Flow-induced Platelet Activation in a St. Jude Mechanical Heart Valve, a Trileaflet Polymeric Heart Valve, and a St. Jude Tissue Valve

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Abstract: Polymer heart valves have been under investigation since the 1960s, but their success has been hampered by an overall lack of durability mainly due to calcification of the leaflets and a relatively high rate of thromboembolic complications. A new polymer (Quatromer) trileaflet design was tested for its thrombogenic potential and was compared to that of existing prosthetic heart valves routinely implanted in patients: a St. Jude Medical bileaflet mechanical heart valve (MHV) and a St. Jude porcine bioprosthetic tissue valve. The valves were mounted in a left ventricular assist device and the procoagulant activity of the platelets was measured using a platelet activation state (PAS) assay. The PAS measurements indicated that the platelet activation level induced by the polymeric valve was very similar to that induced by the St. Jude Medical MHV and the St. Jude tissue valve. No significant difference was observed between the three valves, indicating that they have a comparable thrombogenic potential. Key Words: Thrombogenic—Polymer trileaflet valve—Bioprosthetic tissue valve—Left ventricular assist device—Mechanical heart valve.

INTRODUCTION

Valve replacement is a necessary treatment for patients with severe valvular diseases. The choice of a mechanical heart valve (MHV) or a bioprosthetic heart valve depends on the patient’s age and condition, valve type, and the feasibility of anticoagulation (1). According to the World Medical Market Fact File published in 2001, 60% of the prosthetic heart valve (PHV) market was occupied by MHVs and 40% by tissue valves.

MHVs have better durability and lower reoperation rates (2), but patients with MHVs must undergo a life-long anticoagulation treatment, which has an attendant risk of hemorrhage (3). The implantation of MHVs may induce nonphysiological flow patterns and cause platelet activation, potentially leading to thromboembolism and cardioembolic stroke, which are the major complications of MHVs (4). The most widely used MHV in the US market is the St. Jude Medical bileaflet MHV. Its three-orifice design enables a lower transvalvular pressure gradient and a decreased thrombosis potential (5). Clinical studies indicated that St. Jude Medical bileaflet MHVs have satisfactory hemodynamic performance (6).

Compared to MHVs, bioprosthetic heart valves have better biocompatibility and can eliminate the need for anticoagulation (7). However, they may have compromised hemodynamic performance (8,9), the potential for tissue deterioration, and a higher risk of structure failure (10). Valve leaflet or vessel wall calcification is always a significant complication in bioprosthetic heart valves, leading to durability problems; for example, Koutsopoulos et al. indicated that bioprosthetic heart valves have limited long-term durability due to calcification problems (11).
However, recently it was reported that tissue valves and MHVs share similar long-term survival and thromboembolism rates (1), and that, for example, the durability of porcine valves is more related to the patient’s age (12).

Polymeric heart valves have started to draw more attention in recent years due to their potentially improved hemodynamic performance and mechanical properties. Their geometric similarity to the natural valves provides a large orifice in the ejection phase and helps keep the aortic blood flow less disturbed. In vitro animal studies conducted by Daebritz et al. (13) indicated that a polycarbonateurethane heart valve can achieve thrombosis rates comparable to bioprosthetic heart valves. However, the major impediment to the success of the polymeric heart valves is the valve material degradation and calcification rates (14,15). To make the polymeric valves a viable alternative to MHVs and tissue valves, newly developed materials are needed to solve the degradation and calcification problems. In late 1990s, Pinchuk et al. developed a new polymeric heart valve made of a novel polyolefin (polystyrene-b-isobutylene-b-styrene, also known as polysytrene-polysobutylene-polostyrene, or “SIBS,” and trademarked by Innovia LLC (Miami, FL, U.S.A.) as Quatromer. Their in vivo studies indicated that this new material is oxidatively stable within the physiological environment, and therefore will not degrade during long-term implantation (16).

Wheatley et al. (17) compared the performance of MHVs, tissue valves, and polymeric heart valves in sheep models. They implanted ATS bileaflet MHVs, polyurethane (PU) valves, and Carpentier-Edwards (CE) porcine valves in sheep and measured high-intensity transient signals (HITS) with transcranial Doppler (TCD) ultrasound, thrombotic potential, aggregation rates, heart tissue calcification, and arterial stenosis after valve implantation. They found that the PU valves had better hemodynamic properties compared to the bioprosthetic ones and also had lower thrombogenicity rates compared to the MHVs. Polymeric heart valves may also be better suited for the emerging percutaneous valve implantation procedure, which requires compressing the valve at the end of a catheter during delivery.

Whether these newly developed polymeric valves have thrombogenic potential that is comparable to other prosthetic valves in the market still remains to be answered.

In this study, we address this by comparing the thrombogenic potential of a St. Jude Medical bileaflet MHV, a St. Jude tissue valve, and a SIBS trileaflet heart valve by measuring in vitro their induced platelet activity state (PAS) in a left ventricular assist device (LVAD).

MATERIALS AND METHODS

Outdated pheresis units of platelets were obtained either from Stony Brook University Hospital blood bank or the Long Island blood service (Melville, NY, U.S.A.). One hundred milliliters of platelet-rich plasma was gel filtered at a flow rate of 800 mL/h through a 1000 mL column of coarse Sepharose 2B beads (2% agarose; Amersham-Pharmacia, Sigma Chemical, St. Louis, MO, U.S.A.). After gel filtration, the collected platelets were counted (Z1 particle counter; Coulter, Hialeah, FL, U.S.A.) and adjusted to a concentration of 100 000/μL. Dextran (MW = 5 000 000, Sigma Chemical, St. Louis, MO, U.S.A.) was added to the platelet buffer to increase the viscosity to 3.5 cp. The platelets were kept on a gentle shaker in room temperature and were used within 6 h.

The thrombogenic potential of the three prosthetic heart valves was measured in an LVAD, which is the implantable part of a pneumatic heart-assist system developed by Prof. Klaus Affeld (Biofluidmechanics Laboratory, Clinic of Cardiovascular Surgery, University Hospital Charité, Humboldt University of Berlin, 14 050, Berlin, Germany). The LVAD chamber (70-mm diameter clear diecast polyvinyl chloride [PVC] 0.6-mm-thick polyurethane diaphragm with a blood-compatible inner coating) has a 65 mL stroke volume and a total volume of about 100 mL. Two PHVs were mounted in the LVAD as outflow and inflow valves to control flow direction. The outflow and inflow tracts of the LVAD were connected by a compliance reservoir made of a 5-inch clinical grade surgical penrose tubing (Bard Inc., Covington, GA, U.S.A.). To conduct the circulation experiments, the LVAD recirculation loop was placed in an incubator with the temperature of 37 ± 2°C. The LVAD was driven by a pulsatile reciprocating pump (Model 1423 Blood Pump, Harvard Apparatus, Holliston, MA, U.S.A.), which can generate quasi-physiological flow conditions and regulate stroke volume and stroke rate. The flow rate of the system was 4.7 L/min, the stroke rate was 72 beats/min, and the systole/diastole ratio was 0.375. Platelets were circulated in the LVAD for 30 min; aliquots were taken every 5 min and PAS was measured according to the methods described in our previous publications (18,19). Because platelet activation may significantly vary from donor to donor and from day to day, all the PAS measurements were normalized by the full platelet activation achieved by
sonication (Branson Sonifier S150D, Thistle Scientific Ltd, U.K.) for 10 s at 10 watts. For each circulation experiment, the PAS values were normalized by the full activation obtained by sonication at the end of each run. The normalized numbers were then plotted over time (30 min) and fitted with linear regression to obtain the platelet activity rate (PAR). The ratios of the PARs obtained from two different conditions (e.g., PAR_{MHV}/PAR_{tissue}) were computed on a daily basis and compared to 1 using Student’s t-test (Eq. 1), using the following definition:

$$t = \frac{\log(r_m)}{\sqrt{\log(SD_r^2)/n}}$$

where $r_m$ is the average of the ratios, $SD_r^2$ is the standard deviation of the ratios, and $n$ is the sample size.

We have tested the following three PHVs: a St. Jude Medical MHV-SJM Masters Series with a tissue annulus diameter of 27 mm, a St. Jude Toronto SPV stentless porcine bioprosthetic tissue valve with a tissue annulus diameter of 21 mm (St. Jude Medical Inc., St. Paul, MN, U.S.A), and an Innovia LLC Trileaflet (Innovia, Miami, FL, U.S.A.) composite polymeric valve with a tissue annulus diameter of 25 mm. The trileaflet polymeric valve is a newly designed valve, which is manufactured by dip coating in a 15% Quatromer/toluene solution. The leaflets have 3 dip coatings of Quatromer with an embedded polyester mesh and an average leaflet thickness of approximately 240 µm (Fig. 1) (20). The external diameters of all three PHVs were adjusted to an identical LVAD valve holder conduit with an internal diameter of 25 mm, to enable easy mounting and removal. The suture ring of the 27-mm St. Jude MHV was removed to snugly fit the MHV into the LVAD valve holder conduit, resulting in a nominal external diameter of 25 mm. The trileaflet polymeric valve, with a nominal external diameter of 23 mm, was fitted into a high-density polyethylene ring to adjust it to the 25 mm conduit diameter accordingly (corresponding to its target tissue annulus diameter). The stentless tissue valve, with a nominal external diameter of 21 mm, was similarly mounted into the conduit using a thicker high density polyethylene fitting ring. It is important to note that these three PHVs have different internal diameters: 23.6 mm for the MHV, 21 mm for the polymer valve, and 19 mm for the tissue valve. The tissue annulus diameters, the adjusted external diameters, and the nominal internal diameters of these three PHVs are listed in Table 1.

A 25-mm (external diameter) St. Jude Medical bileaflet MHV served as the inflow heart valve in all the measurements. The surfaces of all the valves used in this study were not passivated. PAS measurements, using the same batch of platelets, were performed by comparing two different valve designs on each day, with each recirculation experiment repeated once. To minimize the effect of platelet activation over time, the recirculation runs were performed in palindromic orders (A-B-B-A). Between runs, the PHVs were removed from the LVAD and cleaned thoroughly. For the polymeric valve and the MHV, 10% bleach, deionized water, and 0.01 M HCl and larger quantities of deionized water were used to wash the valves. For the St. Jude tissue valve, nonionic detergent Tween 80 (Sigma Chemical) and large quantities of deionized water were used instead. After cleansing, the tissue valve was kept in 0.9% saline solution until next use.

### RESULTS

PAS measurements were conducted in the LVAD recirculation loop to compare the thrombogenic...
potential of the polymeric valve and the St. Jude MHV. The comparison result is shown in Fig. 2. In both cases, PAS values were normalized with reference to the maximum activation determined by sonication. The PAR (that is, the slope of the thrombin generation in the PAS assay) induced by the polymeric valve was 0.0011/min and that induced by the MHV was 0.0006/min. Although the PAR appears to be higher for the polymeric valve when directly compared to the St. Jude bileaflet valve, statistical analysis of the collected data indicated that there was no significant difference between the two slopes ($n = 8$, $P > 0.1$).

Similarly, PAS was measured in the LVAD to compare the PAR between the St. Jude tissue valve and the St. Jude MHV. The PARs obtained are shown in Fig. 3. The PAR induced by the tissue valve was 0.001/min and that induced by the MHV was also 0.001/min. Statistical analysis showed no significant difference between the two valves ($n = 6$, $P > 0.1$).

The comparison of PAR between the St. Jude tissue valve and the polymeric valve is shown in Fig. 4. The PAR induced by the tissue valve was 0.0012/min and that induced by the polymeric valve was 0.0008/min. Again, statistical analysis indicated that the difference between the two cases was not significant ($n = 6$, $P > 0.1$).

DISCUSSION

PAS assay provides an efficient and relatively inexpensive technique for measuring platelet activation in near-real time in PHV, and it is suitable for time-resolved studies of multiple samples. Gel filtration removes most plasma proteins such as the von Willebrand factors and fibrinogen. The removal of those plasma proteins and the use of acetylated prothrombin (18) enables a one-to-one relationship between the agonist, that is, flow-induced mechanical stresses in the PHVs and the thrombin-generation rates in the PAS assay. The PAS assay provides a reliable and repeatable way to measure platelet activation in blood-recirculating devices (21). The statistical approach in the design of the experiments diminished the inherent variability in platelet behavior, which may change significantly from day to day and from donor to donor. The first comparative measurements were conducted between the polymeric heart valve and the St. Jude MHV, followed by a comparative study between the St. Jude porcine valve and the MHV. The results indicated that there were no significant differences between the two groups.
Meanwhile, PAS measurements obtained from the polymeric heart valve at different times (the group of data obtained from the experiment comparing to the MHV and the group of data obtained from the experiment comparing to the tissue valve) were compared and no significant difference was detected ($P > 0.1$). The same result applied to the tissue valve measurements. The PAS values obtained from the MHV in all three cases were compared and no significant difference was detected, which indicated that the cleaning procedure for the MHV is also valid. These comparisons between experiments conducted with the same prosthetic heart valve at different times demonstrated that the LVAD setup and the PAS assay were able to measure platelet activities in a reliable and consistent manner. It also indicated that the cleaning process between runs (bleach, water, hydrochloric acid, and water for the MHV; the polymeric valve, and the LVAD; Tween 80 and water for the tissue valve) was effective. After cleaning, platelets did not get activated by the potentially deposited platelets from the previous experimental run.

The PHVs were switched between experimental runs and the same LVAD and valve holders were used for all the PAS measurements. This eliminated all the geometry and material factors associated with the LVAD and the valve holders that might cause platelet activation in the comparison. It enabled us to measure directly the contribution of the flow conditions through the outflow prosthetic heart valve to platelet activation.

The PAS measurements indicated that during the 30-min circulation, the thrombogenic potentials of the three PHVs were very similar. Statistical analysis clearly indicated that there was no significant difference in the potential between the MHV and the polymeric valve, the MHV and the tissue valve, and between the polymeric valve and the tissue valve.

However, the three PHVs have different internal diameters, and the PAS measurements were not normalized with respect to the valve opening areas. The same 25-mm external diameter of all these valves facilitated their uniform fitting into the valve holders of the LVAD, but their different internal diameters affected their hemodynamic performance. The St. Jude tissue valve used in this study was a stentless porcine aortic bioprosthetic heart valve with a tissue annulus of 21 mm and an internal diameter of 19 mm. It was sutured into a rigid cylindrical stent with an external diameter of 25 mm in order to allow a comparison between the polymeric and the MHV with the same external dimensions. The internal diameter of the St. Jude MHV was about 23.6 mm, which made the opening area approximately 50% larger than that of the tissue valve. The larger orifice should decrease the thrombogenic potential of the MHV. However, the opening area of a bileaflet MHV is divided into three orifices by the two valve leaflets. The smaller core orifice between the two leaflets may induce flow acceleration, jet flow, and elevated shear stresses, and all these factors may enhance platelet activation (22). The internal diameter of the polymeric valve was 21 mm and the opening area was about 40% less than that of the MHV. However, at opening, the polymeric valve and the tissue valve mimic natural valves, and their aortic flow dynamics are similar to that of native valves. Although the MHV had the assumed advantage of a bigger opening area, which potentially should have translated into a reduced thrombogenic potential, the comparison results clearly indicated that there was no significant difference between these three PHVs. This demonstrated the detrimental effect of the MHV flow dynamics on platelet activation. As mentioned above, this conclusion was drawn without normalizing the PAS measurements with respect to the valve opening area. Following this argument, an MHV with an opening area similar to that of either the tissue or the polymeric valve, may have a higher thrombogenic potential. The actual opening area of these three heart valves could be determined using optical methods. Additionally, techniques such as digital particle image velocimetry (DPIV) and numerical simulations, combined with a platelet stress accumulation model predicting platelet activation should be conducted in each of these valves in order to depict the flow mechanisms that lead to their thrombogenic potential.

Despite these limitations, we were able to control in this study most of the factors affecting PAS measurement. This was achieved by using the same LVAD and materials for the valve holders, by conducting the measurements of the thrombogenic potential using the same batch of platelets for each comparison between any pair of valves, and by conducting the experiments in a palindromic sequence to offset the effect of platelet activation over time. By conducting rigorous statistical analysis, we have clearly demonstrated that the PAS results of the three valves indicated a very similar thrombogenic potential under our experimental conditions. The MHV possibly has a higher thrombogenic potential, had it been of similar size to that of the two other valves. Further, our measurements have demonstrated that the PAS assay is a robust method that is particularly suited for studies of the thrombogenic potential in blood-recirculating devices such
as PHVs by quantifying the procoagulant properties of platelets activated by flow stresses in these devices.

The tissue and the polymeric valves have been shown to share a very similar thrombogenic potential, indicating that the novel material, Quatromer, in addition to its proven biostability has the potential of minimizing thrombus formation. One may speculate that like their bioprosthetic counterparts, this new generation of polymeric valves may not require the anticoagulation drug regimen that MHV do.

Acknowledgments: This work is supported in part by an SBIR grant from the NHLBI to Innovia LLC, an Established Investigator Award from the American Heart Association (D.B.), and by the National Science Foundation under Grant no. 0302275 (D.B.).

REFERENCES