path. The other two had isolated clots in the space between the outer housing and the outer layer of the fiber bundle and the steps between the connectors and tubing. Figure 9 shows a sample image of the unfolded HFM sheet with an insert of enlarged view of an area with an isolated thrombus and the percentage of the areas of significant gross thrombotic deposition in the hollow membranes of the five devices. The highest percentage of the thrombus covered area happened in one device ($4.35\pm0.31\%$ gross thrombosis). The other four devices had less than 3% area with visible thrombus deposition.

Figure 10 shows sample SEM images of the hollow fibers from one explanted device. SEM images revealed that there were minimal visible deposits on the HFM surface (Figure 10(a)). The insert shows the enlarged view of the membrane surface. There were isolated thrombi on the weft of the fiber sheet (Figure 10(b)). A higher magnification of inset shows that there were many morphological changed and activated platelets with other blood cells. In most fibers adjacent to the weft strands, only scattered lamellar fibrin and small platelet aggregates were observed (Figure 10(c)). Some cluster of cellular elements and fibrin adhered to the weft of the fiber sheet (Figure 10(d)).

Discussion

To address the long-term respiratory support with the newly developed APL device, we assessed the biocompatibility of the APL in a clinically relevant large animal model (ovine). The platelet activation over the 30 day study period and thrombosis in the blood contacting surface of the main flow path and the HFMs after the 30 day in-vivo operation were

examined. Two commonly used platelet activation markers (surface P-selectin and soluble P-selectin) were utilized to indicate platelet activation. To better relate the measured indices of the platelet activation markers, a sham animal experiment and a series of in-vitro platelet activation experiments were carried out.

Major surgery wound can disturb the regulation of coagulation and thrombosis with high level of markers for platelet activation through the extrinsic pathway [18, 19]. In both the device group and sham group, CPK reached its peak in first 24 hours then fall back to normal levels in first week. Although the surfaced expressed CD62P of both the device and sham groups did not vary significantly while the soluble P-selectin rose significantly (more than 10 fold) during the first 2 to 10 hours after surgery. The sharp rise and drop of the CPK were also well correlated with the initial drop in the platelet number and rise during the first week. This may indicate there was an impact of surgical trauma on the initial platelet consumption and subsequent production [20]. However, the extent and recovery of the platelet number are different between the APL and sham groups. The recovery of the platelets from the sham surgery occurred immediately for the sham group while there was some delay for the device group. The elevation in the platelet number during the first week in both of the two groups may due to the acute inflammatory responses associated with vascular injury and wound healing [21].

It has been well known that both mechanical stress and artificial surface during extracorporeal life support lead to activation of various blood components [22]. High mechanical stress encountered in device assisted circulation causes platelet activation, resulting in structural and morphological changes, secretion of intracellular content (ADP, ATP, serotonin, etc) and aggregation [23,24]. The APL devices were implanted in sheep and provided up to 30 days of respiratory support. There was no drastic change in the surface expressed CD62P over the 30-day study period in the device and sham groups. Except for the sharp rise during the first 24 hours, the soluble P-selectin concentration of the device group elevated slightly compared to the baseline value while that of the sham group fluctuated between the baseline value and the slightly elevated level. However, these elevated soluble P-selectin concentration values are close to the baseline values from six additional sheep from which blood was collected for the in-vitro platelet activation experiments as described early (Figures 5 and 6).

The results from in-vitro platelet activation experiments showed that the surface expressed CD62P might not a sensitive measure to mechanical shear-induced platelet activation. There was almost no increase in the percentage of the CD62P positive platelets after blood was sheared with a high speed drill bit at 22,000 rpm for 5 minutes while the soluble P-selectin increased 3 folds after the mechanical shearing. It is interesting to note that the surface expressed CD62P with soluble P-selectin remained to be sensitive to chemically induced platelet activation by PMA, ADP, thrombin and collagen. It is known that platelet surface P-selectin molecules can be cleared from the circulation less than 2 hours with a concomitant increase in soluble P-selectin concentration [25]. Soluble P-selectin has been found to rise at the time of surface P-selectin began to decrease in the clinical setting during the surgery with the use of cardiopulmonary bypass [26, 27]. The results from our in-vitro platelet activation experiments suggest that P-selectin molecules on the surface of activated platelets can be easily cleaved off as soluble P-selectin by mechanical forces while they remain partially on platelet surface during chemically induced activation. The observation of P-selectin shedding is consistent with the early reports on P-selectin shedding from activated platelets in circulation by others [28–30]. The measured soluble P-selectin concentration in the animals implanted with the APL device suggests that the APL device cause minimal platelet activation. This may be attributed to the unique design of the APL devices. In the APL device, the only moving component is the magnetically levitated impeller. The

magnetic levitation eliminates problems associated with valves, seals, mechanical bearings. The flow path was optimized to guide the blood flow from the impeller smoothly to HFMs in order to minimize the flow-induced blood cell damage.

The housing components and impeller of the APL are made of biocompatible polycarbonate and titanium. The oxygenation component is made of polymethylpentene (PMP) HFM, which have been used in various blood contacting devices. However, cellular deposition still develops on these materials under unfavorable flow conditions [31–33]. The APL features a magnetically levitated impeller with computationally optimized geometry and a PMP fiber bundle with a unique circumferential-radial, uniform outside-in flow path design to achieve sufficient oxygen transfer without significant flow stagnancy. As a result, there were only few and scattered visible thrombus deposits on the blood-contacting surface of the APL and PMP fiber membranes. However, the nylon weft strands of the fiber sheets were found to be nidus for thrombus formation and then lead to major thrombotic deposition on the fibers adjacent to them. Nevertheless, the optimized design of the magnetically levitated impeller pump and distinctive flow path configuration of the fiber bundle may contribute to the insignificant platelet activation during the long-term study period and the limited thrombotic deposition in the APL device after the long-term use.

Conclusion

The newly developed wearable APL was evaluated for biocompatibility in the ovine animal model. The APL exhibited excellent biocompatibility with an insignificant level of platelet activation over the 30 day study period and without function impaired thrombosis. The unique flow path and small HFM surface area of the APL design mitigated the potential of the thrombosis. The study also revealed that soluble P-selectin is more relevant indicator for evaluation of platelet activation in the ovine animal model.

Acknowledgments

This work was supported partially by the National Institutes of Health grants (R01HL082631, R42 HL084807, and R01HL088100).

References

1. Florchinger B, Philipp A, Klose A, et al. Pumpless extracorporeal lung assist: a 10-year institutional experience. *Ann Thorac Surg.* 2008; 86(2):410–7. discussion 417. [PubMed: 18640306]
2. Wang D, Lick SD, Zhou X, Liu X, Benkowski RJ, Zwischenberger JB. Ambulatory oxygenator right ventricular assist device for total right heart and respiratory support. *Ann Thorac Surg.* 2007; 84(5):1699–703. [PubMed: 17954089]
3. Sato H, Hall CM, Lafayette NG, et al. Thirty-day in-parallel artificial lung testing in sheep. *Ann Thorac Surg.* 2007; 84(4):1136–43. discussion 1143. [PubMed: 17888959]
4. Goodman SL. Sheep, pig, and human platelet-material interactions with model cardiovascular biomaterials. *J Biomed Mater Res.* 1999; 45(3):240–50. [PubMed: 10397982]
5. Chandler AB, Earhart AD, Speich HE, et al. Regulation of CD40L (CD154) and CD62P (p-selectin) Surface Expression upon GPIIb-IIIa Blockade of Platelets from Stable Coronary Artery Disease Patients. *Thromb Res.* 2009
6. Hubl W, Assadian A, Lax J, et al. Assessing aspirin-induced attenuation of platelet reactivity by flow cytometry. *Thromb Res.* 2007; 121(1):135–43. [PubMed: 17467777]
7. Maeda NY, Carvalho JH, Otake AH, Mesquita SM, Bydlowski SP, Lopes AA. Platelet protease-activated receptor 1 and membrane expression of P-selectin in pulmonary arterial hypertension. *Thromb Res.* 2009

8. Niimi Y, Ishiguro Y, Nakata Y, Goto T, Morita S, Yamane S. Platelet adhesion to heparin coated oxygenator fibers under in vitro static conditions: impact of temperature. *ASAIO J.* 2001; 47(4): 361–4. [PubMed: 11482487]
9. Michelson AD. Methods for the measurement of platelet function. *Am J Cardiol.* 2009; 103(3 Suppl):20A–26A.
10. Snyder TA, Eash HJ, Litwak KN, et al. Blood biocompatibility assessment of an intravenous gas exchange device. *Artif Organs.* 2006; 30(9):657–64. [PubMed: 16934093]
11. Lehle K, Philipp A, Gleich O, et al. Efficiency in extracorporeal membrane oxygenation-cellular deposits on polymethylpentene membranes increase resistance to blood flow and reduce gas exchange capacity. *ASAIO J.* 2008; 54(6):612–7. [PubMed: 19033775]
12. Taskin ME, Zhang T, Fraser KH, Griffith BP, Wu ZJ. Design Optimization of a Wearable Artificial Pump-Lung Device with Computational Modeling. *ASME Journal of Medical Devices.* 2012; 6/031009-1
13. Wu ZJ, Taskin ME, Zhang T, Griffith BP. Computational Model-Based Design of a Paracorporeal Artificial Pump-Lung for Cardiopulmonary/Respiratory Support. *Artif Organs.* 2012; 36(4):387–99. [PubMed: 22145732]
14. Zhang T, Wei X, Bianchi G, Griffith BP, Wu ZJ. A novel wearable pump-lung device: in-vitro and acute in-vivo study. *Journal of Heart and Lung Transplantation.* 2012; 31(1):101–5. Epub 2011 Oct 20. [PubMed: 22014451]
15. Zhang T, Cheng G, Koert A, et al. Functional and biocompatibility performances of an integrated Maglev pump-oxygenator. *Artif Organs.* 2009; 33(1):36–45. [PubMed: 19178439]
16. Massaguer A, Engel P, Tovar V, et al. Characterization of platelet and soluble-porcine P-selectin (CD62P). *Vet Immunol Immunopathol.* 2003; 96(3–4):169–81. [PubMed: 14592730]
17. Wu ZJ, Zhang T, Bianchi G, Wei X, Son H-S, Zhou K, Taskin ME, Wang P, Bianucci B, Garcia J, Griffith BP. 30-Day In-vivo Performance of a Wearable Artificial Pump-Lung for Ambulatory Respiratory Support. *Ann Thorac Surg.* 2012; 93(1):274–81. [PubMed: 22115337]
18. Tanaka KA, Key NS, Levy JH. Blood coagulation: hemostasis and thrombin regulation. *Anesth Analg.* 2009; 108(5):1433–46. [PubMed: 19372317]
19. Achneck HE, Sileshi B, Lawson JH. Review of the biology of bleeding and clotting in the surgical patient. *Vascular.* 2008; 16 (Suppl 1):S6–13. [PubMed: 18544308]
20. Hiippala ST, Myllyla GJ, Vahtera EM. Hemostatic factors and replacement of major blood loss with plasma-poor red cell concentrates. *Anesth Analg.* 1995; 81(2):360–5. [PubMed: 7542432]
21. Lo B, Fijnheer R, Castiglione D, Borst C, Kalkman CJ, Nierich AP. Activation of hemostasis after coronary artery bypass grafting with or without cardiopulmonary bypass. *Anesth Analg.* 2004; 99(3):634–40. table of contents. [PubMed: 15333385]
22. Alpard SK, Zwischenberger JB. Extracorporeal gas exchange. *Respir Care Clin N Am.* 1998; 4(4): 711–38. ix. [PubMed: 9881401]
23. Hathcock JJ. Flow effects on coagulation and thrombosis. *Arterioscler Thromb Vasc Biol.* 2006; 26(8):1729–37. [PubMed: 16741150]
24. Cook KE, Maxhimer J, Leonard DJ, Mavroudis C, Backer CL, Mockros LF. Platelet and leukocyte activation and design consequences for thoracic artificial lungs. *ASAIO J.* 2002; 48(6):620–30. [PubMed: 12455772]
25. Michelson AD, Barnard MR, Hechtman HB, et al. In vivo tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. *Proc Natl Acad Sci U S A.* 1996; 93(21):11877–82. [PubMed: 8876231]
26. Ferroni P, Pulcinelli FM, Lenti L, Gazzaniga PP. Is soluble P-selectin determination a more reliable marker of in vivo platelet activation than CD62P flow cytometric analysis. *Thromb Haemost.* 1999; 81(3):472–3. [PubMed: 10102487]
27. den Oever MG, Schoorl M, Schoorl M, Bartels PC, Grooteman MP, Nube MJ. The role of the extracorporeal circuit in the trapping and degranulation of platelets. *Blood Purif.* 2009; 28(3):253–9. [PubMed: 19684392]
28. Leytin V, Allen DJ, Gwozdz A, Garvey B, Freedman J. Role of platelet surface glycoprotein Ib α and P-selectin in the clearance of transfused platelet concentrates. *Transfusion (Paris).* 2004; 44(10):1487–95.

29. Berger G, Hartwell DW, Wagner DD. P-Selectin and platelet clearance. *Blood*. 1998; 92(11): 4446–52. [PubMed: 9834252]
30. Ushiyama S, Laue TM, Moore KL, Erickson HP, McEver RP. Structural and functional characterization of monomeric soluble P-selectin and comparison with membrane P-selectin. *J Biol Chem*. 1993; 268(20):15229–37. [PubMed: 7686912]
31. Addonizio VP. Platelet function in cardiopulmonary bypass and artificial organs. *Hematol Oncol Clin North Am*. 1990; 4(1):145–55. [PubMed: 2179210]
32. Misawa Y, Konishi H, Kawahito K, Fuse K. Platelet activation and aggregation during normothermic cardiopulmonary bypass. *Jpn J Thorac Cardiovasc Surg*. 2001; 49(1):21–8. [PubMed: 11233238]
33. Paparella D, Brister SJ, Buchanan MR. Coagulation disorders of cardiopulmonary bypass: a review. *Intensive Care Med*. 2004; 30(10):1873–81. [PubMed: 15278267]

(b) Exchangeable APL device

(c) Controller & motor drive

(a) Ambulatory patient with APL

Fig. 1. The newly developed wearable APL for ambulatory respiratory support: (a) Ambulatory patient with implanted APL. (b) Disposable pump-oxygenator. (c) Controller-motor drive assembly.

Fig. 2. Hematological data of the APL and sham sheep over the 30-day study period: (a) hematocrit and (b) platelet counts at the pre-surgical and post-operative time points.

Fig. 3. Indices for blood cell damage and tissue injury: (a) PFH for hemolysis and (b) LDH for tissue injury.

Fig. 4. CD62P expression on platelet surface in the APL study and sham sheep: (a) percentage of CD62P positive platelets over the 30-day study period; (b) percentage of CD62P positive platelets during the first 24 hours.

Fig. 5. Soluble P-selectin concentration in the APL and sham sheep: (a) normalized soluble P-selectin concentration over the 30-day study period; (b) normalized soluble P-selectin concentration during the first 24 hours.

Fig. 6. Platelet activation markers in ovine activated blood by mechanical shearing and chemical agonist: (a) percentage of CD62P positive platelets in the activated blood; (b) normalized soluble P-selectin concentration in the activated ovine blood.

Fig. 7.
The total amount of P-selectin in platelets.

Fig. 8. Photographs of the five explanted APL devices, ring-thrombosis at the steps between connectors and tubing and bottom view of the impeller/diffuser section of one explanted APL device.

Fig. 9. Analysis of hollow of the fiber membranes from explanted APL devices: (a) Sample images of the unfolded fiber membrane sheet from after the 30 day study; and (b) The percentage of the membranes with visible thrombotic deposition in the fiber membranes of the five APL devices.

Fig. 10. SEM microphotographs of the fiber membranes from one explanted APL device: (A) images of several fibers and surface of one sample fiber; (B) images of deposits on the weft strands and enlarged view of the deposits; (C) image of surface of one sample fiber adjacent to the weft strands; (D) image of thrombosis on the weft strands.