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Application of Drag-Reducing Polymer Solutions as Test Fluids for *In Vitro* Evaluation of Potential Blood Damage in Blood Pumps

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Abstract

In vitro evaluation of the potential of a circulatory-assist device to damage blood cells has generally been performed using blood from various species. Problems with this approach include the variability of blood sensitivity to mechanical stress in different species, preparation of blood including the adjustment of hematocrit to a standard value, changes in the mechanical properties of blood that occur during storage, and necessity to pool blood samples to obtain an adequate amount of blood for *in vitro* circulating systems. We investigated whether the mechanical degradation of a drag-reducing polymer (DRP) solution resulting in the loss of drag-reducing ability can indicate the degree of shear-induced blood damage within blood pumps. DRP solution (polyethylene oxide, 4,500 kDa, 1,000 ppm) or porcine blood were driven through a turbulent flow system by a centrifugal pump, either the Bio-Pump BPX-80 (Medtronic, Inc.) or CentriMag (Levitronix LLC) at a constant pressure gradient of 300 mm Hg for 120 minutes. DRP mechanical degradation was evaluated by reduction of flow rate and solution viscosity. A proposed index of DRP mechanical degradation (PDI) is similar to the normalized index of hemolysis (NIH) typically used to quantify the results of *in vitro* testing of blood pumps. Results indicate that the mechanical degradation of DRP solutions may provide a sensitive standard method for the evaluation of potential blood trauma produced by blood pumps without the use of blood.

Blood damage is recognized as one of the main problems associated with mechanical circulatory support. Newly developed or modified ventricular assist devices (VADs) require *in vitro* testing to sufficiently demonstrate blood biocompatibility, a minimum trauma to red blood cells (RBCs), and other blood components before conducting animal studies and ultimately translating the devices for clinical use. The generally accepted manifestation of mechanical blood damage is the amount of hemoglobin released into plasma, or hemolysis. 1–5 Assessment of hemolysis has been performed using animal blood from different species (mostly bovine blood) in mock circulation systems with the tested blood pump. 6–14 Despite the existence of the American Society for Testing and Materials recommendations on the standard assessment of blood trauma in medical devices *in vitro* [e.g., Standard Practice for Assessment of Hemolysis in Continuous Flow Blood Pumps American Society for Testing,

and Materials F1841-97 (2005)], the variations in testing conditions, sensitivity to mechanical stress of RBCs from different species, and dependence of RBC mechanical fragility on storage time thus precludes the comparison of hemolysis test results.^{15–19} Therefore, a standard test fluid for the evaluation of potential blood damage within blood pumps would be beneficial for such a comparison.

Suspensions of polyurethane microcapsule filled with leuco dye have been proposed previously as test solutions for the evaluation of hemolytic characteristics of circulatory-assist devices.^{20,21} According to the authors, microcapsule suspensions were proposed due to their nonbiological nature and ability to “simulate the static mechanical and fatigue properties” of RBCs.²⁰ However, this method has several disadvantages, such as the intricate preparation of microcapsule suspensions and very complex analysis of test results. Moreover, the major problem of using this method is that even fine suspensions of polyurethane capsules have a broad size distribution (from ~70 to ~200 μm) with the largest concentration of capsules at 100 μm . Consequently, these suspensions cannot be used to test axial blood pumps and, in general, cannot simulate the hemodynamics in circulatory-assist devices.

Polyacrylamide (PAA) solutions were proposed as a potential test fluid for evaluation of circulatory-assist devices due to their pronounced non-Newtonian properties and the shear-induced degradation of PAA molecules over time.²² A solution of PAA with molecular weight (MW) 10^7 Da at 300 ppm concentrations was a suitable fluid with comparable rheological behavior as that of blood. The authors tested their hypothesis of the similarity of PAA degradation and hemolysis using a specifically designed shearing device in which PAA solution and blood were exposed to a constant shear stress during a certain time. They characterized the shear-induced degradation of PAA by measurement of viscosity over a wide range of shear rates. Zero-shear viscosity obtained using the generalized Quemada model was applied to calculate a “loss of viscosity” parameter, which was compared with a specially designed index of hemolysis only applicable to the hemolysis generated in their shearing device. The proposed method was not well justified because the observed changes in low shear viscosity of PAA solution without significant changes in viscosity at high shear rates may be related to disentanglement and disaggregation of the polymer molecules and not to their physical degradation. Since the authors did not test PAA solutions in a flow system with a circulatory-assist device, it is not clear how relevant their method of testing the potential damage to PAA molecules during a very short exposure time in a shearing device is for *in vitro* hemolysis testing of actual blood pumps. The authors did not discuss how to apply their method for this purpose nor did they prove whether a damage index based on changes of low shear viscosity would be informative for *in vitro* testing of circulatory-assist devices.

In this report, we hypothesized that solutions of water-soluble drag-reducing polymer (DRP) with non-Newtonian flow behavior and viscosity similar to that of blood can replace human or animal blood for the *in vitro* assessment of potential mechanical blood damage in circulatory-assist devices. DRPs are soluble polymers that reduce resistance to turbulent flow in a pipe when added to flowing fluid at minute concentrations; phenomenon known as the Toms Effect.²³ A slight modification of the traditional hemolysis test circuit is needed to facilitate real-time observation and recording of polymer mechanical degradation.

Materials and Methods

DRP Solution

To test our hypothesis, we proposed to use for the *in vitro* assessment of potential mechanical blood damage a DRP solution of polyethylene oxide (PEO), with an average

MW of ~4,500 kDa at a concentration of 1 mg/mL. PEO solutions have many advantages, such as easy standard preparation and simple detection of mechanical degradation during tests using the loss in PEO drag-reducing ability. Moreover, PEO is a nontoxic and readily available DRP, which is known to be the most effective drag reducer and concurrently most fragile compared with other DRPs.^{24–27} Previous studies of PEO performed in our laboratory using a Viscotek Triple Detector Array gel permeation chromatography (GPC) system (Viscotek, Houston, TX) have found that the average MW of this polymer was $4.4 \times 10^6 \pm 0.2 \times 10^6$ Da, and the polydispersity index was 1.7 ± 0.4 .²⁸ In this study, a stock PEO solution of 2,500 ppm concentration was prepared from Polyox WSR-301 (Dow Chemical, Midland, MI) by slowly dissolving the polymer powder in saline over several hours to avoid molecular degradation during the preparation procedure. Before testing, the stock solution was diluted with saline to 1,000 ppm and gently mixed for 2 hours. A fresh stock solution was prepared 1 to 2 days before each test.

Blood

Porcine blood was collected in plastic containers with 10% acid-citrate-dextrose anticoagulant (ACD; Gambro BCT, Lake-wood, CO) from a local abattoir. Blood was filtered through 40- μ m pore size filters (Pall Biomedical, SQ49S, Fajardo PR) to remove white blood cells and platelet aggregates. Gentamicin (0.25 g/L; American Pharmaceutical Partners, Schaumburg, IL) was added to prevent bacterial growth. Blood hematocrit (Ht) was measured using microhematocrit centrifugation (IEC MB Centrifuge, International Equipment Company, Needham Heights, MA) at 13,000g for 4 minutes. Ht was adjusted to a standard value of $30.0\% \pm 1.0\%$ using autologous plasma. All blood testing was completed within 3 days of blood collection.

Viscometric Measurements

A Wells-Brookfield Cone/Plate viscometer (Model LVDV-IIIUCP, Middleboro, MA) with CPE-40 cone (cone radius = 2.4 cm, cone angle = 0.8° , sample volume = 0.5 mL) was used to measure viscosity of PEO solutions and blood samples. The viscosity of tested fluids was measured over a range of shear rates (40–400 s^{-1}) at a temperature of 25°C. A circulating water bath (Neslab RTE7, Thermo Fisher Scientific, Waltham, MA) was used to maintain constant temperature. Figure 1 shows the similar rheological behavior of 1,000 ppm PEO solutions ($n = 4$) and 30% Ht porcine samples ($n = 4$) at a temperature of 25°C.

Turbulent Flow System

The turbulent flow circulating system used in these studies is shown schematically in Figure 2. The system consisted of a centrifugal pump, either the Bio-Pump BPX-80 (Medtronic, Inc., Minneapolis, MN) or CentriMag (Levitronix LLC, Waltham, MA), a glass tube [0.44 cm internal diameter (ID), 91.5 cm length], and a 1 L open fluid reservoir. All parts of the flow system were interconnected with 3/8 inch ID Tygon tubing (Cole-Parmer, Vernon Hills, IL). The fluid reservoir was immersed in a water bath to maintain constant temperature. A clamp-on flow probe (8CB9, Transonic Systems Inc., Ithaca, NY) and pressure transducer (PCB Piezotronics, Inc., Depew, NY) were used to record flow rate and pressure. Equivalent wall shear stress in the glass tube was maintained during all tests.

DRP Degradation Studies

One liter of 1,000 ppm PEO solution was driven through the flow system for 120 minutes. The pressure gradient across the glass tube was maintained constant at 300 mm Hg, and flow rates were recorded. Since polymer drag reduction (DR) occurs only at turbulent flow conditions, the presence of turbulence in the system was verified by calculation of Reynolds number (Re):

$$Re = \frac{4Q}{\pi d \nu} \quad (1)$$

where Q is volumetric flow rate (cm^3/s), d is glass tube diameter (cm), and ν is kinematic viscosity (cm^2/s).

Mechanical degradation of DRP is defined as the loss of effectiveness in polymer drag-reducing ability that is not regained once mechanical stress is removed.²⁹ In our study, PEO degradation was evaluated via reduction in drag-reducing efficiency and polymer solution viscosity. Four experiments were performed (two for each pump), and small samples (2 mL) of PEO solution were collected from the reservoir at 0, 3, 15, 30, 60, and 120 minutes to measure viscosity. DR was calculated as a percentage:

$$DR = \frac{Q_{\text{DRP}} - Q_{\text{saline}}}{Q_{\text{saline}}} \times 100 \quad (2)$$

where Q_{DRP} is the flow rate of the DRP solution (L/min) and Q_{saline} is the flow rate of saline at the same pressure (L/min).

DRP Degradation Index

Mechanical degradation of PEO solution was quantified using the DRP mechanical degradation index (PDI) defined as:

$$PDI = \frac{DR_0 - DR_{120}}{\int Q dt} \quad (3)$$

where Q is flow rate (L/min), DR_0 is the original DR (%) of the PEO solution, and DR_{120} is the DR (%) of the PEO solution after 120 minutes of testing.

Hemolysis Studies

For each centrifugal pump, hemolysis tests ($n = 4$) were performed using the same flow circuit and conditions as in DRP degradation studies to directly compare the mechanical degradation of PEO with hemolysis generated by the pumps during 120-minute studies.

Temperature in the flow system was maintained at $23^\circ\text{C} \pm 2.0^\circ\text{C}$. Blood samples were obtained every 30 minutes from the reservoir for measurement of plasma-free hemoglobin (plfHb) concentration. Briefly, sample tubes were centrifuged for 15 minutes at 2,200g to obtain plasma. Plasma was transferred to microcentrifuge tubes and centrifuged at 21,000g for 20 minutes in a microcentrifuge (Eppendorf 5417R, Eppendorf North America). Plasma was then transferred to disposable semimicro cuvettes (Thermo Fisher Scientific, Waltham, MA). PlfHb was assessed for each sample using a spectrophotometer (Spectronic GENESYS 5, Thermo Fisher Scientific Inc., Waltham, MA) at 540 nm wavelength. The spectrophotometer was calibrated to zero using a blank solution. A standard curve of bovine Hb concentrations *versus* absorbance values was used to calculate plfHb. The plfHb value used for subsequent blood damage quantification was the difference between the plfHb concentration in the 120-minute sample.

Blood damage was characterized by the normalized index of hemolysis (NIH):

$$NIH = \frac{\Delta \text{freeHb} \times V \times \frac{(100 - Ht)}{100}}{Q \times T} \times 100 \quad (4)$$

where ΔfreeHb is plasma-free hemoglobin (g/L) released during the test period T , V is blood volume (L) of the circuit; Ht is hematocrit (%), Q is flow rate (L/min), and T is test time (min).

Statistical Analysis

Values are presented as mean $\pm$ SD. Two-tailed Student's t -tests for paired observations were performed to determine the statistical significance of the difference in mean DR, MW, DRP degradation index (PDI), plfHb concentration, and NIH for the two flow systems. Linear regression analysis was performed to determine the correlation between the PDI and NIH for the Bio-Pump and CentriMag flow systems.

Results

Pressure-Flow-RPM Characteristic of the Centrifugal Pumps

To ensure comparable flow fields within the centrifugal pumps studied, pressure-flow (H - Q) testing was performed on the Bio-Pump and CentriMag flow systems using water as a test fluid. The H - Q behavior was very similar in both systems (Figure 3).

DRP Degradation Studies

Reynolds numbers in the glass tube ranged from 7,000 to 11,000 in the DRP experiments characterizing a fully developed turbulent flow in the tube. Flow rate of the PEO solution in the flow system at the beginning of each study was 5.0 L/min. After 2 hours, flow rate in both the Bio-Pump and CentriMag flow systems decreased to 4.05 ± 0.01 and 4.17 ± 0.03 L/min, respectively. The decrease in flow rate represented the decrease in PEO drag-reducing ability, hence indicating polymer mechanical degradation (Figure 4). Calculated DR of the PEO solution declined during 120-minute tests in the two flow systems (Figure 5). Analysis revealed a statistically significant difference ($p = 0.005$) between the mean DR calculated for the two flow systems.

A direct measurement of PEO mechanical degradation was attained by evaluating the changes in PEO MW and solution viscosity during degradation tests. PEO MW was determined by extrapolation of MW *versus* viscosity values obtained for several commercial PEO with known MW (data not shown). At 120 minutes, PEO MW was calculated as 868 kDa and 1,410 kDa in the Bio-Pump and CentriMag flow systems, respectively. Statistical analysis revealed a significant difference ($p = 0.01$) between the MW calculated for the two flow systems. Viscosity of PEO solution (150 s^{-1} shear rate, 25°C temperature) decreased in both flow systems during tests. At baseline, PEO solution viscosity was 3.80 ± 0.12 cP in each flow system and decreased to 1.79 ± 0.05 cP and 2.19 ± 0.08 cP after 120 minutes in the Bio-Pump and CentriMag flow systems, respectively (Figure 6).

DRP Degradation Index

Mean PDI of the Bio-Pump and CentriMag flow systems at 120 minutes was calculated as $0.049 \pm 0.001\%$ and $0.041 \pm 0.002\%$, respectively. Statistical analysis revealed a significant difference ($p = 0.03$) between the mean PDI calculated for the two flow systems.

Hemolysis Studies

Change in plfHb concentration from baseline to end of study was 29.8% higher in the Bio-Pump than the CentriMag flow system (Figure 7). Mean plfHb concentration values were significantly different ($p = 0.048$) in the two flow systems.

Mean NIH of the CentriMag and Bio-Pump flow systems at 120 minutes was calculated as 0.005 ± 0.003 g/100 L and 0.018 ± 0.003 g/100 L, respectively. Analysis revealed a significant difference ($p = 0.006$) between the mean NIH calculated for the two flow systems.

Correlation Between Hemolysis and DRP Degradation

Linear regression of the PDI and the NIH at 120-minute time points for the CentriMag and Bio-Pump flow systems is shown in Figure 8. The coefficient of determination for this analysis is 0.991.

Discussion

Development of mechanical circulatory support devices requires extensive *in vitro* testing before conducting animal studies and the ultimate realization of clinical use. Due to the variations in testing conditions, different sensitivity to mechanical stress of RBCs from various species, and dependence of RBC mechanical fragility on storage time, the comparison of results of different blood pump tests is difficult. We proposed to use a solution of water-soluble DRP with rheological properties and shear stress sensitivity similar to that of blood as a substitute for human or animal blood in the *in vitro* assessment of potential mechanical blood damage in circulatory-assist devices.

Herein, we investigated whether the mechanical degradation of a DRP solution resulting in the loss of drag-reducing ability can indicate the degree of shear-induced blood damage within blood pumps. DRP mechanical degradation was determined empirically by recording the decrease in fluid flow rate at constant pressure gradient and in calculation of DR after 120 minutes of circulation in the two flow systems (Figures 4 and 5). In addition, PEO mechanical degradation was confirmed by reduction in PEO MW and solution viscosity (Figure 6). PEO solution viscosity, MW, and drag-reducing ability exhibited a larger decrease in the Bio-Pump system than in the CentriMag flow system, signifying that the Bio-Pump exerted greater mechanical stress on PEO molecules.

The calculated PDI, based on the change in PEO DR per passing volume, was statistically significantly higher in the Bio-Pump than the CentriMag flow system at 120 minutes of testing. Hemolysis testing revealed that mean plfHb concentration and NIH in the Bio-Pump flow system were also statistically significantly higher than corresponding values for the CentriMag flow system, thereby confirming a higher degree of mechanical stress in the Bio-Pump than the CentriMag.

With improvements in biocompatibility of novel circulatoryassist devices, *in vitro* hemolysis testing becomes more challenging due to the reduction in mechanical blood damage produced by these devices. Thus, hemolysis testing of these devices would require prolonged test times to detect blood damage with inevitable artifacts. Our results showed that the mechanical degradation of DRP can predict the potential mechanical hemolysis produced in blood pumps. The PDI may be used as a sensitive indicator of hemolysis produced in the pumps without the use of blood and with faster response to mechanical stress. Therefore, PEO solutions may provide simple standard test fluids for the *in vitro* evaluation of potential blood trauma produced in circulatory-assist devices.

Conclusion

DRP mechanical degradation in a flow system with an incorporated blood pump may provide useful information on the device performance predicting potential hemolysis produced by the pump without the use of blood. A simple, reliable method for characterization of DRP mechanical degradation can be realized by recording the decrease in DRP solution flow rate at a constant pressure and measurement of polymer solution viscosity in samples collected from the flow system during testing. A calculated polymer degradation index (PDI) can be regarded as an analog of the hemolysis index (NIH) traditionally used for evaluation and comparison of circulatory-assist devices. Further optimization of the proposed test by screening of various drag-reducing polymers is possible.

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Figure 1.
Viscosity of 30% hematocrit (Ht) porcine blood (squares) and 1,000 ppm polyethylene oxide (PEO) solution (triangles) measured at 25°C.

Figure 2.

Schematic of the flow system used for studies of drag-reducing polymer (DRP) mechanical degradation and shear-induced hemolysis in the centrifugal pumps studied. (A) flow probe, (B) glass tube (0.44 cm internal diameter, 91.5 cm length), (C) pressure transducer, (D) centrifugal pump (Bio-Pump BPX-80 or CentriMag), (E) open reservoir, and (F) water bath.

Figure 3. Flow-pressure characteristics of the flow system and rotational speed of the Bio-Pump and CentriMag centrifugal pumps. Bio-Pump (white triangles), Bio-Pump krpm (gray triangles), CentriMag (white squares), and CentriMag krpm (gray squares).

Figure 4. Changes in polyethylene oxide (PEO) solution flow rate in Bio-Pump (triangles) and CentriMag (squares) flow systems.

Figure 5. Changes in polyethylene oxide (PEO) solution drag reduction in Bio-Pump (triangles) and CentriMag (squares) flow systems.

Figure 6.
Changes in asymptotic viscosity of polyethylene oxide (PEO) solution in Bio-Pump (triangles) and CentriMag (squares) flow systems.

Figure 7. Changes in plasma-free hemoglobin concentration of 30% hematocrit (Ht) porcine blood in Bio-Pump (triangles) and CentriMag (squares) flow systems.

Figure 8. Linear regression of the drag-reducing polymer (DRP) degradation index and normalized index of hemolysis at 120 minutes of testing for the Bio-Pump (triangles) and CentriMag (squares) flow systems.