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Ex vivo assessment of erythrocyte tolerance to the HeartWare ventricular assist device operated in three discrete configurations

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Abstract

Despite technological advances in ventricular assist devices (VADs) to treat end-stage heart failure, hemocompatibility remains a constant concern, with supraphysiological shear stresses an unavoidable reality with clinical use. Given that impeller rotational speed is related to the instantaneous shear within the pump housing, it is plausible that modulation of pump speed may regulate peak mechanical shear stresses and thus ameliorate blood damage. The present study investigated the hemocompatibility of the HeartWare HVAD in three configurations typical of clinical applications: standard systemic support left VAD (LVAD), pediatric support LVAD, and pulmonary support right VAD (RVAD) conditions.

Two *ex vivo* mock circulation blood loops were constructed using explanted HVADs, in which pump speed and external loop resistance were manipulated to reflect the flow rates and differential pressures reported in configurations for standard adult LVAD (at 3150 rev·min⁻¹), pediatric LVAD (at 2400 rev·min⁻¹), and adult RVAD (at 1900 rev·min⁻¹). Using bovine blood, the mock circulation blood loops were tested at 37°C over a period of 6 hours (consistent with

ASTM F1841-97) and compared with a static control. Hemocompatibility assessments were conducted for each test condition, examining hematology, hemolysis (absolute and normalized index), osmotic fragility, and blood viscosity.

Regardless of configuration, continuous exposure of blood to the VAD over the 6-hour period significantly altered hematological and rheological blood parameters, and induced increased hemolysis when compared with a static control sample. Comparison of the three operational VAD configurations identified that the adult LVAD condition – associated with the highest pump speed, flow rate, and differential pressure across the pump – resulted in increased normalized hemolysis index (NIH; 0.07) when compared with the lower pump speed ‘off-label’ counterparts (NIH of 0.04 in pediatric LVAD and 0.01 in adult RVAD configurations).

After normalizing blood residence times between configurations, pump speed was identified as the primary determinant of accumulated blood damage; plausibly, blood damage could be limited by restricting pump speed to the minimum required to support matched cardiac output, but not beyond.

Keywords: blood damage, blood viscosity, pediatric support, pulmonary support, systemic support

Introduction

The use of left ventricular assist devices (LVADs) has become common practice over recent decades in the management of patients with advanced stage heart failure (1). Typically, patients are implanted short-term with an LVAD while awaiting transplantation of a donor heart; however, due to continued development and improved reliability, LVAD therapy is increasingly considered a feasible long-term strategy for patients that are ineligible for transplant (1, 2). While LVADs appear to have become a progressively successful therapeutic option, prolonged reliance on these systems has resulted in the emergence of seemingly diverse secondary complications; i.e., stroke, infection, and multi-organ failure are frequently observed, arising independent of patients’ initial pathology (3). The precise mechanisms and processes that govern these secondary complications are poorly understood; however, blood trauma related to blood-device interactions has been implicated where artificial environments (e.g., foreign materials, surfaces) (4) and non-physiological fluid properties (e.g., excessive shears) are dominant (for review, see 5).

While blood may tolerate physiological ranges of shear stress (i.e., 0-10 Pa) (6), computational modelling of clinically relevant VADs (specifically the HeartWare HVAD) operating under normal conditions has identified maximum scalar stress generated inside the pump exceeds 1000 Pa (7). This 100-fold shear increase above the upper limits of the normal physiological range is suspected to induce damage to the constituents of blood: excessive shear stress exposure can cause hemolytic destruction and sub-hemolytic trauma of red blood cells (RBC) (8-10), pathological activation of platelets and hyper-coagulopathy (11-13), von Willebrand factor degradation and hypo-coagulopathy (14, 15), and leukocyte destruction (16). This blood damage is likely associated with altered rheology, ischemic complications, angiodysplasia, and the vascular-related incidence of neurological complications (3, 17). Indeed, alterations in the physical properties of RBC caused by supraphysiological shear stress propagate hyperviscosity, which in turn has profound implications for vascular health (for a recent review, see 18). Further improvement of VAD clinical outcomes may be achieved by optimizing operational functioning for greater hemocompatibility and minimizing peak shear stress exposure of blood components.

Although current VAD operating parameters have been established to provide adequate blood flow, due to the compact design, versatility, and accessibility of VADs, devices such as the HeartWare HVAD are being used 'off-label'. The HVAD may be operated at lower pump speeds, for example, to accommodate the lower flow rate and pressure required for pediatric specific LVADs (19, 20) and pulmonary appropriate right ventricular specific VADs (21, 22). Pump rotational speeds within these alternative applications have been reported to be at least 25% lower than those within standard adult systemic support, and computational models predict a 50% decrease in the blood volume exposed to shear stresses above 50 Pa (23). While operating adult HVADs at these lower pump speeds has seen varying degrees of clinical success, the major adverse events that plague standard VAD use are still prevalent and remain unresolved clinical concerns (20, 21). Given that impeller rotational velocity is related to the peak instantaneous shear within the pump housing, it is plausible that decreasing pump speed may ameliorate acute blood trauma owing to reduced peak shear stresses, albeit at the expense of potentially increased flow stagnation and recirculation. Thus, using an *ex vivo* design (following the ASTM F1841-97 standard for assessing blood damage in continuous-flow pumps (24)), the present study aimed to investigate the hemocompatibility of three different pump configurations of the HeartWare HVAD

that are representative of off-label clinical practice (Adult LVAD, Pediatric LVAD, and Adult RVAD).

Method

Experimental design

Two explanted HVADs were obtained for the present study and integrated into two *ex vivo* mock circulation blood loops (MCLs; Fig. 1), which were used for hemocompatibility testing of three modified pumping configurations representative of adult LVAD, pediatric LVAD, and adult RVAD. Briefly, the MCLs were assembled from a combination of 3/8" and 1/2" Tygon tubing (Saint-Gobain, Paris, France), with a total length of 1200 mm, and connected to a custom-built blood reservoir and compliance chamber. The current study was conducted as an independent measures trial with concurrent assessments; that is, blood from each donor was used within discrete MCLs for two of the three pump configurations on a given test day. Blood from each donor was split and allocated to the conditions at random (total n=12; n=6 per pump configuration), with each half of a sample used only for a single testing procedure, while a volume aliquot was kept at rest to serve as a static control. The total blood volume in each MCL was 450 ± 50 mL. Throughout hemocompatibility testing and pump operation, the MCLs and static control sample were maintained at $37 \pm 1^\circ\text{C}$ in a water bath. Pump flow rate was recorded in each MCL using an ultrasonic flow meter (ME9PXL1153, Transonic Systems Inc., NY, USA), and pressure differential across the pump (ΔP : pressure out – pressure in) was determined as the difference between the inlet and outlet pump pressure, monitored with piezoresistive pressure transducers (PX181B-015C5V, Omega Engineering, CT, USA). Flow rates and pressures within each MCL rig were manipulated with external resistive clamps and maintained within operating parameters throughout each test. Between each test, the MCLs were extensively washed and cleaned to remove residual cellular components. The MCL cleaning procedure required: rinsing with saline, washing with an enzymatic cleaner (0.6% Medizyme solution, Whiteley Corporation, Sydney Australia) for 60 min, flushing with deionized water for four cycles, and finally running with isotonic saline solution for at least 20 min prior to the next test.

FIGURE 1 ABOUT HERE

Blood loop hemodynamics

Due to lower cardiac output requirements within the pediatric circulation, and lower pressure requirements of the pulmonary circulation, clinical ‘off-label use’ of the HeartWare HVAD requires pump speeds to be maintained within suitable flow ranges and pressures. To simulate the clinical VAD use for pediatric and pulmonary support, pump speed and loop resistance (controlled with a pressure restriction clamp) within the MCL were manipulated to reflect average reported values for: i) pediatric LVAD (19, 20)); ii) adult RVAD (21); and iii. standard systemic LVAD support (25). An overview of pump parameters is presented in Table 1.

TABLE 2 ABOUT HERE

Blood sample collection and processing

Bovine blood samples were sourced from live cows (Serum Australis Pty Ltd, New South Wales, Australia) via standard venipuncture techniques, collected into a 450 mL blood bag containing anticoagulant (14% citrate phosphate dextrose adenine; SSS Australia Health Supplies, NSW, Australia). Subsequently, a small dose of antibiotics (50 mg L⁻¹ gentamycin; Sigma-Aldrich, St. Louis, USA) was added to each blood bag to prevent bacterial contamination. Prior to infusion of blood into each MCL, blood samples were filtered through a 40 µm transfusion filter (SQ40, Haemonetics, Braintree, USA) to prevent influx of potential contaminants (e.g., animal fur, skin particles).

MCL blood sampling

Throughout operation of the MCLs, blood was collected from each test rig using sterile syringes through a sampling port embedded in the custom-built reservoir. The first 1 mL of each blood sample extracted from the loop was discarded, and immediately following a fresh syringe was used to aspirate 5 mL of blood from each loop to be processed for subsequent analyses. Blood samples were taken at the following time intervals after commencement of pump operation: 5 min, 30 min, 90 min, 120 min, and 360 min. Hematological assessments were performed on samples obtained directly from the MCL. Samples were then centrifuged at 1500 × g for 10 min to separate the plasma, some of which was used for hemolysis quantification. The hematocrit of the remaining sample was adjusted to 0.3 L L⁻¹ by removing excess plasma for all subsequent measurements.

Hematology

Hematological assessments were performed using the native blood samples obtained directly from the MCL. An automated analyzer, employing the coulter cell counting principle (DxH 600, Beckman Coulter Inc., Brea, USA), was used to carry out hematological measurements.

Hemolysis and N.I.H.

Given hemoglobin is typically located inside RBC, complete RBC destruction (i.e., hemolysis) is marked by increased presence of hemoglobin in the surrounding medium (i.e., plasma). In the present study, the concentration of hemoglobin in plasma was quantified using the Harboe spectrophotometric method (26). Briefly, plasma was isolated from blood samples by centrifugation at $1500 \times g$ for 10 min. The supernatant was then diluted 1:6 in a 0.01% Na_2CO_3 solution. Resultant solutions were then mixed and loaded into a microtiter plate in duplicates. Light absorbance (*Abs*) was measured for each plate at three discrete wavelengths ($\lambda = 380; 415; 450 \text{ nm}$) using an automated reader (FLUOstar Omega, BMG Labtech, Mornington, Australia). The following equation was used to determine the concentration of hemoglobin (Hb; $\text{mg}\cdot\text{dL}^{-1}$):

$$[\text{Hb}] (\text{mg}\cdot\text{dL}^{-1}) = \left(\frac{167.2 (Abs_{415 \text{ nm}}) - 83.6 (Abs_{380 \text{ nm}}) - 83.6 (Abs_{450 \text{ nm}})}{10} \right) \times \frac{1}{\text{Na}_2\text{CO}_3 \text{ dilution factor}} \quad (\text{Eq. I})$$

Using the determined hemoglobin concentration (Eq. I), the normalized index of hemolysis (N.I.H.) was also calculated using Equation II (27), where Hct is the hematocrit, Q is the measured flow rate in the MCL, and t is the sampling timepoint:

$$N.I.H. (g \cdot 100 L^{-1}) = \Delta[\text{Hb}] \times \text{loop volume} \times \frac{100 - \text{Hct}}{100} \times \frac{100}{Q \times t} \quad (\text{Eq. II})$$

Whole blood viscosity

Following standardization of hematocrit to $0.3 \text{ L}\cdot\text{L}^{-1}$ for all samples, a rotational cone-and-plate viscometer (DV-II+Pro, Brookfield Engineering Labs, Middleboro, MA) equipped with a CPE-40 spindle, operating at 37°C , was used to determine blood viscosity at discrete shear rates between 75 and 1500 s^{-1} .

RBC osmotic fragility

Osmotic fragility of isolated packed RBC was assessed using a modified version of the assay established by Parpart *et al.* (28). Briefly, isolated RBC were resuspended at a low hematocrit ($0.02 \text{ L} \cdot \text{L}^{-1}$) into eleven solutions containing increasing concentrations of NaCl (0-0.9% w/v). RBC-NaCl suspensions were incubated for 30 min at 37°C . Thereafter, RBC-NaCl suspensions were centrifuged at $1500 \times g$ for 10 min to precipitate the cell pellet. The concentration of free hemoglobin in the supernatant was determined using the Harboe spectrophotometric method as described above.

Data processing and statistical analysis

Comparison of baseline blood parameters was performed using one-way analysis of variance (ANOVA) (IBM SPSS Statistics for Windows, version 26, IBM Corp., Armonk, New York, USA), with post-hoc comparisons adjusted using the Bonferroni correction, where appropriate. It is important to note that the results of the current study were limited for statistical power due to the inter-donor variability in baseline blood parameters observed in commercially obtained bovine blood samples (Table 1). It appears, for example, that some of the donors experienced pronounced immunological responses at the time of sample retrieval, despite being confirmed by the veterinary phlebotomist as being in “good health”, resulting in large variance of white cell counts. Given that only two of the three pump configurations could be compared during a given test, these baseline differences introduced significant variability in hematological blood parameters (e.g. a donor with extremely abnormal values was only represented in two out of the three testing conditions). Thus, to adequately assess the effect of blood exposure to each HeartWare HVAD configuration, we normalized the change in hematological blood parameters over time relative to the first sample obtained from the MCLs (i.e., 5 min).

Raw osmotic fragility data, displaying free hemoglobin in response to incubation with media of decreasing tonicity compared with free hemoglobin in isotonic medium, were plotted against the concentration of NaCl in the respective solution, resulting in a sigmoidal curve. The point representing 50% hemolysis (EC_{50}) was identified through non-linear curve-fitting of the following equation:

$$Y = Bottom + \frac{(Top - Bottom)}{1 + \left(\frac{EC_{50}}{X}\right)^{Hill\ slope}} \quad (Eq. III)$$

Top and bottom refer to the respective Y-unit plateaus, while the Hill slope reflects the shared slope of the curves. Alterations of hematological parameters and osmotic fragility (EC_{50}) were expressed relative to baseline of each sample, and comparisons between the three pump configurations and the static control were performed using a two-way ANOVA with repeated measures. Viscosity, raw osmotic fragility, and free hemoglobin data were also compared *via* repeated measures two-way ANOVA. Normalized index of hemolysis data was compared using one-way ANOVA. The F-ratio of each ANOVA, calculated as the ratio of the mean square values, is provided in-text to indicate effect size. Post-hoc comparisons were carried out using the Bonferroni correction throughout. An alpha level of 0.05 was applied to determine statistically significant differences; a commercial software package (Prism, GraphPad Software Inc., Release 8.1.1, La Jolla, CA, USA) was utilized to perform these analyses.

Results

Baseline blood parameters

Comparison of hematological parameters of the samples used for all three pump conditions revealed no statistical differences at baseline ($p > 0.05$; Table 2). Leukocyte count was significantly increased in all conditions (mean $\pm$ s.d.: $44.7 \pm 55.0 \times 10^3 \mu L^{-1}$) when compared with normal ranges reported for bovine blood samples ($5.9\text{--}13.3 \times 10^3 \mu L^{-1}$ (29)). Moreover, red cell distribution width was elevated (grouped mean $\pm$ s.d.: $25.9 \pm 3.1\%$) compared to normal ranges (15.5–20.0%).

TABLE 1 ABOUT HERE

Exposure to pump induced significant alterations of blood parameters over time

Leukocyte count significantly decreased by ~35–55% in the three investigated pump speed configurations when compared with static control ($F = 15.06$, $p < 0.001$; Fig. 2A). A near-linear decrease of leukocytes was observed throughout the 360 min of pump exposure in the adult and pediatric LVAD configurations, respectively. In the adult RVAD configuration, however, a more pronounced decrease in leukocyte count was observed between 90 and 120 min on-pump.

Mean corpuscular volume (MCV) was observed to significantly increase over time on-pump when compared with the static control sample ($F=21.46$, $p<0.001$; Fig. 2B), wherein the adult LVAD configuration precipitated the earliest and most pronounced alterations in MCV, and the adult RVAD pump-speed condition exhibited the least pronounced increase over 360 min of exposure (~1%).

Mean corpuscular hemoglobin (MCH) mass did not statistically significantly change following 6 h of pump-exposure (Fig. 2C), which is likely due to variability observed in the present study. Moreover, mean corpuscular hemoglobin concentration (MCHC; a parameter indicating the cellular hemoglobin concentration normalized for cell volume) significantly increased over the 6-h time period on-pump for the adult RVAD configuration ($F=3.07$, $p<0.05$; Fig. 2D) in comparison to static control. Operation of the pump in adult LVAD configuration resulted in a significant decrease in MCHC when compared with adult RVAD pump speed ($p<0.05$).

The standard deviation of red cell distribution width (RDW-SD; an index describing the heterogeneity of the cell population) significantly increased with time on-pump in all pump configurations ($p<0.001$; Fig. 2E), while RDW-SD of the static control decreased by ~2%. The increase on-pump, however, was most pronounced in the pediatric LVAD configuration (~7%), compared with the adult LVAD (~4%; $p<0.05$) and adult RVAD (~5%) configurations.

Collectively, significant alterations in hematological parameters of RBC were observed on-pump when compared with the static control RBC, generally irrespective of the pump speed.

FIGURE 2 ABOUT HERE

Hemolysis is most pronounced when the pump is operated in adult LVAD configuration

Hemolysis significantly increased in all pump configurations when compared with the static control sample after 6 h of continuous exposure (Fig. 3A). The most pronounced increase in free-hemoglobin concentration was observed when the pump was operated in the adult LVAD configuration, which exhibited a non-linear increase from $5.3 \text{ mg} \cdot \text{dL}^{-1}$ at 120 min to $46.6 \text{ mg} \cdot \text{dL}^{-1}$ at 360 min. When operated in pediatric LVAD or adult RVAD configuration, however, free-hemoglobin concentration remained unaltered in comparison to the static control for 300 min of exposure. Upon 360 min of exposure, free-hemoglobin concentration significantly increased to

12.8 mg dL⁻¹ (p<0.05) and 6.6 mg dL⁻¹ (p<0.01) in pediatric LVAD and adult RVAD configurations, respectively.

Hemolysis expressed as N.I.H., which normalizes measured hemolysis for loop volume, pump exposure, and flow rate (Eq. II), was significantly different among configurations. Specifically, N.I.H. was significantly increased (Fig. 3B) when the pump was operated in the pediatric LVAD (0.004 g 100 L⁻¹; p<0.05) or adult LVAD (0.007 g 100 L⁻¹; p<0.05) configurations, compared with the adult RVAD configuration (0.001 g 100 L⁻¹; p<0.05).

FIGURE 3 ABOUT HERE

Osmotic fragility of RBC decreased over time on-pump

RBC exposed to the adult RVAD pump speed exhibited significantly decreased hemolysis in response to hypoosmotic solutions containing 0.45% (p<0.05) and 0.5% NaCl (p<0.01), respectively (Fig. 4A). While RBC from the static control sample exhibited respective hemolytic rates of 80.7% and 43.4%, RBC exposed to the adult RVAD pump configuration showed hemolytic rates of 68.5% and 28.4% at each of these NaCl-concentrations. Samples exposed to the pediatric and adult LVAD configurations also presented with decreased osmotic fragility, albeit less pronounced and not statistically significant.

EC₅₀ significantly decreased with 6 h of exposure to the pump operated in the RVAD configuration when compared to the static control and other pump configurations (p<0.05; Fig. 4B).

FIGURE 4 ABOUT HERE

Blood viscosity is elevated in samples exposed to adult LVAD pump configuration

Blood viscosity quantified at five discrete shear rates (75 to 1500 s⁻¹) after 360 min of pump-exposure is depicted in Figure 5. Viscosity was significantly increased when the pump was operated in the adult LVAD configuration compared to the static control ($F=3.06$, p<0.05). Increased blood viscosity was also observed in samples exposed to adult RVAD and pediatric LVAD configurations when compared with the static control; however, the effect was less pronounced, thus statistical significance was not reached given the limited sample size and considerable variance.

FIGURE 5 ABOUT HERE

Discussion

The salient findings of the present study emphasize that while VADs and artificial circulatory support devices are invaluable tools for extending patient lives, the hemocompatibility of these devices remains an ongoing issue that may be responsible for precipitating the plethora of secondary complications associated with VAD use. Continuous exposure of fresh blood samples to the HeartWare HVAD for six hours altered hematological (Fig. 2) and rheological (Fig. 4) blood parameters, while also inducing hemolysis (Fig. 3) compared with the static control blood sample. Comparison of the three operational configurations investigated in the current study (i.e., adult LVAD, pediatric LVAD, and adult RVAD) yielded the observation that the adult LVAD configuration – which was associated with the highest flow rate, pump pressure, and pump speed – resulted in more significant alterations of blood parameters and hemolysis, especially when compared with the ‘off-label’ adult RVAD configuration, which was associated with a similarly high flow rate, but significantly lower pump pressure and speed. Collectively, the results of the present study indicate that while blood exposure to any VAD configuration generally impaired functional properties of RBC, distinct differences were observed when the VAD was operated in clinically relevant ‘off-label’ configurations with lower operational pump speed.

Exposure to the VAD operated in any configuration resulted in highly pronounced loss of leukocytes (~45-60%), while the static control exhibited no alteration in leukocyte count. Leukocytes are thought to be more susceptible to mechanical shear stresses than RBC (30), and have been reported to decline and fragment following prolonged exposure to *in vitro* testing with rotary blood pumps (31, 32). The present study confirms these prior reports, showing a linear decrease in leukocyte count after 30 min of exposure (Fig. 2A) prior to the occurrence of any significant hemolysis, which occurred after 180-240 min (Fig. 3). This acute loss of leukocytes may have an impact on immediate post-surgical recovery of patients, compounding the already elevated risk of infection during invasive procedures (3), albeit the *in vitro* model fails to reflect the increased WBC activation and migration observed in patients in the days post-surgery (33).

Hematological parameters reflecting RBC volume and hemoglobin content (i.e., MCV, MCH, MCHC) exhibited varied sensitivity to the different pump configurations employed in the current study (Fig. 2B, 2C, 2D). Specifically, MCV significantly increased over the course of the 6-h test in all pump configurations, albeit to a lesser extent in the adult RVAD configuration. In

congruence with the present study, Yokoyama *et al.* (34) deployed an *in vivo* bovine model equipped with an extracorporeal centrifugal blood pump, and reported increased MCV and decreased MCH occurring with concurrent hemolytic events. Previous *ex vivo* blood loop experiments with rotary blood pumps (35) have identified that sub-populations of RBC have increased sensitivity to mechanical shear; those that are more dense with lower MCV and higher MCH (i.e., presumably older RBC) are likely preferentially lysed, thus shifting the composition of the remaining population towards presumably younger cells that are more shear-tolerant and have larger cytosolic volume (36, 37).

Red cell distribution width (RDW) significantly increased in all conditions with time on-pump (Fig. 2E), which occurred in the absence of significant alterations in hemolysis (Fig. 3A), indicating that the RBC population becomes increasingly heterogeneous with accumulated shear history. Given the level of hemolysis in the adult RVAD configuration was lower when compared with the high pump speed conditions, it appears that blood-exposure with low pump-speed ameliorated hemolysis, yet still induced pre-hemolytic alterations of the native RBC population, and propagated the development of a more heterogeneous (and likely fragile) cell population. Shear-induced sublethal functional impairments of vital RBC properties have been increasingly defined in the past decade (6, 38), and could provide a more sensitive measure of blood trauma. Alterations of RBC sub-populations in response to high mechanical stress may also be explored as an additional sensitive pre-hemolytic biomarker of blood trauma. The observation of decreased hemolysis with decreased pump speed being an ‘improvement’ requires careful interpretation; that is, although hemolysis may be ameliorated, decreasing pump speed may increase the risk of adverse events associated with low flow, low pressure, and recirculation, including thrombotic events and insufficient opening of the aortic valve (23).

Osmotic fragility was decreased in blood samples exposed to the pump when compared with the static control (Fig. 4A), indicating that RBC had a higher capacity to tolerate hypoosmotic environments. Significant alterations did not occur prior to 360 min of pump-exposure, which coincided with major hemolysis. This observation was unexpected, given the increase in MCV (Fig. 2B) would decrease the average surface-area-to-volume ratio, and thus would be expected to decrease the capacity of RBC to take up water without undergoing lysis (39). It appears that prior exposure of RBC to mechanical shear stress may modulate the osmotic fragility of RBC independent of MCV (40). Given VAD pump speed governs, in part, shear stress within the device, the present observation that exposure to the lowest pump speed configuration

(i.e., adult RVAD) produced the least osmotically fragile RBC supports a positive relation between shear history and osmotic sensitivity. Further investigations are required to define the underlying mechanisms governing this observation; however, we speculate that a common mechanism of shear-induced damage – potentially altering membrane permeability to ions that mediate volume homeostasis (41, 42) – occurred in RBC exposed to the pump in the adult RVAD configuration.

Blood viscosity, standardized to a hematocrit of $0.3 \text{ L}\cdot\text{L}^{-1}$, was increased in response to pump-exposure when compared with the static control; this effect was most pronounced in the adult LVAD configuration. Several *in vitro* and *in vivo* studies have shown hyperviscosity of blood following exposure to excessive mechanical stress; elevated blood viscosity has major implications for blood flow, oxygen transport to working tissues, and endothelial function (43-46). It appears that the magnitude of hyperviscosity was dependent on the VAD pump speed, while insensitive to flow rate and differential pressure. When standardizing for hematocrit, the intrinsic physical properties of RBC – i.e., cellular deformability and their tendency to form reversible multi-cell aggregates – govern blood fluidity. Previous experimentation has established that exposure to supraphysiological shear environments may cause both increased RBC aggregation and decreased RBC deformability in human blood samples (47, 48). Normal bovine RBC do not readily form aggregates; however, have been shown to aggregate following exposure to a centrifugal blood pump *in vivo* (48). Further exploration of underlying RBC alterations following exposure to high mechanical shear stresses could reveal sublethal specific alterations, and potentially identify new markers sensitive to detecting pre-hemolytic blood trauma.

Collectively, the results of the present study demonstrate shear-induced alterations of RBC properties which occur prior to hemolysis. Moreover, our data indicate that pump speed is a critical determinant of blood damage, while flow rate and differential pump pressure may play a minor role, which could indicate that shear stress is the dominant force in inducing mechanical blood trauma to RBC, at least in *ex vivo* systems lacking biological buffering and filtering processes. Operation of mechanical circulatory support devices at lower pump speeds, however, may increase the risk of thrombus-formation, given the associated decrease in washout, recirculation, and flow rates (for review, see 49).

Conclusion

The requirement for improving hemocompatibility of future generation mechanical circulatory support devices is evident by the significant prevalence of secondary complications associated with clinical VAD use. The salient findings of the current study provide insight into the shear-related effects of VADs on the physical properties of blood, where operational VAD pump speed appears to be a predominant factor contributing to accumulated blood trauma. While the inherent inter-donor variability observed in the commercially obtained bovine blood samples limited the detection of statistical significance in some experimental measures herein, distinct differences between blood samples exposed to the VAD (in any configuration) *versus* the static control sample were observed. Therefore, decreasing pump speed where possible may prevent excessive blood damage, potentially promoting improved circulatory health in those requiring mechanical circulatory support.

References

1. Rogers JG, Pagani FD, Tatroles AJ, Bhat G, Slaughter MS, Birks EJ, et al. Intrapericardial left ventricular assist device for advanced heart failure. *New England Journal of Medicine*. 2017;376(5):451-60.
2. Teuteberg JJ, Cleveland Jr JC, Cowger J, Higgins RS, Goldstein DJ, Keebler M, et al. The society of thoracic surgeons intermacs 2019 annual report: the changing landscape of devices and Indications. *The Annals of thoracic surgery*. 2020;109(3):649-60.
3. Kirklin JK, Pagani FD, Kormos RL, Stevenson LW, Blume ED, Myers SL, et al. Eighth annual INTERMACS report: special focus on framing the impact of adverse events. *The Journal of Heart and Lung Transplantation*. 2017;36(10):1080-6.
4. Jung F, Wischke C, Lendlein A. Degradable, multifunctional cardiovascular implants: challenges and hurdles. *MRS bulletin*. 2010;35(8):607-13.
5. Faghih MM, Sharp MK. Modeling and prediction of flow-induced hemolysis: a review. *Biomechanics and modeling in mechanobiology*. 2019:1-37.
6. Baskurt OK, Meiselman HJ. Red blood cell mechanical stability test. *Clinical Hemorheology and Microcirculation*. 2013;55(1):55-62.
7. Chen Z, Jena SK, Giridharan GA, Sobieski MA, Koenig SC, Slaughter MS, et al. Shear stress and blood trauma under constant and pulse-modulated speed CF-VAD operations: CFD analysis of the HVAD. *Medical & biological engineering & computing*. 2019;57(4):807-18.
8. Suter SP. Flow-induced trauma to blood cells. *Circulation Research*. 1977;41(1):2-8.
9. Simmonds MJ, Meiselman HJ. Prediction of the level and duration of shear stress exposure that induces subhemolytic damage to erythrocytes. *Biorheology*. 2016;53(5-6):237-49.
10. McNamee AP, Tansley GD, Simmonds MJ. Sublethal mechanical shear stress increases the elastic shear modulus of red blood cells but does not change capillary transit velocity. *Microcirculation*. 2020:e12652.
11. Chan CHH, Inoue M, Ki KK, Murashige T, Fraser JF, Simmonds MJ, et al. Shear-Dependent Platelet Aggregation Size. *Artificial Organs*. 2020.
12. Bakir I, Hoylaerts MF, Kink T, Foubert L, Luyten P, Van Kerckhoven S, et al. Mechanical stress activates platelets at a subhemolysis level: an in vitro study. *Artificial Organs*. 2007;31(4):316-23.

13. Wurzinger L, Opitz R, Wolf M, Schmid-Schönbein H. Ultrastructural investigations on the question of mechanical activation of blood platelets. *Blut*. 1987;54(2):97-107.
14. Horobin JT, Simmonds MJ, Nandakumar D, Gregory SD, Tansley G, Pauls JP, et al. Speed Modulation of the HeartWare HVAD to Assess In Vitro Hemocompatibility of Pulsatile and Continuous Flow Regimes in a Rotary Blood Pump. *Artificial Organs*. 2018.
15. Chan CHH, Pieper IL, Fleming S, Friedmann Y, Foster G, Hawkins K, et al. The Effect of Shear Stress on the Size, Structure, and Function of Human von Willebrand Factor. *Artificial organs*. 2014;38(9):741-50.
16. Carter J, Hristova K, Harasaki H, Smith W. Short exposure time sensitivity of white cells to shear stress. *ASAIO Journal*. 2003;49(6):687-91.
17. Papp J, Toth A, Sandor B, Kiss R, Rabai M, Kenyeres P, et al. The influence of on-pump and off-pump coronary artery bypass grafting on hemorheological parameters. *Clinical Hemorheology and Microcirculation*. 2011;49(1-4):331-46.
18. Richardson KJ, Kuck L, Simmonds MJ. Beyond oxygen transport: Active role of erythrocytes in the regulation of blood flow. *American Journal of Physiology-Heart and Circulatory Physiology*. 2020.
19. D'Alessandro D, Forest SJ, Lamour J, Hsu D, Weinstein S, Goldstein D. First reported use of the heartware HVAD in the US as bridge to transplant in an adolescent. *Pediatric transplantation*. 2012;16(8):E356-E9.
20. Adachi I, Burki S, Zafar F, Morales DLS. Pediatric ventricular assist devices. *Journal of Thoracic Disease*. 2015;7(12):2194.
21. Karimov JH, Sunagawa G, Horvath D, Fukamachi K, Starling RC, Moazami N. Limitations to chronic right ventricular assist device support. *The Annals of thoracic surgery*. 2016;102(2):651-8.
22. Enciso JS, Tran HA, Brambatti M, Braun OÖ, Pretorius V, Adler ED. Management of RVAD thrombosis in biventricular HVAD supported patients: case series. *ASAIO Journal*. 2019;65(4):e36-e41.
23. Granegger M, Thamsen B, Schlöglhofer T, Lach S, Escher A, Haas T, et al. Blood trauma potential of the HeartWare Ventricular Assist Device in pediatric patients. *The Journal of Thoracic and Cardiovascular Surgery*. 2020;159(4):1519-27. e1.
24. ASTM. Standard practice for assessment of hemolysis in continuous flow blood pumps. *Annual Book of ASTM Standards, F1844-97*. 2017;13:1-5.
25. Chorprenning K, Brown MC, Voskoboynikov N, Reyes C, Dierlam AE, Tamez D. HeartWare controller logs a diagnostic tool and clinical management aid for the HVAD pump. *Asaio Journal*. 2014;60(1):115-8.

26. Harboe M. A method for determination of hemoglobin in plasma by near-ultraviolet spectrophotometry. *Scandinavian Journal of Clinical and Laboratory Investigation*. 1959;11:66-70.
27. Naito K, Mizuguchi K, Nosé Y. The need for standardizing the index of hemolysis. *Artificial Organs*. 1994;18(1):7-10.
28. Parpart AK, Lorenz PB, Parpart ER, Gregg JR, Chase AM. The Osmotic Resistance (Fragility) of Human Red Cells. *Journal of Clinical Investigation*. 1947;26(4):636-40.
29. Roland L, Drillich M, Iwersen M. Hematology as a diagnostic tool in bovine medicine. *J Vet Diagn Invest*. 2014;26(5):592-8.
30. Takami Y, Yamane S, Makinouchi K, Glueck J, Nosé Y. Mechanical white blood cell damage in rotary blood pumps. *Artificial organs*. 1997;21(2):138-42.
31. Chan CH, Hilton A, Foster G, Hawkins KM, Badiei N, Thornton CA. The evaluation of leukocytes in response to the in vitro testing of ventricular assist devices. *Artificial organs*. 2013;37(9):793-801.
32. Kusserow BK, Larrow R. Studies of leukocyte response to prolonged blood pumping-effects upon phagocytic capability and total white cell count. *ASAIO Journal*. 1968;14(1):261-3.
33. Dieleman S, Jacob K, Nathoe H, Ten Berg M, Van Osch D, Frencken J, et al. White blood cell count and new-onset atrial fibrillation after cardiac surgery. *Critical Care*. 2014;18(1):1-182.
34. Yokoyama N, Sakota D, Nagaoka E, Takatani S. Alterations in red blood cell volume and hemoglobin concentration, viscoelastic properties, and mechanical fragility caused by continuous flow pumping in calves. *Artificial Organs*. 2011;35(8):791-9.
35. Sakota D, Sakamoto R, Sobajima H, Yokoyama N, Waguri S, Ohuchi K, et al. Mechanical damage of red blood cells by rotary blood pumps: selective destruction of aged red blood cells and subhemolytic trauma. *Artificial Organs*. 2008;32(10):785-91.
36. McNamee AP, Tansley GD, Sabapathy S, Simmonds MJ. Biphasic impairment of erythrocyte deformability in response to repeated, short duration exposures of supraphysiological, subhaemolytic shear stress. *Biorheology*. 2016;53(3-4):137-49.
37. McNamee AP, Richardson K, Horobin J, Kuck L, Simmonds MJ. Susceptibility of density-fractionated erythrocytes to subhaemolytic mechanical shear stress. *The International Journal of Artificial Organs*. 2019;42(3):151-7.
38. Simmonds MJ, Atac N, Baskurt OK, Meiselman HJ, Yalcin O. Erythrocyte deformability responses to intermittent and continuous subhemolytic shear stress. *Biorheology*. 2014;51(2-3):171-85.
39. Van Der Vegt S, Ruben AT, Werre J, De Gier J, Staal G. Membrane characteristics and osmotic fragility of red cells, fractionated with anglehead centrifugation and counterflow centrifugation. *British journal of haematology*. 1985;61(3):405-13.

40. Orbach A, Zelig O, Yedgar S, Barshtein G. Biophysical and biochemical markers of red blood cell fragility. *Transfusion Medicine and Hemotherapy*. 2017;44(3):183-7.
41. Kuck L, Peart JN, Simmonds MJ. Calcium dynamically alters erythrocyte mechanical response to shear. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*. 2020:118802.
42. Johnson RM, Gannon SA. Erythrocyte cation permeability induced by mechanical stress: a model for sickle cell cation loss. *American Journal of Physiology-Cell Physiology*. 1990;259(5):C746-C51.
43. Baskurt OK, Meiselman HJ, editors. Blood rheology and hemodynamics. *Seminars in thrombosis and hemostasis*; 2003: New York: Stratton Intercontinental Medical Book Corporation, c1974-.
44. Gnasso A, Cacia M, Cutruzzolà A, Minieri M, Carallo C, Cortese C, et al. Influence of acute reduction of blood viscosity on endothelial function. *Clinical hemorheology and microcirculation*. 2019;72(3):239-45.
45. Forconi S, Gori T. The evolution of the meaning of blood hyperviscosity in cardiovascular physiopathology: Should we reinterpret Poiseuille? *Clinical hemorheology and microcirculation*. 2009;42(1):1-6.
46. De Simone G, Devereux RB, Chien S, Alderman MH, Atlas SA, Laragh JH. Relation of blood viscosity to demographic and physiologic variables and to cardiovascular risk factors in apparently normal adults. *Circulation*. 1990;81(1):107-17.
47. McNamee AP, Tansley GD, Simmonds MJ. Sublethal mechanical trauma alters the electrochemical properties and increases aggregation of erythrocytes. *Microvasc Res*. 2018;120:1-7.
48. Kameneva MV, Antaki JF, Butler KC, Watach MJ, Kormos RL, Griffith BP, et al. A sheep model for the study of hemorheology with assisted circulation. Effect of an axial flow blood pump. *ASAIO J*. 1994;40(4):959-63.
49. Blitz A. Pump thrombosis—a riddle wrapped in a mystery inside an enigma. *Annals of cardiothoracic surgery*. 2014;3(5):450.

Tables

Table 1. Simulated pumping configurations of the HeartWare HVAD and corresponding mock circulation loop hemodynamics.

Simulated pump condition	Flow rate (Q; L·min ⁻¹)	Differential pump pressure (ΔP; mmHg)	Pump speed (rev·min ⁻¹)
Adult LVAD	5.0 ± 0.02	100.4 ± 0.2	3166 ± 10
Pediatric LVAD	2.5 ± 0.01	68.7 ± 0.3	2408 ± 8
Adult RVAD	4.5 ± 0.06	19.6 ± 0.4	1900 ± 34

Data presented as mean ± standard error. LVAD = left ventricular assist device. RVAD = right ventricular assist device.

Table 2. Baseline hematology data ($t = 5 \text{ min}$) for each experimental mock circulation loop condition.

Parameter	Unit	Adult LVAD	Pediatric LVAD	Adult RVAD	F	p -value
Red blood cell count	10 ⁶ □ μL ⁻¹	5.2 ± 1.2	4.8 ± 1.0	5.5 ± 1.0	0.610	0.556
White blood cell count	10 ³ □ μL ⁻¹	35.7 ± 30.0	44.4 ± 68.5	54.0 ± 66.9	0.149	0.863
Hematocrit	L □ L ⁻¹	0.25 ± 0.0	0.30 ± 0.0	0.27 ± 0.0	0.443	0.650
Mean corpuscular volume (MCV)	fL	49.3 ± 6.9	54.3 ± 6.7	49.6 ± 7.4	0.982	0.398
Mean corpuscular hemoglobin (MCH)	pg	16.4 ± 3.4	17.6 ± 3.5	15.4 ± 2.6	0.704	0.510
Mean corpuscular hemoglobin concentration (MCHC)	g □ dL ⁻¹	33.1 ± 2.3	32.2 ± 3.8	31.1 ± 2.7	0.722	0.502
Red blood cell distribution width (RDW)	%	26.8 ± 3.3	25.1 ± 2.9	25.7 ± 3.3	0.438	0.653
Red blood cell distribution width – standard deviation	fL	45.7 ± 4.7	47.4 ± 2.4	44.4 ± 4.2	0.583	0.571

(RDW-SD)

Platelet count

 $10^3 \mu\text{L}^{-1}$ 356.7 ± 273.9 249.2 ± 203.4 334.8 ± 252.2

0.278

0.762

Data is presented as mean $\pm$ standard deviation. LVAD = left ventricular assist device. RVAD = right ventricular assist device.

Figure legends

Figure 1. Experimental setup of the mock circulation blood loops using the HeartWare HVAD ventricular assist device to simultaneously simulate adult left ventricular support, pediatric left ventricular support, and/or adult right ventricular support.

Figure 2. Hematological parameters exposed to the HeartWare HVAD operated in different configurations (adult LVAD, pediatric LVAD, adult RVAD) are presented across 360 min of exposure time, and compared with a static control. Significant alterations in most parameters were observed with pump exposure relative to baseline. **** $p < 0.0001$, *** $p < 0.001$, * $p < 0.05$: significant effect of pump exposure. Significant differences detected in post-hoc comparisons of ^aadult RVAD and adult LVAD; ^badult RVAD and pediatric LVAD; ^cpediatric LVAD and adult LVAD. *WBC*: leukocyte count; *MCV*: mean corpuscular volume; *MCH*: mean corpuscular hemoglobin; *MCHC*: mean corpuscular hemoglobin concentration; *RDW-SD*: standard deviation of red cell distribution width.

Figure 3. Hemolysis is presented as free hemoglobin concentration (A) of samples exposed to the three different configurations of the HeartWare HVAD and as normalized hemolysis index (N.I.H.; B). Hemolysis was observed in all samples exposed to each pump configuration, wherein exposure to the adult LVAD configuration precipitated most pronounced increases in free hemoglobin concentration and N.I.H. ** $p < 0.01$, * $p < 0.05$: significantly different to the static control (in panel A) or the adult RVAD condition (in panel B).

Figure 4. Hemolytic rates of red blood cell (RBC) samples in decreasingly hypoosmotic solutions after 360 min of exposure to the different pump speeds decreased compared with the static control (A). Osmotic fragility of RBC exposed decreased following 360 min of exposure to the HeartWare HVAD (B) operated in adult RVAD configuration. ** $p < 0.01$, * $p < 0.05$: significantly different to static control.

Figure 5. Whole blood viscosity of samples exposed to the HeartWare HVAD operated in three different configurations (i.e., adult LVAD, pediatric LVAD, adult RVAD) for 360 min. Blood sample hematocrit

was standardized to 0.3 L L⁻¹ and viscosity was quantified across an increasing shear rate regime. *p<0.05: significantly different to static control.

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