METHODS ARTICLE

Acute Swine Model for Assessing Biocompatibility of Biomedical Interface Materials

Tohru Takaseya, MD, PhD,¹ Hideyuki Fumoto, MD,¹ Junmin Zhu, PhD,² Akira Shiose, MD, PhD,¹ Mariko Kobayashi, MD,¹ Masako Fujiki, MD,¹ Yoko Arakawa, MD,¹ Raymond Dessoffy, AA,¹ Larry D. Kramer, Jr.,¹ and Kiyotaka Fukamachi, MD, PhD¹

We established an acute animal model for early, straightforward, and reproducible assessment of a biocompatible material interface. Bilateral femoral artery-to-vein shunts were created in 12 pigs: two tubes per shunt, the left two coated and the right two uncoated. We evaluated two groups: uncontrolled flow (UF; shunt flow unregulated) and controlled flow (CF; shunt flow ~ 50 mL/min). For each case on each side, two shunts were evaluated: one for 1 h and the other for 3 h. Arterial blood gas and complete blood count were recorded at baseline, 1, and 3 h. Mean shunt flows were 532 ± 88 mL/min UF and 52 ± 8 mL/min CF. Differences in flow were much smaller in CF (0.5 mL/min; 1% of mean flow) than UF (24.8 mL/min; 5% of mean flow). In UF, significant changes occurred: in pH, from start of shunting through 1 h; in pO₂ and pCO₂, from start through 3 h. This swine model using bilateral femoral shunts with controlled blood flow provides a reliable, reproducible, easily implemented method by which to evaluate biocompatibility of device coatings at an early stage of investigation.

Keywords: arteriovenous shunt, biocompatible materials, blood coagulation, device coating, swine model

Introduction

BIO MATERIALS ARE USED in the manufacturing and construction of biomedical devices that sustain, augment, or completely replace diseased human organs: vascular grafts, heart valves, ventricular assist devices, orthopedic devices, drug delivery systems, extracorporeal systems, and a wide range of invasive treatment and diagnostic systems.^{1,2} Current biomaterials are still largely based on the type of thermoplastics called “commodity polymers,” introduced over 40 years ago (e.g., polyethylene [PE], polyvinyl chloride). Unfortunately, these biomaterials are associated with well-known problems, including thrombosis, intimal hyperplasia, and infection.^{3,4}

The material interface between a biomaterial and its biological environment is a critical factor in evaluating the likelihood of protein and platelet interactions, thrombus formation, and cell–surface interactions. Consequently, approaches that can provide a reliable, reproducible, and easily implemented assessment of the interface of cells and tissues with a new or improved biocompatible material would have a positive impact and facilitate development of medical devices that are not currently feasible.

We chose an acute swine model in this study for screening purposes to obtain preliminary data about platelet adhesion

in the early phase, without any anticoagulation therapy. We have reported on the use of a bilateral femoral shunt with controlled shunt flow in swine as the most appropriate animal model in which to study a biomaterial coating that mimics the cell glycocalyx and the extracellular matrix.^{5–8} The ability to mimic both the functional and nonadhesive properties of a glycocalyx and extracellular matrix in swine provides a route by which to solve numerous clinical problems with biomaterials. Evaluation of antithrombotic properties was the key issue in this biocompatibility study. The purpose of this study was to present the methodology used to obtain the animal experimental data needed to address these issues.

Materials and Methods

Animal model

Twelve male pigs (Yorkshire cross; Michael Fanning Farms, Howe, IN) weighing 41.0–53.5 kg (mean: 49.5 ± 3.3 kg), naive to any prior procedures and kept in standard pens with access to food and water *ad libitum*, were used for an acute evaluation of the biocompatibility of a new biomedical interface material through use of bilateral femoral shunts. The study was approved by the Institutional Animal Care and Use Committee, and all animals received humane care in

¹Department of Biomedical Engineering, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio.

²Chemical Abstracts Service, Columbus, Ohio.

compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996.

Experimental design

The porcine animal model was chosen for our study since the coagulation and fibrinolytic systems as well as the cardiovascular system of pigs have significant similarities to those of humans, a critical factor for this hemocompatibility study.^{9,10} To improve our femoral shunt experimental model, we changed the surgical procedure after the first four cases to lower the shunt flow and to better control the variability of flow. We divided the 12 cases into two groups: the uncontrolled flow (UF) group ($n=4$), in which shunt flow was not limited or was kept at ~ 500 mL/min in the first four cases; and the controlled flow (CF) group ($n=8$), in which shunt flow was decreased and maintained at ~ 50 mL/min by a tourniquet in the last eight cases. No anti-coagulation agents were used during the surgical procedure.

Anesthesia and surgical procedure

Study animals were anesthetized with an intramuscular injection of xylazine (1–2 mg/kg) and, after intubation, were ventilated through an endotracheal tube by a respirator. General anesthesia was maintained with isoflurane (0.5–2.5%). A venous catheter was placed in a peripheral vein to allow administration of fluids.

Animals were placed on the surgical table in the supine position, and the surgical procedure was performed under sterile conditions. A right lateral neck incision was made to isolate the right carotid artery for arterial pressure monitoring and the jugular vein for insertion of a venous infusion line. Arterial blood gas (ABG) samples and complete blood cell counts (CBCs) were taken as baseline data at this point, before shunting.

Longitudinal bilateral inguinal incisions were made to isolate the femoral arteries and veins. The left femoral artery and vein were used with the coated tubes (polyvinylamine dextran/hexyl interface materials).⁷ The right femoral artery and vein were used with the uncoated grafts as a control group. A perivascular 3.0-mm ultrasonic flow probe (Model SB-3.0 mm; Transonic Systems, Inc., Ithaca, NY) was placed on each artery proximal to the site to be used for shunting, and blood flow was recorded as baseline data before shunting.

In the UF group, the left and right femoral arteries and veins were then cannulated with coated PE tubes (inner diameter 1/8 in., outer diameter 3/16 in.; Polymer Plastics Corp., Reno, NV) and uncoated PE tubes, respectively. These shunted the femoral arterial flow directly to the femoral vein (artery-to-vein [A-V] shunt) through the tubing being evaluated (Fig. 1A). Shunt flow was recorded every 15 min for all tubes; however, the flow level was not adjusted in the UF group. ABG and CBC were taken at baseline before shunting and at the end of each shunt flow period (1 or 3 h). When the tubes were removed from the circuit, they were immediately transferred into a phosphate-buffered saline solution with minimal blood–air contact. Each tube was cut into nine parts, washed with the saline solution, and fixed with 2.5% glutaraldehyde. Samples were stored at 4°C for future analysis.

FIG. 1. (A) The tube was inserted into the femoral artery and vein in the uncontrolled flow group. A flow probe was placed at the femoral artery proximal to the site to be used for shunting. (B) Femoral arteries and veins were cannulated by inserting PU tubes containing a female Luer connector and a stopcock in the controlled flow group. A tube was connected to the stopcocks, which were attached to the inserting tubes. Shunt flows were adjusted to between 40 and 60 mL/min by a tourniquet. After the first set of tubes was tested for 1 h on each side, they were removed and a second set of tubes was tested for a 3-h period. PU, polyurethane.

In the final eight animals (CF group), several changes were incorporated: (1) We placed a tourniquet on each proximal femoral vein to decrease and control shunt flow to ~ 50 mL/min, with shunt flow adjusted every 15 min with the recorded tube flow measurements; (2) we changed the tubing material from PE to polyurethane (PU; inner diameter 1/8 in., outer diameter 3/16 in.; NewAge Industries, Southampton, PA); and (3) we divided the tubes into three parts, including two inserting tubes and one tested PU tube. The inserting parts were placed into the femoral artery and vein, and both ends of the test tube were connected to the inserting tubes using a female Luer connector and a stopcock to quickly change out the PU tubes to be tested for the 1- or 3-h periods (Fig. 1B).

Data analysis

All values are expressed as mean $\pm$ standard deviation. For paired data analysis, a paired t test was used. Comparisons of CBC and ABG data among baseline, 1, and 3 h were performed by repeated-measures analysis of variance using SPSS statistical software (SPSS v. 15.0; SPSS, Inc., Chicago, IL). A p -value of <0.05 was considered statistically significant.

Results

Modification of shunt flow management

Figure 2 shows the flow levels recorded every 15 min in both groups. Average shunt flow in the UF group was

FIG. 2. Shunt flows of each group during a 1-h trial (A) and a 3-h trial (B).

>400 mL/min without the use of a tourniquet, with significant variability in the flow levels over the 1- and 3-h periods. Shunt flows in the CF group were stable at ~50 mL/min using the tourniquets, reducing the standard deviation of the flows from 17% of mean flow in the UF group to 15% of mean flow in the CF group. There was no significant difference in flow between coated and uncoated tubes in both groups; however, the difference in flow was much larger in the UF group (24.8 mL/min; 5% of the mean flow) than in the CF group (0.5 mL/min; 1% of the mean flow).

Modification of materials

We switched to PU tubing from PE tubes in the CF group because the hardness of the PE material made it difficult to insert tubes into the vessels without the tips pushing against the vessel wall, resulting in occlusion of

the tips by the arterial wall or in inadvertent injury to the intima of vessels. In one case in the UF group, total bleeding volume reached 5000 mL, primarily because of bleeding around the tube insertion sites during frequent adjustments of the positions of the tubes. We, therefore, had to terminate that case early.

CBC and ABG

CBC and ABG data (Table 1) were successfully obtained from all pigs in both groups, except for the one pig with excessive bleeding in the UF group. In the CF group, there was no significant change in CBC and ABG data from baseline through the end of the 1- and 3-h runs. In the UF group, there was a significant change in pH from the start of shunting to the end of the 1-h shunt period. There were also significant changes in pO_2 and pCO_2 from the

TABLE 1. ARTERIAL BLOOD GAS AND COMPLETE BLOOD CELL COUNT RESULTS

	UF			CF		
	Baseline	1 h	3 h	Baseline	1 h	3 h
WBC (K/ μ L)	17.6 $\pm$ 3.4	12.1 $\pm$ 4.8	13.6 $\pm$ 1.6	18.0 $\pm$ 4.3	17.4 $\pm$ 5.8	15.8 $\pm$ 6.2
RBC (M/ μ L)	5.6 $\pm$ 0.8	4.3 $\pm$ 1.4	4.4 $\pm$ 0.4	5.8 $\pm$ 0.6	5.6 $\pm$ 0.4	5.6 $\pm$ 0.4
Hg (g/dL)	9.3 $\pm$ 1.4	7.3 $\pm$ 2.4	7.5 $\pm$ 0.9	10.0 $\pm$ 0.8	9.7 $\pm$ 0.5	9.5 $\pm$ 0.5
Ht (%)	31.8 $\pm$ 4.6	25.0 $\pm$ 8.1	25.7 $\pm$ 2.7	33.7 $\pm$ 2.5	33.5 $\pm$ 2.0	32.9 $\pm$ 1.8
Plt (K/ μ L)	17.0 $\pm$ 4.4	15.3 $\pm$ 7.7	16.5 $\pm$ 5.4	34.5 $\pm$ 14.2	31.4 $\pm$ 11.6 ^a	29.3 $\pm$ 10.3
pH	7.53 $\pm$ 0.05	7.50 $\pm$ 0.07 ^b	7.41 $\pm$ 0.09	7.52 $\pm$ 0.08	7.51 $\pm$ 0.07	7.50 $\pm$ 0.08
pCO ₂ (mm Hg)	34.5 $\pm$ 4.3	36.8 $\pm$ 4.8	42.6 $\pm$ 3.6 ^b	34.4 $\pm$ 10.0	35.3 $\pm$ 8.9	36.7 $\pm$ 9.8
pO ₂ (mm Hg)	461 $\pm$ 58	299 $\pm$ 160	251 $\pm$ 71 ^b	453 $\pm$ 121	457 $\pm$ 124	437 $\pm$ 125

^aVersus UF $p < 0.05$.^bVersus Baseline $p < 0.05$.

CF, controlled flow group; g/dL, grams per deciliter; Hg, hemoglobin; Ht, hematocrit; K/ μ L, thousands of cells per microliter; M, molar; mm Hg, milligrams of mercury as a unit of pressure; pCO₂, partial pressure of carbon dioxide in blood; pH, measure of acidic/basic quality of water; Plt, platelet; pO₂, partial pressure of oxygen as a measure of the amount of oxygen dissolved in blood; RBC, red blood cell count; UF, uncontrolled flow group; WBC, white blood cell count.

start of shunting to the end of the 3-h shunt period in the UF Group.

Discussion

This study demonstrated that the swine bilateral femoral A-V shunt model with tourniquet-CF and use of an inserting tube interface between the vessel and test shunt is a stable acute shunt flow model, and we were able to change the tested tubes without surgical complications. There were no statistical differences between coated and uncoated samples or between the 1- and 3-h experiments in both UF and CF groups. We were able to keep stable flow in all tested tubes and evaluate two different tubes simultaneously. However, bleeding and establishing control of platelet adhesion were two major complications in the UF group.

Swine were chosen for this shunt protocol because it has been established that the blood-biomaterial interaction and the activation of the clotting cascade in pigs best approximates analogous responses in humans. The vessels of pigs, however, are very fragile. The PE tubes, because of their hardness and stiffness, were difficult to insert, easily became occluded by the vessel wall, and frequently induced vessel wall trauma. We changed to using PU tubes because it is softer and more pliable than PE tubes. Softer materials performed better in our swine model because the vessels of the animals are fragile. The decreased red blood cell counts, hemoglobin values, and hematocrit in the UF group can be attributed to bleeding at the cannulation site because of the use of PE shunt tubes.

In addition, our having to administer a considerable quantity of fluid to maintain arterial pressure could have resulted in changes in the pH, pO₂, and pCO₂ levels only in the UF group. In the CF group, we added insertion tubes between the vessels and the test tubing to eliminate manipulation at the cannulation site during attachment and detachment in the 1- and 3-h runs. There were no significant changes in CBC and ABG results in the CF group because of reduced bleeding and lower shunt flow levels.

Platelet adhesion is sensitive to shunt flow and shear stress. The A-V shunt flow in the UF group was originally >400 mL/min. We were not able to see platelet adhesion at this high flow rate using scanning electron microscopy.

When shunt flow was reduced and kept to 40–60 mL/min in the CF group, we were able to see platelet adhesion in tested shunt tubes (these results will be reported separately). The tubes selected had an inner diameter of 1/8 inch (3.2 mm) because this experiment simulated the use of similar tubes in coronary artery bypass graft surgery.

We decided to keep shunt flow at 40–60 mL/min in the CF group, because blood flow during coronary artery bypass graft procedures is about 50 mL/min. If shunt flow had been lower than 40 mL/min or the duration had been longer than 3 h, we might have seen more platelet adhesion in the tubes. This swine model holds great potential for quickly and reliably assessing biocompatibility.

Cikirikcioglu *et al.*⁹ performed chronic bilateral carotid graft interposition for 30 days in swine with uncoated ($n=5$) and titanium-coated ($n=5$) expanded polytetrafluoroethylene grafts (internal diameter, 4 mm; length, 5 cm). Their patency rate was 80% for all grafts. Measured graft blood flow after completion of graft interposition was around 200 mL/min. Ueberrueck *et al.*¹⁰ coated polyester (Dacron) vascular prostheses with titanium ($n=7$) and implanted these into swine infrarenal aorta for 3 months; however, all grafts became occluded.

Several acute arteriovenous shunt models similar to ours have been reported.^{11–13} Handa *et al.*¹¹ built the arteriovenous shunt in rabbits by inserting 16-gauge and 14-gauge angiocatheters into the left carotid artery for inflow and the right external jugular vein for outflow, respectively. The investigators removed the vinyl chloride shunt tube after 4 h, and animals received no systemic anticoagulation throughout the experiment. Seven control tubes clotted within 3 h, whereas all seven coated tubes remained patent after 4 h. As the shunt tube was not replaceable, they had to use 14 rabbits to evaluate 14 tubes, which may have induced the animals' individual variability.

Hagen *et al.*¹² used an acute baboon thrombogenicity model to assess biocompatibility. The investigators used only one animal and implanted a chronic arteriovenous shunt between the femoral artery and femoral vein, and for each acute study, the shunt loop was extended with silicone tubing. The investigators removed the silicone shunt tubes (inner diameter, 3.18 mm) after 1 h, and the animals received no systemic anticoagulation throughout the experiment. This method is unique, as all tested materials can be

evaluated in a single animal, avoiding the issue of animals' individual variability.

Otsuka *et al.*¹³ used an acute swine thrombogenicity model to test for biocompatibility; their method consists of a carotid-to-jugular arteriovenous shunt model involving a test circuit of three in-line stents. They removed the silicone shunt tubes (inner diameter, 2.7 mm) after 1 h. Target blood-activated clotting times between 150 and 190 s were achieved with intravenous heparin (100 IU/kg) dosing in this experiment. The investigators had planned to evaluate two shunts in each animal; however, one animal completed only the first shunt experiment because its general condition deteriorated during the second shunt experiment.

The advantage of our swine model is that we can change tubes as many times as needed, using stopcocks to maintain stable hemodynamics without hemodynamic deterioration. Also, by using a bilateral shunt model, we can test two materials simultaneously in the same animal without changing tubes. Another advantage of our method is that shunt flow can be controlled in the CF group, as platelet adhesion is sensitive to shunt flow and shear stress.

We used PE or PU tubes inserted into the femoral A-V shunt with CF levels, adjusted through a tourniquet, in an acute feasibility study. We were able to easily measure and control the flow as needed. In our protocol, biocompatibility of the coated and uncoated tubes was assessed quickly, within only 3 h. Platelet adhesion to biomedical materials occurs within the first 30 min of blood exposure and slowly progresses thereafter. Using this method, potential candidate biomaterials intended for longer-term preclinical chronic device studies can be easily and quickly screened by comparing them to known control materials. Our results suggest that the biocompatibility of the coated tube is at least equivalent to that of the control uncoated PE and PU tubes.

Conclusion

Our swine bilateral femoral A-V shunt model with PU tubing, tourniquet-CF and use of an inserting tube interface between the vessel and test shunt provides a stable shunt flow model. We changed the tested tubes without surgical complications. This model is useful and efficient for rapidly testing the biocompatibility of tubes coated with a specific biomaterial.

Acknowledgments

The work reported here was funded by the National Heart, Lung, and Blood Institute of the National Institutes of Health (Bethesda, MD) under grant 2R01EB002067-15A1 to Roger E. Marchant, PhD (deceased), formerly of the Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio. The authors are grateful for his supervision and valuable guidance in the course of this study.

Disclosure Statement

No competing financial interests exist.

References

- Jaganathan, S.K., Supriyanto, E., Murugesan, S., Balaji, A., and Asokan, M.K. Biomaterials in cardiovascular research: applications and clinical implications. *Biomed Res Int* **2014**, 459465, 2014.
- Karimov, J.H., Horvath, D., Sunagawa, G., *et al.* K. Post-explant visualization of thrombi in outflow grafts and their junction to a continuous-flow total artificial heart using a high-definition miniaturized camera. *J Artif Organs* **18**, 354, 2015.
- Lamba, N.M., Baumgartner, J.N., and Cooper, S.L. The influence of thrombus components in mediating bacterial adhesion to biomaterials. *J Biomater Sci Polym Ed* **11**, 1227, 2000.
- Tang, C., Kligman, F., Larsen, C.C., Kottke-Marchant, K., and Marchant, R.E. Platelet and endothelial adhesion on fluorosurfactant polymers designed for vascular graft modification. *J Biomed Mater Res A* **88**, 348, 2009.
- Zhu, J., Tang, C., Kottke-Marchant, K., and Marchant, R.E. Design and synthesis of biomimetic hydrogel scaffolds with controlled organization of cyclic RGD peptides. *Bioconj Chem* **20**, 333, 2009.
- Sagnella, S., Anderson, E., Sanabria, N., Marchant, R.E., and Kottke-Marchant, K. Human endothelial cell interaction with biomimetic surfactant polymers containing peptide ligands from the heparin binding domain of fibronectin. *Tissue Eng* **11**, 226, 2005.
- Zhu, J., Gosen, C., and Marchant, R.E. Synthesis and characterization of poly(vinyl amine)-based amphiphilic comb-like dextran glycopolymers by a two-step method. *J Polym Sci A Polym Chem* **44**, 192, 2006.
- Gupta, A.S., Wang, S., Link, E., *et al.* Glycocalyx-mimetic dextran-modified poly(vinyl amine) surfactant coating reduces platelet adhesion on medical-grade polycarbonate surface. *Biomaterials* **27**, 3084, 2006.
- Cikirikcioglu, M., Sedelnikov, N., Osorio-Da Cruz, S., *et al.* Improved neo-endothelialization of small diameter ePTFE grafts with titanium coating. *Int J Artif Organs* **29**, 990, 2006.
- Ueberrueck, T., Meyer, L., Zippel, R., and Gastinger, I. Characteristics of titanium-coated polyester prostheses in the animal model. *J Biomed Mater Res B Appl Biomater* **72**, 173, 2005.
- Handa, H., Brisbois, E.J., Major, T.C., *et al.* *In vitro* and *in vivo* study of sustained nitric oxide release coating using diazeniumdiolate-oped poly(vinyl chloride) matrix with poly(lactide-co-glycolide) additive. *J Mater Chem B Mater Biol Med* **1**, 3578, 2013.
- Hagen, M.W., Girdhar, G., Wainwright, J., and Hinds, M.T. Thrombogenicity of flow diverters in an ex vivo shunt model: effect of phosphorylcholine surface modification. *J NeuroIntervent Surg* **9**, 1006, 2017.
- Otsuka, F., Cheng, Q., Yahagi, K., *et al.* Acute thrombogenicity of a durable polymer everolimus-eluting stent relative to contemporary drug-eluting stents with biodegradable polymer coatings assessed ex vivo in a swine shunt model. *JACC Cardiovasc Interv* **8**, 1248, 2015.

Address correspondence to:

Kiyotaka Fukamachi, MD, PhD

Cardiovascular Dynamics Laboratory

Department of Biomedical Engineering/ND20

Lerner Research Institute

Cleveland Clinic

9500 Euclid Avenue

Cleveland, OH 44195

E-mail: fukamak@ccf.org

Received: June 27, 2017

Accepted: September 27, 2017

Online Publication Date: December 5, 2017