A Comparative *In Vitro* Hemolysis Study of a Pulsating Intravenous Artificial Lung

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A study was conducted to measure and compare the levels of hemolysis generated by an intravenous membrane oxygenation device referred to as the Intravenous Membrane Oxygenator (IMO) in previous literature. The device is comprised of several hundred hollow fiber membranes of approximately 40 cm in length that are woven in a fabric and wrapped around a centrally positioned balloon. The balloon, which is similar in shape and volume to an intra-aortic balloon, is rapidly inflated and deflated up to 300 bpm to augment gas exchange. To evaluate the hemolytic nature of this device, an *in vitro* test system was developed, consisting of two identical test loops, each incorporating a device test section of 1 inch in diameter, a heat exchanger, a Biomedicus pump head, a compliance bag, a venous reservoir bag, and Tygon tubing. Both loops were primed with 1.5 L of a bovine blood solution and run simultaneously at 37°C for 6 hours at 4 L/min. Hematocrit and plasma free hemoglobin concentration were measured every 30 minutes to monitor hemolysis within each loop. This methodology was used to compare the hemolysis of the device at maximal pulsation with that of the control loop with an empty test section, as well as with a pulsing balloon of the same volume without any fibers. The results suggest that the hemolytic nature of the pulsating intravenous oxygenator is consistent with that of an intra-aortic balloon, a clinically used device not associated with any complications due to hemolysis. *ASAIO Journal* 2002; 48:631–635.

An intravenous respiratory support catheter (RSC) is under development in our laboratory to provide a means of temporary support for patients suffering from acute respiratory distress syndrome (ARDS) or acute exacerbations of chronic respiratory disorders. Currently, the primary method of support for such patients is mechanical ventilation. Despite many advancements in administration of this therapy, however, the high tidal volumes and oxygen concentrations necessary to provide adequate oxygenation and carbon dioxide removal lead to further damage of the ailing lungs.[1] An estimated 150,000 patients in the United States develop ARDS every year, and mortality rates remain as high as 50%.[2,3]

In the early 1990s, a clinical trial was conducted to evaluate an intravenous membrane oxygenation device (IVOX) as a method for providing temporary respiratory support and allowing the lungs of ARDS patients to heal.[4] The IVOX established the feasibility of intravenous respiratory support despite limited gas exchange, which prevented further progress of this device.[5] We have conducted extensive *in vitro* and *in vivo* studies of a next generation intravenous gas exchange device, or RSC, which employs a mechanism for active mixing of the blood to augment gas exchange from the limited amount of membrane surface area that can be positioned within the vena cava.[6–9] This device has been referred to in previous manuscripts as the University of Pittsburgh Intravenous Membrane Oxygenator (IMO).

Like the IVOX, the RSC is designed to be inserted through the right femoral vein and positioned within the inferior and superior vena cava, spanning the right atrium. It is composed of several hundred hollow fiber membranes manifolded to gas flow pathways that extend from the femoral vein insertion site and channel oxygen gas through the fiber lumens. As venous blood passes over the fibers, concentration gradients drive diffusion of oxygen across the membrane wall into the blood and diffusion of carbon dioxide from within the blood into the fiber lumens. The mechanism for active mixing of the blood as it passes over the fibers is an elongated balloon positioned centrally within the fiber bundle. The balloon, which is similar in size and shape to an intra-aortic balloon (IAB) pump (IABP), is filled and deflated several hundred times per minute to draw blood in and out of the fiber bundle (**Figure 1**), augmenting gas exchange by as much as 2 to 3 times.[10] The gas exchange capacity of the RSC is directly proportional to the rate of balloon pulsation, and thus the balloon is operated at the maximal rate at which it can be fully inflated and deflated by an external drive system. This rate is dependent upon several factors, including the balloon volume, the balloon gas pathway dimensions, and the drive system pump capacity; currently, rates of 300 bpm are reached for balloons with volumes as great as 25 ml.

The high rates of balloon pulsation, however, naturally raise questions about the potential for this device to cause hemolysis. Preliminary chronic 4 day *in vivo* tests in calves with continuous balloon pulsation at 120 bpm have shown negligible hemolysis (**Figure 2**);[11] however, more extensive *in vivo* testing will be required before concerns can be fully addressed. This article presents the results of a comparative *in vitro* hemolysis study of the RSC conducted to address hemolysis by the RSC in a more immediate and controlled fashion. A primary goal of this research was to use *in vitro* testing as a means of approximating levels of hemolysis that might be encountered clinically. Tests were conducted using identical test circuits perfused simultaneously but separately with bovine blood from the same preparation. We found that *in vivo* hemolysis of the RSC could be inferred by direct *in vitro*
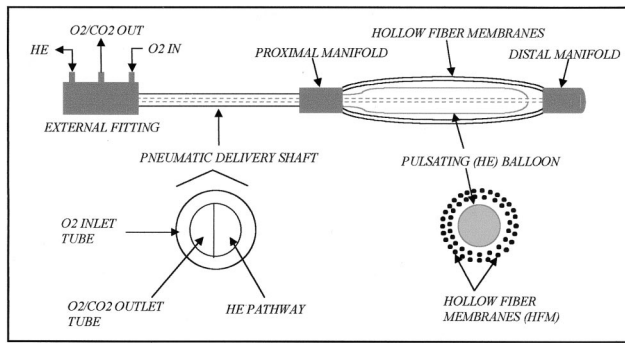
Figure 1. Schematic of the respiratory support catheter.

Figure 3. Schematic of hemolysis test circuit.

comparison of the RSC to an IABP, a cardiovascular device of known hemolytic behavior. The IABP is similar in shape, size, and mode of operation to the balloon used in the RSC and is operated in a vessel of similar size to the vena cava. IABs are used in approximately 100,000 patients per year and are not associated with complications due to hemolysis.[12–14]

Methods

Two identical test circuits were constructed consisting of a reservoir bag, a Medtronics Biomedicus centrifugal pump head, a pediatric heat exchanger (Medtronic Electromedics Model D1078E, Minneapolis, MN), a compliance bag, and a 1 inch inner diameter Tygon test section 70 cm long. The circuit components were connected with $3/8$ inch Tygon laboratory tubing (formulation R3603). Flow was measured in each loop with a Transonics flow meter, and temperature was measured with a thermocouple. The pressures within each circuit were governed by the height of the reservoir and the compliance bag. The magnitudes of the pressure within each circuit were consistent with each other and were maintained below 100 mm Hg so as not to prevent maximum balloon filling (as confirmed with a plethysmograph). A schematic of the test

circuit configuration is shown in **Figure 3**. Before each test, all loop components and the device were sterilized with ethylene oxide at a temperature of 104°F for 6 hours.

Fresh bovine blood was collected from a slaughterhouse the morning of each test. Anticoagulant citrate dextrose (ACD) was used to anticoagulate the large volumes of blood collected to reduce the risk of clot development during and after the collection process. The ratio of blood to ACD was 9:1. The blood was also treated with penicillin (500,000 U/ml) and Gentamicin (0.1 g/ml) then filtered, and its hematocrit and protein concentration measured. Using these measurements, 2.5 L blood mixtures were prepared for each circuit using saline and bovine albumin to have a hematocrit of 30% and a protein concentration of 7.0 g/dl. Protein concentration was controlled to be at a physiologic level not only to maintain consistency between tests, but primarily because diluted protein concentrations were found to affect blood fragility (see Discussion).

Each circuit was then primed and adjusted so that the final volume of blood filling the circuit was 1.5 L. Before the start point, the blood was circulated for 5 minutes at 1 L/min to allow the temperature to reach 37°C. To begin the test, the flow was set to 4 L/min, and the test conditions were established. Blood samples were drawn every 30 minutes for the first 2 hours of the test, and then every hour. The test was run for a total time period of 6 hours.

Each sample taken during the course of the 6 hour test period was used to measure hematocrit and plasma free hemoglobin. Hematocrit was measured with a capillary tube spun for 5 minutes in a microhematocrit centrifuge (International Equipment Co.). Plasma free hemoglobin was measured using a spectrophotometer (Spectronic Genesys 5) at a wavelength of 540 nm, with saline used as the zero control. Blood samples were centrifuged at 3,800 rpm for 15 minutes, the plasma was removed, and the blood was again centrifuged at 10,000 rpm for 15 minutes. The centrifuged plasma was then transferred to a cuvette for measurement in the spectrophotometer.

Using these methods, three comparative tests were conducted:

1. An empty test section *versus* a device with a fiber surface area of 0.17 m^2 and a balloon size of 25 ml (12.5 mm diameter) pulsing at 300 bpm.

2. A 25 cc balloon *without* fibers pulsing at 300 bpm *versus* a device with a fiber surface area of 0.17 m^2 and a balloon size of 25 ml (12.5 mm diameter) pulsing at 300 bpm.
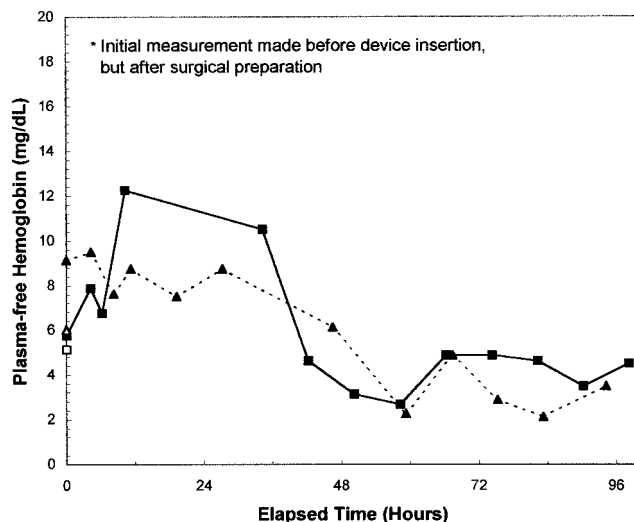

Figure 2. Plasma free hemoglobin levels measure during 4 day chronic *in vivo* trials of RSC devices pulsing at 120 bpm in the inferior vena cava of a calf.
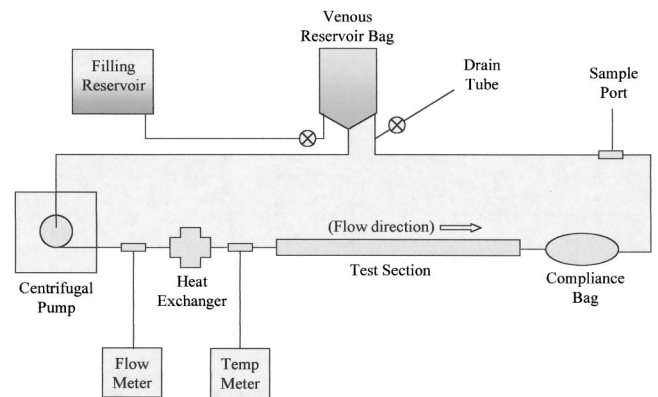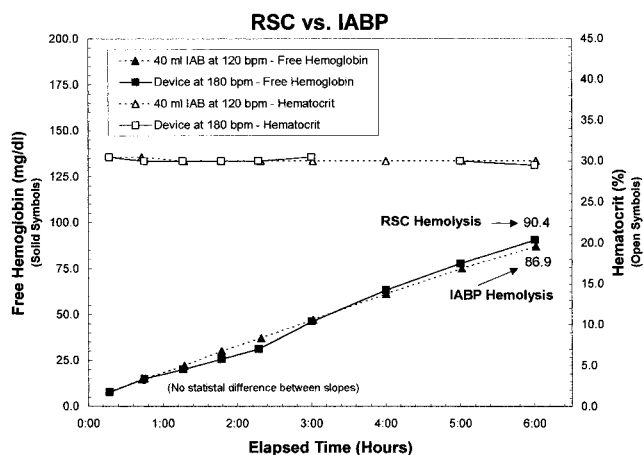
Figure 4. A 40 ml Datascope intra-aortic balloon (IAB) pulsing at 120 bpm (solid triangle, open triangle) *vs.* a respiratory support catheter (RSC) device with a 40 ml balloon pulsing at 180 bpm (solid square, open square). Blood flow rate was maintained at 4 L/min and temperature at 37°C in both circuits.

3. A 40 cc Datascope IAB pulsing at 120 bpm *versus* a device with a fiber surface area of 0.17 m² and a balloon size of 40 ml (15.0 mm diameter) pulsing at 180 bpm.

The first test was conducted to determine what portion of the measured hemolysis was generated by the test circuit itself compared with a standard size RSC device. The purpose of the second test was to isolate whether the hemolysis generated by the device was due to shear forces from balloon generated flow past the fibers or to secondary flow shear forces generated by the balloon motion alone. The final test was to compare a Datascope IAB directly with a RSC device with a similar balloon size. Each of these tests was conducted once, after a series of preliminary tests performed for protocol development. The Datascope balloon was driven with a Datascope IAB pump at 120 bpm, which is approximately the maximum rate that would be used clinically, whereas the RSC device was driven at 180 bpm, which is the maximum rate that our laboratory drive system could fully inflate and deflate the 40 ml balloon. The RSC was tested at its highest rate, as opposed to the same as that of the IAB, because that is the rate at which it would be operated clinically. Gas exchange has been found to be directly proportional to balloon pulsation rate, and using the maximum rate would give a worst case hemolysis comparison to the Datascope balloon at its clinically used maximum rate.

Results

The hemolysis test results are plotted in **Figures 4, 5, and 6**. In **Figure 4**, it is clearly shown that in the test comparing a Datascope IAB with an RSC device with a balloon of similar size, the levels of hemolysis were found to be essentially the same. In this test, the level of free hemoglobin in the circuit with the Datascope balloon reached 86.9 mg/dl after 6 hours, and the level in the circuit with the RSC device reached 90.4 mg/dl. A statistical analysis comparing the slopes of the hemolysis measurements over time showed no statistical difference. The hematocrit for both circuits remained within the range of 29.5% to 30.5%.
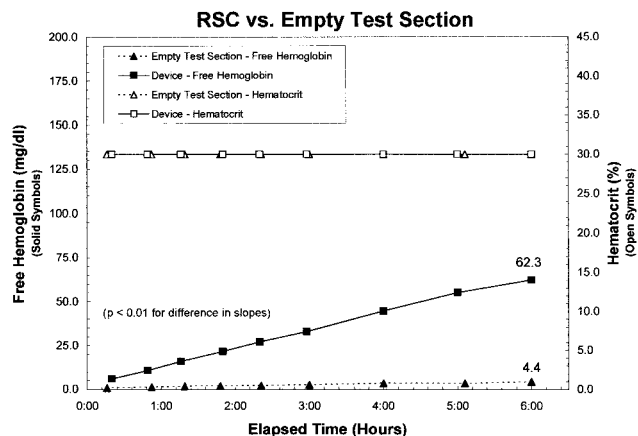
Figure 5. Empty test section (solid triangle, open triangle) *vs.* device with 25 ml balloon pulsing at 300 bpm (solid square, open square). Blood flow rate was maintained at 4 L/min and temperature at 37°C in both circuits.

Figure 5 shows the results of the test comparing the RSC device with an empty test section. The hemolysis generated by a standard size device at maximum pulsation rose at a constant rate to a level significantly higher than that measured within the empty test section. The hematocrit measurements are included on this graph to validate consistency over the course of the experiment, as well as between test circuits. For the empty test section, the plasma free hemoglobin level reached only 4.4 mg/dl after 6 hours, whereas for the circuit with the device, the levels of plasma free hemoglobin rose to a final value of 62.3 mg/dl after 6 hours.

Finally, the levels of hemolysis generated by a standard device were compared with the hemolysis generated by an equivalent sized balloon without fibers and were found to be approximately 25% less, as shown in **Figure 6**. In this test, the level of hemoglobin after 6 hours in the circuit with the RSC device with a 25 ml balloon pulsing at 300 bpm reached 77.0 mg/dl, whereas the level of free hemoglobin in the circuit with
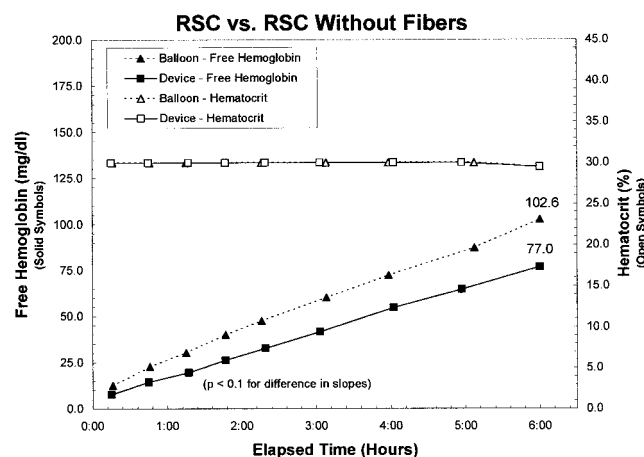
Figure 6. A 25 ml balloon without fibers pulsing at 300 bpm (solid triangle, open triangle) *vs.* a respiratory support catheter (RSC) device with a 25 ml balloon pulsing at 300 bpm (solid square, open square). Blood flow rate was maintained at 4 L/min and temperature at 37°C in both circuits.

the 25 ml balloon with no fibers pulsing at 300 bpm reached 102.6 mg/dl. The hematocrit remained at 30.0% in both circuits for the duration of the test.

Discussion

An intravenous RSC composed of hollow fiber membranes is being developed to provide temporary support for patients suffering from acute respiratory failure. This device uses a pulsating balloon within the fiber bundle to augment oxygen and carbon dioxide exchange across the hollow fiber membranes. An *in vitro* hemolysis study was conducted to evaluate the potential hemolysis that might be generated by this device. Identical test circuits were constructed so that measurements of hemolysis could be made simultaneously to compare hemolysis of the RSC with that of other devices or test conditions. This method provided a means for inferring the levels of hemolysis that will occur clinically through direct comparison with devices of known clinical hemolytic behavior.

In comparison, the *in vitro* hemolysis generated by the RSC device and an IABP were found to be equivalent. The test comparing the two devices was conducted simultaneously within identical test circuits primed with blood from the same preparation. The volume, hematocrit, and protein concentration in each circuit were confirmed to be the same. Blood flow in each circuit was maintained at 4 L/min for a duration of 6 hours. The volume of both the IAB and the RSC balloon was 40 ml; however, the IAB was pulsed at 120 bpm, which is the maximum rate used clinically, while the RSC balloon was pulsed at 180 bpm. This is the maximum rate at which a 40 ml balloon in the RSC could be fully inflated and deflated (as determined before testing) and is the rate that would be used clinically for maximal gas exchange.

The equivalent rate of *in vitro* hemolysis demonstrated by the RSC and the IAB implies that the RSC will not cause unacceptable levels of hemolysis in humans. Leinbach *et al.*[13] evaluated the hematologic effects of patients implanted with IABs and found no evidence of balloon induced hemolysis. Similarly, Schneider *et al.*[14] found no increase of plasma free hemoglobin in a study of IABs used in calves. The *in vitro* tests show that both devices can, in fact, cause hemolysis; however, based on *in vivo* and clinical studies of the IAB, the rate of hemolysis is within the range that can be compensated for by the body.

The differences between the conditions of balloon pulsation in the aorta *versus* in the vena cava are not expected to alter the association between the equivalence in *in vitro* hemolysis and ultimate clinical hemolysis of the RSC and the IAB. In an *in vitro* study of the effect of IAB parameters on hemolysis, Heuser[15] showed that the rates of hemolysis were not affected by the stiffness of the aorta. Therefore, the difference in compliance of the vena cava and the aorta should not change the conclusion. With respect to differences in blood flow, the RSC would be positioned so that it spans the right atrium, and thus the flow rate past the device at the proximal end in the inferior vena cava and at the distal end in the superior vena cava will be a fraction of the total cardiac output. Similarly, flow in the portion of the aorta where an IAB is positioned is approximately 60% of the total cardiac output, and thus the actual flow conditions past both devices would be similar. The pressure of the blood in the aorta is greater both in magnitude and fluctuation than in the vena cava, which translates to larger

longitudinal flow rate fluctuations. This difference would intuitively lead to larger shear rates in the aorta and thus greater levels of hemolysis implying, if anything, the RSC in the vena cava will generate less hemolysis than the IAB in the aorta.

An *in vitro* test was also conducted to isolate the factors of RSC performance primarily responsible for causing hemolysis. The rates of hemolysis between a RSC device with a 25 ml balloon pulsing at 300 bpm was compared with a 25 ml balloon with no fibers pulsing at the same rate. The results of this test (**Figure 6**) showed that the levels of hemolysis generated by the RSC device were slightly *less* than that of the "naked" balloon. This suggests that the cause of hemolysis is the balloon itself and not the shear forces established by balloon generated flow across the fiber bundle. Placement of a pulsating balloon in a vessel increases shear forces because of the increase in velocity of the blood flow parallel to the fibers as it passes over the balloon and from the secondary flows generated by balloon pulsations.

That the levels of *in vitro* hemolysis could be primarily attributed to the devices tested rather than the circuit was confirmed by simultaneously comparing the hemolysis of a circuit with a RSC device with a circuit having an empty test section. The hemolysis generated by the components of the test circuit was very little in comparison to that generated by the RSC device (**Figure 5**). It was also shown that consistent hematocrit could be maintained between circuits over the course of the 6 hour test period. From these results, it was concluded that the test methods were effective for characterizing hemolysis of the RSC device and comparing it with cardiovascular devices of known clinical behavior.

The tests were designed based on the guidelines recommended by the Association for the Advancement of Medical Instrumentation Standards and the Food and Drug Administration Guidance for Cardiopulmonary Bypass Oxygenators 510(k) Submissions.[16,17] In developing our protocols, however, we found that certain test conditions were not addressed by these guidelines. For example, both Kameneva *et al.*[18] and Butler *et al.*[19] demonstrated a protective effect against mechanical hemolysis of plasma proteins, yet a recommended protein concentration is not specified in the standards or in reported *in vitro* hemolysis studies. In preliminary trials during development of the protocols used for the tests shown in **Figures 4, 5, and 6**, we found that certain factors not addressed by *in vitro* hemolysis test standards seemed to have a significant effect on measured hemolysis levels. Specifically, we explored the effects of protein concentration and osmolality on the fragility of blood. Blood fragility tests were conducted by gently rocking 7 ml vials containing 3 ml of blood and 5 stainless steel ball bearings for 1 hour and comparing measured plasma free hemoglobin levels to control vials without the ball bearings. A fragility index was used to normalize for hematocrit and control sample plasma free hemoglobin levels. The fragility index was calculated using the following equation:

$$FI = \left[\frac{(fHb_{balls} - fHb_{control})100}{(Hct/3)1000 - fHb_{control}} \right] \left[\frac{30}{Hct} \right]^2 \tag{1}$$

where fHb is plasma free hemoglobin concentration in mg/dl and Hct is hematocrit.

Anticoagulation of the blood with ACD resulted in dilution of the protein concentration, as well as reduction of the blood pH.
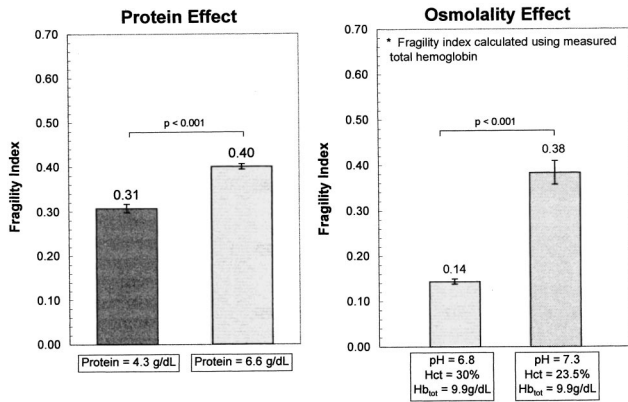
Figure 7. Measurements of blood fragility comparing samples with a physiologic protein concentration of 6.6 g/dl with samples with a diluted protein concentration of 4.3 g/dl (left) and comparing samples with low pH having a normal osmolality (~300 mOsm) to samples with pH adjusted with sodium bicarbonate resulting in a raised osmolality, as evidenced by the difference in hematocrit measured despite equivalent levels of total hemoglobin.

The fragility of blood with the protein concentration reduced by anticoagulation with ACD to a ratio of 9:1 (blood:ACD) was compared with blood of the same anticoagulation but with its protein concentration adjusted to physiologic levels by addition of bovine albumin. The fragility tests showed that the reduction in protein concentration caused a 25% increase in fragility (**Figure 7**), which is consistent with the protective effect of proteins documented by previous investigations.[18,19]

Likewise, fragility tests were conducted to compare blood with its pH adjusted to 7.4 using sodium bicarbonate to blood without a pH adjustment. The osmolality of the blood without pH adjustment was within a physiologic range, while that of the blood adjusted with sodium bicarbonate was elevated. The increase in osmolality was found to increase fragility by more than two-fold, as shown in **Figure 7**. These results underscore the necessity for more detailed *in vitro* hemolysis standards. The dependency of the levels of hemolysis measured in an *in vitro* circuit on such factors render comparison with or replication of other published *in vitro* hemolysis studies inconclusive. Furthermore, such variability makes any kind of correlation between *in vitro* and *in vivo* results elusive.

Conclusion

The *in vitro* hemolytic nature of the RSC device was shown to be comparable with that of an IABP, which is not associated clinically with hemolysis. Accordingly, the RSC device is not expected to generate unacceptable levels of hemolysis when tested and used in the clinical setting. This conclusion is consistent with preliminary chronic *in vivo* trials of the RSC device in calves where levels of plasma free hemoglobin were below 5 mg/dl after 4 days. The levels of hemolysis generated by the RSC device in a noncompensating *in vitro* test circuit were shown to be related, not to the shear stress associated with balloon generated flow past the fiber surfaces, but rather to the shear associated with the changes in velocity pattern created by placement of a pulsating balloon in a cylindrical vessel. The method of characterizing hemolysis using two identical test loops simultaneously perfused with blood from the same pool proved to be an effective tool for predicting *in vivo* hemolysis by comparison with devices of known clinical behavior. Further refinement and understanding of *in vitro* test conditions is necessary before *in vivo* hemolysis can be predicted directly from levels measured *in vitro*.

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