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Determination of Reynolds Shear Stress Level for Hemolysis

Choon-Sik Jhun¹, Megan A. Stauffer¹, John D. Reibson¹, Eric E. Yeager¹, Raymond K. Newswanger¹, Joshua O. Taylor², Keefe B. Manning², William J. Weiss^{1,2}, and Gerson Rosenberg^{1,2}

¹Department of Surgery, The Pennsylvania State University College of Medicine, Hershey, PA

²Department of Bioengineering, The Pennsylvania State University, College of Engineering, University Park, PA

Abstract

Reynolds shear stress (RSS) has served as a metric for the effect of turbulence on hemolysis. Forstrom (1969) and Sallam (1984) determined the RSS threshold for hemolysis to be 50,000 dyne/cm² and 4,000 dyne/cm², respectively, using a turbulent jet. Despite the order of magnitude discrepancy, Sallam's threshold has been frequently cited for hemolytic potential in blood pumps. We recreated Sallam's apparatus (SA) to resolve this discrepancy and provide additional data to be used in developing a more accurate hemolysis model. Hemolysis was measured over a large range of Reynolds numbers (Re) (Re=1,000 – 80,000). Washed bovine red blood cells (RBCs) were injected into the free jet of phosphate buffered saline, and hemolysis was quantified using a percent hemolysis, $H_p = h(100 - HCT)/Hb$, where, h [mg/dl] is free hemoglobin and Hb [mg/dl] is total hemoglobin. RSS was calculated using two-dimensional laser Doppler velocimetry. RSS of 30,000 dyne/cm² corresponding to Re of 60,000 appeared to cause hemolysis ($p < 0.05$). This RSS is an order of magnitude greater than the RSS threshold that Sallam suggested, and it is similar to Forstrom's RSS threshold. This study resolved a long-standing uncertainty regarding the critical values of RSS for hemolysis, and may provide a foundation for a more accurate hemolysis model.

Keywords

hemolysis; Reynolds Shear Stress; turbulence jet; laser doppler velocimetry; Kolmogorov scale

Introduction

Over the years there have been numerous studies that point to the significant contribution of turbulence on hemolysis.^{1–3} These studies focused on the Reynolds shear stress (RSS) as a metric for the effect of turbulence on hemolysis. However, there is poor agreement on the threshold RSS level for red blood cell (RBC) damage in turbulent flow with values of 2,500

Corresponding Author and Request for Reprints: Gerson Rosenberg, PhD, The Pennsylvania State University, College of Medicine, Department of Surgery, Division of Applied Biomedical Engineering, 500 University Drive, Hershey, PA 17033, Tel #: (717) 531-6301, Fax #: (717) 531-4464, grosenberg@hmc.psu.edu.

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to 50,000 dyne/cm² reported.^{3–10} The RSS threshold levels of red blood cell damage at given exposure times from selected literature are shown in Table 1. Note that the threshold values vary by an order of magnitude, but exposure time is generally the same order of magnitude except for Sutura and Mehrjardi.⁶

Blackshear⁴ and Forstrom⁵ used an equation, developed by Schlichting, that predicts RSS based upon mean jet exit velocity.¹¹ They used different experimental constants (i.e., 0.03 and 0.017, see Stress Measurement in Table 1) likely due to the difference in fragility of human and canine erythrocytes.¹² Using a turbulent jet with canine and human blood, Blackshear⁴ and Forstrom⁵ reported the hemolytic RSS threshold to be 30,000 dyne/cm² and 50,000 dyne/cm², respectively.

Sallam used one-dimensional (1D) laser Doppler anemometry (LDA) to determine RSS⁷ and determined the RSS threshold for hemolysis to be 4,000 dyne/cm². It is of note, however, that 1D LDA has been shown to cause an underestimation due to experimental method and tensor-invariance.¹³ Grigioni *et al*⁹ and Lu *et al*¹⁰ both reevaluated Sallam's RSS calculations without performing any hemolysis experiments. Grigioni *et al*⁹ also worked under the false belief that Sallam had used two-dimensional (2D) LDA as opposed to a single component LDA. Yen *et al*⁸ used 2D particle image velocimetry (PIV) to determine the RSS in a turbulent jet and showed a hemolytic RSS threshold of 5,170 dyne/cm².

Despite the threshold values varying by an order of magnitude, Sallam's threshold of 4,000 dyne/cm² has been cited most frequently in the evaluation of hemolytic potential in blood pumps^{14–16} and prosthetic blood-contacting mechanical devices.^{9,17,18} Note that Sallam elaborated on explaining the earlier discrepancy on a RSS threshold level especially emphasizing Forstrom's⁵ and Blackshear's⁴ potential overestimation in their RSS levels for hemolysis.¹⁹

Hemolysis in Blood Pumps

The recently published 7th annual report of the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) showed that, although the overall adverse events are significantly lower in the most recent era (2012 – 2014), increasing event rates were observed for hemolysis, stroke, renal dysfunction, and respiratory failure, with hemolysis being the second of those four increasing adverse events.²⁰ While the effect of turbulent flow on the physical mechanism of hemolysis still remains unanswered, understanding the precise range of hemolytic RSS as an initial stage to develop a better hemolysis model is crucial.

The goal of this study is to resolve the discrepancy of the threshold RSS level for RBC damage in turbulent flow and provide additional data to be used in developing a more accurate hemolysis model. This work also evaluates the relationship between RSS and hemolysis as a continuous function, as the term threshold value is poorly defined.²¹

Materials and Methods

Sallam Apparatus (SA)

A recreated version of the SA, shown in Figure 1, was used to carry out the hemolysis experiments over a large range of Re , as shown in Table 2. The Re is defined as follows:

$$Re = U_e d_e / \nu \quad (1)$$

where U_e is the jet exit velocity, d_e is the jet exit diameter (3 mm), and ν is the kinematic viscosity of the fluid. The fixture has dimensions of 51 mm $\times$ 51 mm $\times$ 254 mm and is made from acrylic and stainless steel. The jet entrance, where phosphate buffered saline (PBS) enters the fixture, has a diameter of 3 mm. A beveled 17G needle is used for the infusion of washed bovine RBCs into the fixture. An aspirator with a 1 mm diameter opening is used to collect samples at a non-dimensional downstream distance of $x/d_e=6.5$ and $y/r_e=1.2$, where r_e is the jet exit radius (1.5 mm). Two syringe pumps were used to maintain constant flow rates for blood infusion (Harvard Apparatus, Holliston, MA) and sample collection (New Era Pump Systems, Inc., Farmingdale, NY). Sallam's original design was modified for these experiments by the addition of a support beam for the aspirator, which was necessary to eliminate vibration and movement at high Re . In addition, a hydrophone was added on the top surface of the chamber in order to monitor the presence of cavitation in the jet.

Blood Sample Preparation

Whole bovine blood was collected by LAMPIRE Biological Laboratories (LAMPIRE Biological Laboratories, Inc., Pipersville, PA) in CPDA-1 collection bags, and delivered overnight in a freezer-packet cold box. An additional 1.5 mL of Heparin (10,000 USP units/mL) and 1 mL of Tobramycin (40 mg/mL) were added to each unit of blood to ensure no clotting during testing. Washed RBCs were obtained by centrifuging the whole blood at $1,000 \times g$ for 10 minutes and replacing the plasma with PBS. Washed bovine RBCs were resuspended in PBS to achieve a hematocrit of 50%. All blood samples were used within two days after the date of harvest.

Hemolysis Methods

At each given Re , washed RBCs resuspended in PBS were injected into the free jet of PBS through a beveled 17G needle at a flow rate of 54 mL/min. Once blood began to enter the chamber, 10 mL of waste was drawn over 10 seconds, followed by 20 mL of sample drawn over 3.5 seconds.

Lysed Sample Collection

Samples were collected through an aspirator located at $x/d_e=6.5$, $y/r_e=1.2$ at a rate of 60 mL/min. The sampling location of $x/d_e=6.5$, $y/r_e=1.2$, which is believed to be a peak hemolytic location, was selected based on the study of Sallam and Hwang.⁷ A hydrophone and high speed video detected cavitation at $Re = 80,000$, however, the pressure was raised to 2.37 atm and the cavitation signal disappeared. Either a stainless steel Dayton gear pump (W. W. Grainger, Inc., Lake Forest, IL) or a plastic centrifugal pump (MD-70RZ(T), Iwaki America,

Holliston, MA) was used to circulate PBS through the flow loop connecting a 40L tank with the fixture.

Measure of Hemolysis

The free and total hemoglobin analysis was performed using Harboe's spectrophotometric technique.²² For free hemoglobin measure, the 2 mL tubes were centrifuged for 10 minutes at $1,000 \times g$. For each experiment, four 300 μ L samples were placed in a 96 well plate for analysis. Initial samples were further diluted by adding an equal amount of PBS to the samples. For total hemoglobin determination, 0.5 mL of sample was added to 2 mL of deionized water. The mixture then sat for at least 10 minutes followed by centrifugation at $1,500 \times g$ for 30 minutes to separate out cell membranes. After centrifugation, 100 μ L of sample was added to 15 mL PBS. Free hemoglobin and total hemoglobin were measured using a spectrophotometer (BioTek Instruments, Inc., Winooski, VT) and characterized using Harboe's equation shown in Eq. (2):

$$h \left[\frac{mg}{dl} \right] = 16.72 * A_{415} - 8.36 * (A_{380} + A_{450}) \quad (2)$$

where A_{380} , A_{415} , and A_{450} are the light absorbance at wavelengths of 380 nm, 415 nm, and 450 nm, respectively.²² To measure hematocrit on each sample of 50 μ L, an automated hematology analyzer (Sysmex KX-21N, Sysmex America, Inc., Lincolnshire, IL) employing a dynamic focusing (DC) detection method was used to measure hematocrit. Finally, hemolysis was quantified using a percent hemolysis parameter, H_p shown in Eq. (3):

$$H_p = \frac{h * (100 - HCT)}{Hb} \quad (3)$$

where h [mg/dl] is free hemoglobin in suspending medium, HCT [%] is hematocrit, and Hb [mg/dl] is the total concentration of hemoglobin when all RBCs in the sample have been lysed.²³

Statistical Analysis

All values reported in the table and plotted in the figures are expressed as mean $\pm$ standard deviation. Statistical significance was assessed using the Student's t -test. Significance level was set as $p < 0.05$.

Laser Doppler Velocimetry (LDV)

We manufactured an acrylic model of the SA for use in LDV experiments to determine the Reynolds normal and shear stresses. Since it is known that the measure of a non-dimensionalized RSS is constant over Re (i.e., RSS for the jet is linearly dependent on Re), LDV data for the Reynolds stresses was collected only at $Re = 20,000$.^{7,10} Specifics of the LDV system can be found in Taylor *et al.*²⁴

Experimental Conditions—A fluid composed of 37% H₂O/63% NaI by weight was used to match the refractive index of acrylic ($n = 1.49$). The fluid had a kinematic viscosity (ν) of 1.45 cSt at 25°C and a density of 1891 kg/m³. A heat exchanger was used in the flow loop to ensure the fluid remained at 25°C for the duration of the experiment, and a centrifugal pump (Cole-Parmer, Vernon Hills, IL) was used to produce a steady flow rate of 4.1 L/min. This yielded an average nozzle exit velocity (U_e) of 9.67 m/s and a nozzle Re of 20,000.

Data Collection—Despite examining the hemolysis only at $x/d_e = 6.5$ and $y/r_e = 1.2$, we mapped the entire flow field in our LDV experiment for a rigorous comparison to Sallam's study. As illustrated in Figure 2, coincident axial and radial velocities were collected at 7 downstream locations, i.e., $x/d_e = 1, 2, 3, 4.5, 5.5, 6.5$, and 8.5 . Coincidence was established using a 100% gate scale setting. At each downstream location, velocity data were collected at 14 radial locations, starting at the center of the jet and moving radially, i.e., y/r_e from 0 to 1.73 in increments of 0.2 mm (i.e., $y = 0 - 2.6$ mm). The probe volume was an ellipsoid with major and minor axes of 275 and 35 μm , respectively, with the minor axes in the axial and radial directions. Ten thousand 2D velocity measurements were made at each spatial location.

Data Filtering—The LDV data was filtered following the procedure of Baldwin *et al.*¹³ with an in-house MATLAB code, using an elliptical filter of 4 standard deviations to remove outliers from the data set. A value of four standard deviations was chosen as this value removed approximately 1% of the data set.

Reynolds Shear Stress (RSS)

A custom MATLAB code with 2D LDV data was used to calculate RSS and principal RSS at $x/d_e = 6.5$, $y/r_e = 1.2$. Note that the Reynolds shear stresses are not invariant to coordinate rotation, thus, it is important to calculate the principal RSS, which is potentially greater than those calculated in the coordinate system used for this experiment. Briefly, the velocity fluctuations u' and v' are defined as the deviations of instantaneous velocities u and v , respectively, from their average values as shown in Eqs. (4) and (5).

$$u' = u - \bar{u} \quad (4)$$

$$v' = v - \bar{v} \quad (5)$$

The RSS and principal RSS shown in Eq. (6) and Eq. (7), respectively, were normalized by U_e^2 , and fluid density, ρ , which is the same procedure that Sallam used,

$$\text{RSS} = \rho \overline{u'v'} \quad (6)$$

$$RSS_{\max} = \sqrt{\left(\frac{\overline{\rho u' u'} - \overline{\rho v' v'}}{2}\right)^2 - (\overline{\rho u' v'})^2} \quad (7)$$

where, $\overline{\rho u' v'}$ is Reynolds shear stress and $\overline{\rho u' u'}$ and $\overline{\rho v' v'}$ are Reynolds normal stresses, calculated using Eqs. (8) – (10).

$$\overline{\rho u' v'} = \frac{1}{N} \sum_{i=1}^N \rho (u - \bar{u})(v - \bar{v}) \quad (8)$$

$$\overline{\rho u' u'} = \frac{1}{N} \sum_{i=1}^N \rho (u - \bar{u})^2 \quad (9)$$

$$\overline{\rho v' v'} = \frac{1}{N} \sum_{i=1}^N \rho (v - \bar{v})^2 \quad (10)$$

Results

Hemolysis Results for SA

Hemolysis results are shown in Table 3. The H_p calculated with the results shown in Table 3 over Re is also shown in Figure 3. Based on the data presented in Figure 3, hemolysis in the SA was not obvious at Re of up to 50,000, however, the inception of hemolysis appeared at Re = 60,000 ($p = 0.004$) and increased exponentially with Re in our experimental conditions.

RSS from LDV—The entire map of normalized principal RSS (i.e., $RSS_{\max}$) and RSS at x/d_e from 1 – 8.5 and y/r_e from 0 – 1.73 for Re = 20,000 is shown in Figure 4. From the LDV, the maximum normalized value of principal RSS and RSS at $x/d_e = 6.5$, $y/r_e = 1.2$ were found as 96 and 75, respectively, and these can be converted into 4,270 dyne/cm² and 3,336 dyne/cm² with a given density of 1,000 kg/m³ and jet exit velocity of 6.67 m/s at Re = 20,000 used in our hemolysis testing. While understanding the principal RSS (or $RSS_{\max}$) being greater than RSS by 30%, we used the normalized RSS value of 75 to calculate the RSS values for rest of the Re conditions shown in Table 2. The RSS over Re is shown in Figure 5.

Based on the results shown in Figure 3 and Figure 5, it should be reasonable to conclude that the inception of hemolysis occurs at RSS equal to or greater than 30,000 dyne/cm². The inception of hemolysis and RSS can be represented as shown in Figure 6. In our study, hemolysis was not apparent at RSS less than 30,000 dyne/cm², however, once the RSS passed 30,000 dyne/cm², hemolysis increased in a nonlinear fashion with increasing RSS.

Discussion

We recreated Sallam's turbulence jet apparatus to investigate the hemolytic RSS values. In our study, a RSS of 30,000 dyne/cm² was found to be hemolytic, which is an order of magnitude greater than the threshold value of 4,000 dyne/cm² reported by Sallam.⁷

Other groups have tried to resolve the discrepancy on the hemolytic RSS values. Grigioni *et al*⁹ applied 3D stress analysis to Sallam's original 1D LDA measurements and found that the principal RSS causing the inception of hemolysis should be *at least* 6,000 dyne/cm². Lu *et al*¹⁰ reinvestigated Sallam's study by using 2D LDA under similar conditions and found, based on the principal RSS, that the hemolytic threshold should actually be *greater than* 8,000 dyne/cm². Note that no blood testing was involved in the studies of Grigioni *et al*⁹ and Lu *et al*¹⁰, and they never provided a definitive number for "threshold" RSS values.

Yen *et al*⁸ suggested a hemolytic RSS threshold of 5,170 dyne/cm² from their *in-vitro* study with the porcine blood. However, the spatial resolution of 190 μ m with 1000 frames of the particle image velocimetry (PIV) may not be sufficient to capture the smaller turbulent eddies in the flow fields that might have played an important role in causing hemolysis. Liu *et al*²⁵ concluded that the smallest turbulent length scale - which offers a more reliable estimate of the effects of turbulence on blood cell damage - was three times the size of a red blood cell (i.e., ~30 μ m) and five times the size of platelets. It is known that when the Reynolds number is sufficiently high, the Kolmogorov (smallest) scale eddies can be roughly the size of the red blood cell (RBC) and blood damage is possible.²⁶ For this study, we characterized the fluctuating flow fields by using a LDV system with an ellipsoidal probe volume with major and minor axes of 275 μ m and 35 μ m, respectively. Having radial resolution of 35 μ m is considered to be much more accurate in capturing small eddies, if not the smallest, affecting blood cell damages. Ten thousand 2D velocity measurements were made at each spatial location, yielding a higher accuracy in measuring RSS values.

It is interesting to note from the study of Lu *et al*¹⁰ that they found the normalized maximum RSS and principal normalized RSS of 75 and 93, respectively, at $x/d_e = 6$, $y/r_e = 1.2$ that are similar to what we found at $x/d_e = 6.5$, $y/r_e = 1.2$ in our study (Figure 4). They found no significant effects on the flow field characteristics including the principal RSS from their three different Reynolds numbers. This confirms that our RSS values derived by using the normalized RSS of 75 (or RSS_{max} of 96) at Re = 20,000 for other Re conditions should be acceptable.

Note that washed RBCs resuspended in PBS were injected into the free jet of PBS. According to Kamenewa *et al*²⁷ and Sumpelmann *et al*²⁸, the fragility of RBCs increases approximately by 3 – 4 times when RBCs are suspended in saline as opposed to plasma, thus, the critical value of RSS found in the present study may increase by the same factor if it were suspended in plasma. Nevertheless, an order of magnitude underestimation of the critical RSS suggested by Sallam⁷ is still noticeable from this comparative study.

Remarks on Reynolds Stress—In fact, Reynolds stress (often used interchangeably with turbulence stress) is not a physical stress³ and only results from the use of the of the

Re-averaged Navier-Stokes (RANS) equation.^{29,30} Under certain conditions there is a correlation between hemolysis and RSS but that correlation does not imply causation. Taskin *et al*³¹ demonstrated that hemolysis index measurements and predictions over different flow rate conditions with different sets of power law constants showed up to two order of magnitude differences in a custom hemolyzer and up to three order of magnitude differences in the CentriMag pump. Along with Ozturk *et al*³², there is strong evidence that the Kolmogorov scale eddies and the attendant dissipation are a better measure of the effects of turbulence on hemolysis and should be included in any advanced model of hemolysis.^{3,33,34} We are currently testing our recently developed physics-based hemolysis model by employing the energy dissipation.

Study Limitations and Future Study—Note that the present study used bovine RBCs and Sallam⁷ used human RBCs. Jikuya *et al*³⁵ found that the mechanical fragility of bovine and ovine RBCs was 0.5 and 1.8 times as large as human RBCs, respectively. Thus, the RSS threshold that the present study found may be lesser with human RBCs at given conditions, but it is not an order of magnitude difference. We used the centrifugation that Sallam used for a fair comparison, however, the centrifugation used in Ziegler *et al*³⁶, for example, may affect the RSS threshold found in the present study. Note that this study used only one peak location (i.e., $x/d_e = 6.5$, $y/r_e = 1.2$) for assessing hemolysis due to turbulence. A second point somewhere else within the turbulent jet is warranted for further validation of the study.

Summary

This study investigated the threshold of 4,000 dyne/cm² for hemolytic RSS suggested by Sallam and Hwang⁷ by characterizing the flow fields under a wide range of Reynolds numbers. In our study, a RSS of 30,000 dyne/cm² was found to be hemolytic, which is an order of magnitude greater than the RSS threshold of 4,000 dyne/cm² reported by Sallam.⁷ The RSS threshold found in our study is consistent with the values reported by Blackshear⁴ and Forstrom⁵ of 30,000 dyne/cm² and 50,000 dyne/cm², respectively. Since the estimation of the critical value of mechanical destruction on RBC damage in terms of both shear stress and exposure time is central, understanding the definite hemolytic RSS value should be an important step in the study of hemolysis. Our experimental results are significant because they have resolved a long-standing discrepancy regarding the critical values of RSS for hemolysis, and may provide a foundation for a more accurate hemolysis model.

References

1. Baldwin JT, Deutsch S, Geselowitz DB, Tarbell JM. LDA measurements of mean velocity and Reynolds stress fields within an artificial heart ventricle. *J Biomech Eng.* 1994; 116(2):190–200. [PubMed: 8078326]
2. Antiga L, Steinman DA. Rethinking turbulence in blood. *Biorheology.* 2009; 46(2):77–81. doi: 21373313756676J7 [pii] 10.3233/BIR-2009-0538. [PubMed: 19458411]
3. Yen JH, Chen SF, Chern MK, Lu PC. The effect of turbulent viscous shear stress on red blood cell hemolysis. *J Artif Organs.* 2014; 17(2):178–85. DOI: 10.1007/s10047-014-0755-3 [PubMed: 24619800]
4. Blackshear PL Jr, Dorman FD, Steinbach JH, Maybach EJ, Singh A, Collingham RE. Shear, wall interaction and hemolysis. *Trans Am Soc Artif Intern Organs.* 1966; 12:113–20. [PubMed: 5960689]

5. Forstrom, RJ. A New Measure of Erythrocyte Membrane Strength - The Jet Fragility Test. Minneapolis: University of Minnesota; 1969.
6. Suter SP, Mehrjardi MH. Deformation and fragmentation of human red blood cells in turbulent shear flow. *Biophysical journal*. 1975; 15(1):1–10. doi: S0006-3495(75)85787-0 [pii] 10.1016/S0006-3495(75)85787-0. [PubMed: 1174639]
7. Sallam AM, Hwang NH. Human red blood cell hemolysis in a turbulent shear flow: contribution of Reynolds shear stresses. *Biorheology*. 1984; 21(6):783–97. [PubMed: 6240286]
8. Tamagawa M, Akamatsu T, Saitoh K. Prediction of hemolysis in turbulent shear orifice flow. *Artificial organs*. 1996; 20(6):553–9.
9. Grigioni M, Daniele C, D'Avenio G, Barbaro V. A discussion on the threshold limit for hemolysis related to Reynolds shear stress. *Journal of biomechanics*. 1999; 32(10):1107–12. doi: S0021929099000639 [pii]. [PubMed: 10476849]
10. Lu PC, Lai HC, Liu JS. A reevaluation and discussion on the threshold limit for hemolysis in a turbulent shear flow. *Journal of biomechanics*. 2001; 34(10):1361–4. doi: S0021929001000847 [pii]. [PubMed: 11522317]
11. Schlichting, H., Gersten, K. *Boundary-layer theory*. Berlin; New York: Springer; 2000. 8th rev and enl ed ed
12. Matsuzawa T, Ikarashi Y. Haemolysis of various mammalian erythrocytes in sodium chloride, glucose and phosphate-buffer solutions. *Lab Anim*. 1979; 13(4):329–31. [PubMed: 43414]
13. Baldwin JT, Deutsch S, Petrie HL, Tarbell JM. Determination of principal reynolds stresses in pulsatile flows after elliptical filtering of discrete velocity measurements. *Journal of biomechanical engineering*. 1993; 115(4A):396–403. [PubMed: 8309234]
14. Untaroiu A, Wood HG, Allaire PE, et al. Computational design and experimental testing of a novel axial flow LVAD. *ASAIO J*. 2005; 51(6):702–10. doi: 00002480-200511000-00019 -00019 [pii]. [PubMed: 16340354]
15. Deutsch S, Tarbell JM, Manning KB, Rosenberg G, Fontaine AA. Experimental fluid mechanics of pulsatile artificial blood pumps. *Annu Rev Fluid Mech*. 2006; 38:65–86.
16. Yang N, Deutsch S, Paterson EG, Manning KB. Comparative Study of Continuous and Pulsatile Left Ventricular Assist Devices on Hemodynamics of a Pediatric End-to-Side Anastomotic Graft. *Cardiovasc Eng Technol*. 2010; 1(1)doi: 10.1007/s13239-010-0006-6
17. Kamenewa MV, Burgence GW, Kono K, Repko B, Antaki JF, Umezu M. Effects of turbulent stresses upon mechanical hemolysis: experimental and computational analysis. *ASAIO J*. 2004; 50(5):418–23. [PubMed: 15497379]
18. Zhang P, Yeo JH, Qian P, Hwang NH. Shear stress investigation across mechanical heart valve. *ASAIO J*. 2007; 53(5):530–6. doi: 10.1097/MAT.0b013e318157c093 00002480-200709000-00002 [pii]. [PubMed: 17885324]
19. Sallam, AM. *An Investigation of the Effect of Reynolds Shear Stress on Red Blood Cell Hemolysis*. Houston, TX: University of Houston; 1982.
20. Kirklin JK, Naftel DC, Pagani FD, et al. Seventh INTERMACS annual report: 15,000 patients and counting. *J Heart Lung Transplant*. 2015; 34(12):1495–504. doi: S1053-2498(15)01450-3 [pii] 10.1016/j.healun.2015.10.003. [PubMed: 26520247]
21. Arwatz G, Smits AJ. A viscoelastic model of shear-induced hemolysis in laminar flow. *Biorheology*. 2013; 50(1–2):45–55. doi: M066312V963M14WN [pii] 0.3233/BIR-130626. [PubMed: 23619152]
22. Harboe M. A method for determination of hemoglobin in plasma by near-ultraviolet spectrophotometry. *Scand J Clin Lab Invest*. 1959; 11:66–70. DOI: 10.3109/00365515909060410 [PubMed: 13646603]
23. Sowemimo-Coker SO. Red blood cell hemolysis during processing. *Transfus Med Rev*. 2002; 16(1):46–60. doi: S0887-7963(02)80006-2 [pii] 10.1053/tmrv.2002.29404. [PubMed: 11788929]
24. Taylor JO, Good BC, Paterno AV, et al. Analysis of Transitional and Turbulent Flow Through the FDA Benchmark Nozzle Model Using Laser Doppler Velocimetry. *Cardiovasc Eng Technol*. 2016; 7(3):191–209. doi: 10.1007/s13239-016-0270-1 10.1007/s13239-016-0270-1 [pii]. [PubMed: 27350137]

25. Liu JS, Lu PC, Chu SH. Turbulence characteristics downstream of bileaflet aortic valve prostheses. *Journal of biomechanical engineering*. 2000; 122(2):118–24. [PubMed: 10834151]
26. Dooley PN, Quinlan NJ. Effect of eddy length scale on mechanical loading of blood cells in turbulent flow. *Ann Biomed Eng*. 2009; 37(12):2449–58. DOI: 10.1007/s10439-009-9789-8 [PubMed: 19757062]
27. Kameneva MV, Antaki JF, Yeleswarapu KK, Watach MJ, Griffith BP, Borovetz HS. Plasma protective effect on red blood cells exposed to mechanical stress. *ASAIO J*. 1997; 43(5):M571–5. [PubMed: 9360109]
28. Sumpelmann R, Schurholz T, Marx G, Zander R. Protective effects of plasma replacement fluids on erythrocytes exposed to mechanical stress. *Anaesthesia*. 2000; 55(10):976–9. doi: ana1531 [pii]. [PubMed: 11012493]
29. Tennekes, H., Lumley, JL. *A first course in turbulence*. Cambridge, Mass: MIT Press; 1972.
30. Schlichting, H., Gersten, K. *Boundary-layer theory*. Berlin; New York: Springer; 2000. 8th revised and enlarged edition
31. Taskin ME, Fraser KH, Zhang T, Wu C, Griffith BP, Wu ZJ. Evaluation of Eulerian and Lagrangian models for hemolysis estimation. *ASAIO journal*. 2012; 58(4):363–72. DOI: 10.1097/MAT.0b013e318254833b [PubMed: 22635012]
32. Ozturk M, O'Rear EA, Papavassiliou DV. Hemolysis Related to Turbulent Eddy Size Distributions Using Comparisons of Experiments to Computations. *Artificial organs*. 2015; 39(12):E227–39. DOI: 10.1111/aor.12572 [PubMed: 26412190]
33. Jones SA. A relationship between Reynolds stresses and viscous dissipation: implications to red cell damage. *Annals of biomedical engineering*. 1995; 23(1):21–8. [PubMed: 7762879]
34. Morshed KN, Bark D Jr, Forleo M, Dasi LP. Theory to predict shear stress on cells in turbulent blood flow. *PLoS One*. 2014; 9(8):e105357. doi: 10.1371/journal.pone.0105357 ONE-D-14-01063 [pii]. [PubMed: 25171175]
35. Jikuya T, Tsutsui T, Shigeta O, Sankai Y, Mitsui T. Species differences in erythrocyte mechanical fragility: comparison of human, bovine, and ovine cells. *ASAIO J*. 1998; 44(5):M452–5. [PubMed: 9804471]
36. Ziegler LA, Olia SE, Kameneva MV. Red Blood Cell Mechanical Fragility Test for Clinical Research Applications. *Artif Organs*. 2016; doi: 10.1111/aor.12826

Figure 1.

(A) SA experimental setup. (B) a schematic representation of SA chamber with jet exit nozzle, (A), blood injection needle, (B), and sample aspirator, (C). (C) Blood entering the free jet with the aspirator at a non-dimensional downstream distance of $x/d_e = 6.5$ and radial distance of $y/r_e = 1.2$ (a dotted box), where d_e is the jet exit diameter (3 mm) and r_e is the jet exit radius (1.5 mm). No RBCs or fluid are recirculated in this one-pass system.

Figure 2.

Schematics of LDV sampling locations: there are 14 radial locations (y/r_e) at each axial location (x/d_e).

Figure 3.

Hemolysis over Re ranging from 1,000 – 117,000 for the SA. The shaded area represents the incipient hemolysis starting from $Re = 60,000$ (* $p < 0.05$).

Figure 4.

(A) Normalized principal Reynolds shear stress (RSS_{max}) and (B) Normalized Reynolds shear stress (RSS) at $Re = 20,000$ calculated from the 2D LDV data. The normalized RSS_{max} and RSS at $x/d_e = 6.5$, $y/r_e = 1.2$ were 96 and 75, respectively, and these correspond to 4,270 dyne/cm² and 3,330 dyne/cm², respectively.

Figure 5.

The RSS values over Re ranging from 1,000 – 117,000 were calculated based on the 2D LDV at Re = 20,000. The shaded area represents the range of RSS causing hemolysis in the SA.

Figure 6.

Hemolysis increases in a nonlinear fashion with RSS. A threshold RSS was not apparent from our study. The shaded area represents the inception of hemolysis. Hemolysis was not apparent at RSS less than 30,000 dyne/cm², however, once RSS passed 30,000 dyne/cm², hemolysis increased in a nonlinear fashion with increase in RSS (* $p < 0.05$)

Table 1

Reynolds shear stress threshold reported by various studies.

Author	RSS Threshold, $\times 10^3$ [dyne/cm ²]	Exposure Time [sec]	Blood	Experiment Type	Stress Measurement	Year
Blackshear <i>et al</i> ⁴	30	–	Canine	Turbulent Jet	$\tau = 0.030\rho U^2$	1966
Forstrom ⁵	50	10^{-6}	Human	Turbulent Jet	$\tau = 0.017\rho U^2$	1969
Sutera and Mehrjardi ⁶	2.5	240	Human	Concentric Cylinder Viscometer	Determined from direct measurements of torque	1975
Sallam and Hwang ⁷	4	10^{-5}	Human	Turbulent Jet	1-D LDA	1984
Tamagawa <i>et al</i> ⁸	18	$10^{-6} - 10^{-5}$	Bovine	Pipe Orifice	Low Re <i>k-e</i> model	1996
Grigioni <i>et al</i> ⁹	6	–	Human	Re-evaluation of Sallam's work	3-D stress analysis	1999
Lu <i>et al</i> ¹⁰	8	–	Human	Re-evaluation of Sallam's work	2 component LDA to determine major principle RSS	2001
Yen <i>et al</i> ⁶	5.17	10^{-5}	Porcine	Turbulent Jet	2-D PIV	2014

Table 2

In vitro SA submerged free jet testing conditions (jet exit diameter, $d_e = 3$ mm).

Condition	Re ($\times 10^3$)	U_e [m/s]
1	1	0.33
2	1.5	0.50
3	2	0.67
4	5	1.67
5	20	6.67
6	32	10.67
7	40	13.33
8	50	16.67
9	60	20.00
10	70	23.33
11	80	26.67
12	90	30.00
13	100	33.33
14	110	36.67
15	117	39.00

Table 3

Hemolysis results over Re for SA.

Re	<i>h</i> [mg/dl]	<i>Hb</i> [g/dl]	<i>HCT</i> [%]	No. of Samples
1,000	0.879±0.261	0.199±0.043	0.460±0.117	10
1,500	0.805±0.169	0.203±0.051	0.500±0.125	10
2,000	0.920±0.218	0.227±0.028	0.550±0.085	10
5,000	0.909±0.198	0.202±0.021	0.630±0.067	10
20,000	0.703±0.730	0.145±0.087	0.434±0.254	44
32,000	0.211±0.072	0.068±0.021	0.211±0.070	27
40,000	0.619±0.632	0.116±0.066	0.357±0.198	30
50,000	0.181±0.060	0.050±0.016	0.167±0.055	27
60,000	0.272±0.223	0.039±0.009	0.127±0.052	30
70,000	0.424±0.231	0.034±0.012	0.113±0.034	16
80,000	0.228±0.164	0.026±0.006	0.097±0.018	30
90,000	0.315±0.153	0.022±0.007	0.100±0.000	20
100,000	0.304±0.123	0.018±0.004	0.097±0.017	34
110,000	0.465±0.158	0.018±0.006	0.090±0.031	20
117,000	0.661±0.179	0.015±0.003	0.096±0.020	24