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Hemolysis in needles.

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HEMOLYSIS IN NEEDLES

By

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A thesis approved on

April 12, 2008

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DEDICATION

This thesis is dedicated to my parents

Mr. Mahipal Reddy

and

Mrs. Surekha Reddy

for their invaluable support, guidance and educational opportunities provided to me.

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I would like to thank many people for helping me in completing my thesis. I would like to begin by thanking my advisor Dr. Keith Sharp for his support, guidance and encouragement that he provided me continuously throughout my graduate study. I would also extend my thanks to Dr. George Pantalos and Dr. Timothy Dowling for participating on my thesis committee. Special thanks to Jason Yeker and Justin Volz for helping me with the 16G needle experiments and Patrick Recktenwald for designing the T-shape fixture. My thanks to the University Blood Bank and all the people at the hospital without whom my experiments would not have been completed in such short time.

I would like to thank my parents, my brother and other family members for their moral support and encouragement that had given me tremendous strength all my life. In addition, thanks to all my undergraduate friends and friends in Louisville for their continued support and confidence in me.

ABSTRACT

HEMOLYSIS IN NEEDLES

Giridhar Kommidi

May 10, 2008

Hemolysis, the major cause for specimen rejection in clinical laboratories is defined as the rupture of red blood cell membranes, resulting in the release of hemoglobin into the plasma. Blood flow in needles causes hemolysis resulting in complications such as incorrect assay results [13,14], repeated painful blood draws [4,9,13] and also the release of hemoglobin into the plasma, which is toxic to the kidneys [18].

Hemolysis mainly occurs due to high fluid stresses that act on the red blood cells in blood flow through the needle [22]. Highest stresses during blood draws act near the downstream edge of the needle entrance. In this project, the needle has been modified with a more rounded entrance to reduce the effect of the stresses acting near the tip. Experiments were also performed with needles of beveled entrance to reduce the hemolysis.

This thesis discusses in detail, experiments conducted with 16G and 20G standard and modified needles in both reverse and normal orientations at three different pressures. Blood was drawn into a syringe, then the needle is attached to it and the assembly is

placed in a fixture. The fixture was designed to hold the syringe in place and apply desired pressure on the syringe. The blood flowing out of the syringe was collected in a test tube and this process was repeated for all the combinations of pressures and needle orientations viz. normal and reverse. The collected test tubes were then centrifuged and the plasma was removed and analyzed in a spectrophotometer. Results were tabulated and graphs were plotted to compare the hemolysis in the test and control needles.

ANOVA p-values clearly indicated that the rounded entrance significantly reduced hemolysis compared to the standard needles for the 20G size in the reverse orientation at all three pressures (p-values at 20psi, 35psi and 50psi were 0.053, 4.60E-08 and 1.00E-08, respectively). There was also a significant relation between the 16G with rounded entrance and the standard needles in the reverse orientation (p-values were 0.017, 0.004 and 0.001 at 15psi, 30psi and 45psi, respectively), but no significant relation for the 16G needles with beveled entrance.

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I. INTRODUCTION

Rejection of blood specimens by venipuncture rejection in many clinical laboratories can be attributed to hemolysis. Hemolysis can be defined as the breakage of red blood cells releasing hemoglobin into the plasma of blood.

Human blood (Fig. 1) is composed mainly of four components:

(i) Red blood cells (erythrocytes): They constitute 40%-50% of blood, the main function is to carry oxygen to various parts of the body. [38]

(ii) White blood cells (leukocytes): They constitute only 1% of blood and they form the immune system of the body. [38]

(iii) Platelets (thrombocytes): They are usually helpful in clotting of blood at the time of injury. [38]

(iv) Plasma: It forms 55% of blood and 99% of plasma is water. It is a medium (liquid like) carrying all the red blood cells, white blood cells and platelets that remove all the waste products of metabolism from different parts of the body. It contains some antibodies and protein. [38]

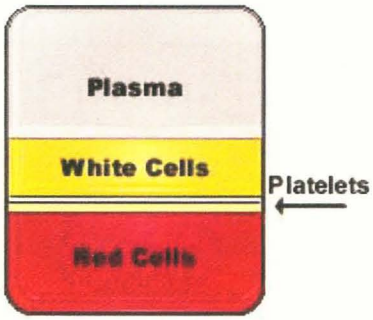

Figure 1.Blood composition- plasma, white cells, platelets, red cells [38

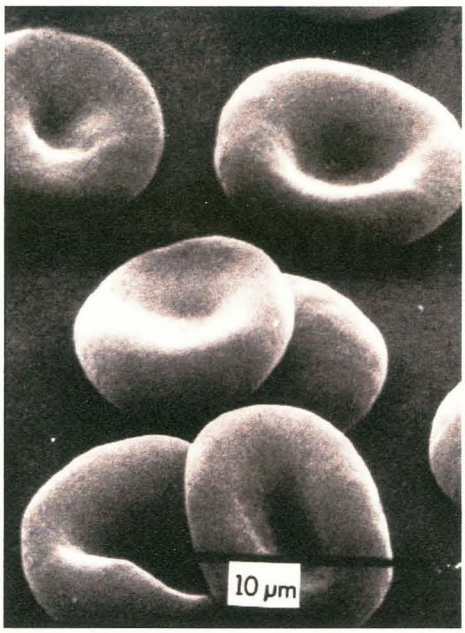

Figure 2. Red blood cell

A red blood cell (Fig. 2) contains hemoglobin that carries oxygen to various parts for the proper functioning of the body. During hemolysis, this hemoglobin is released from the red blood cell into the plasma and this effects the oxygen carrying capacity of RBC. This plasma free-hemoglobin interacts with the assays of a number of metabolites, electrolytes and enzymes like potassium [17], glucose, creatinine, bilirubin and alkaline phosphate [12,13,9]. This leads to incorrect assay results and also in repeated blood draws [13,14] resulting in waste of resources and time [17,4]. especially when the patient's blood results are needed in an emergency condition. It is also a discomfort for the patient to undergo the painful procedure repeatedly [13,9,4]. Also, hemoglobin is toxic to the kidneys and the damaged cells cannot transport oxygen to the various parts of the body [18].

Hemolysis is also observed in hemodialysis procedures. Hemodialysis is a procedure in which metabolic products and impurities are removed from the blood of patients whose kidneys are not functioning properly. This is done by passing the blood along one side of a semi-permeable membrane and the impurities are removed by diffusion through the membrane. Hemolysis during hemodialysis procedure may result in nausea and abdominal or back pain occurring during the later stages of the session of dialysis [21,16].

It is found that plasma-hemoglobin concentration above 250mg/100ml is very toxic, the body degrades these molecules and kidneys cannot dispose of the waste in such quantities [18]. Moreover, a decrease in intracellular hemoglobin concentrations can lead to anemia.

Blood transfusions are common in infants, so hemolysis also has an effect on pediatric patients [1,3]. Blood is injected into the veins in the scalp of under-weight newborns. This is done with small needles that may result in hemolysis and a high level of hemolysis may become dangerous to the patient's life.

One of the main reasons causing hemolysis is the flow of blood through needles. Blood flows through the needle when it is injected into the body or when it is drawn out of the body. Therefore the flow of blood in the needles is studied under two different cases:

(i) when blood is drawn from the body (reverse orientation in our experiment) like sampling for labs, dialysis and apheresis

(ii) when blood is injected into the body (normal orientation in our experiment) like transfusions, dialysis and apheresis.

The main cause of hemolysis in needles is the fluid stress acting on the blood cells [22]. It is found that the maximum stress acting on the blood cells passing through a needle is at the entrance of the needle. So in order to reduce the stress at the needle entrance, a new rounded entrance was proposed and experiments were performed to show that the hemolysis is reduced in the modified needles compared to the standard needles.

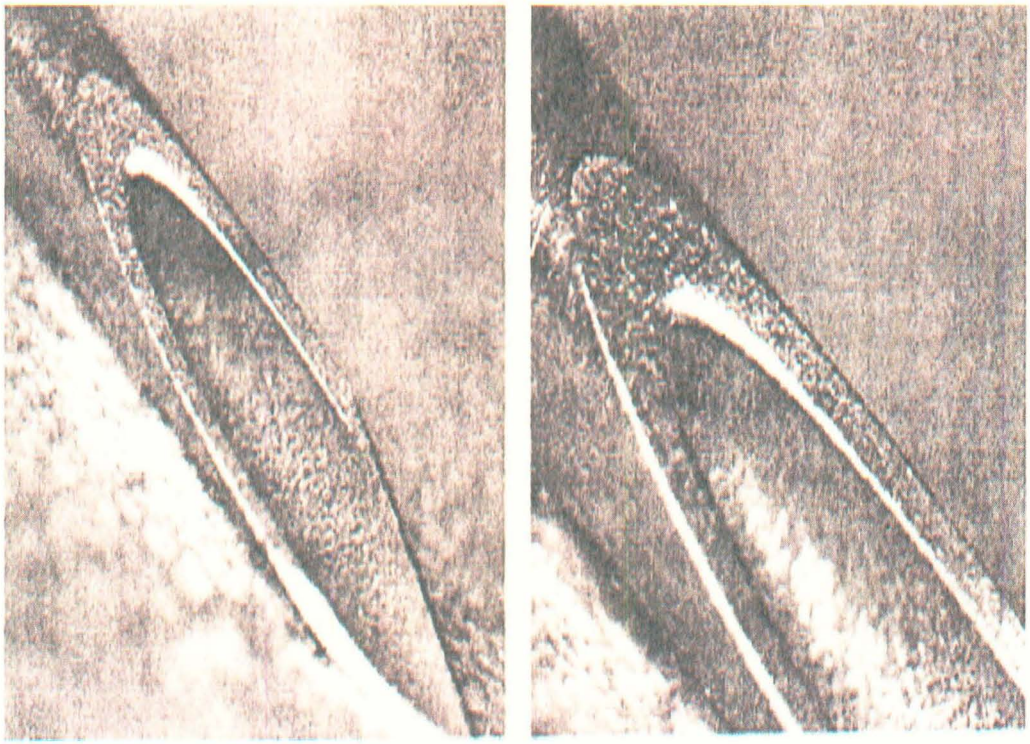

Figure 3. Standard Needle with sharp entrance [15]

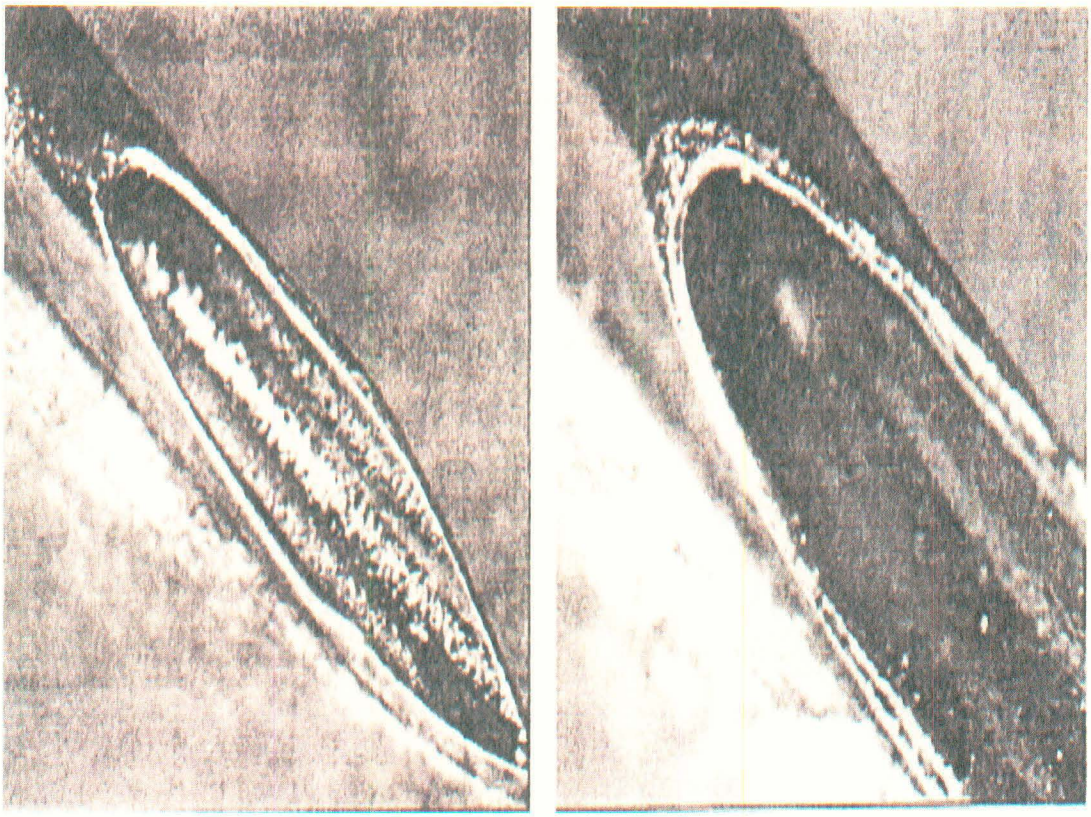

Figure 4. Modified needle with rounded entrance [15]

This project explains in detail the study of hemolysis in two different sets of needles viz. 20G and 16G needles. The needle entrance in both these cases was modified to reduce the effect of stress on the blood cells passing through it compared to that in a standard needle (Figure 3). There were two sets of 16G needles, one with rounded entrance (Fig. 4) manufactured by the electric discharge machining (EDM) method and the other set of needles with beveled entrance by the micro-machining method (Fig. 6). The 20G needles with rounded entrance were manufactured by EDM.

The 20G and 16G needles with rounded entrance were manufactured by wire EDM by Norman Noble Inc. Wire EDM technique is different from conventional vertical EDM in that wire EDM uses electric discharge erosion action with a wire electrode moving longitudinally through the work-piece. Standard 304 stainless steel needles were modified to produce a more rounded entrance compared to the standard needles.

One set of 16G needles was modified on an ultra high precision micro-milling machine that allows small scale machining. A fixture was designed to align the needle in the desired position during the machining process. A photograph of the needle during machining is shown in Fig. 6.

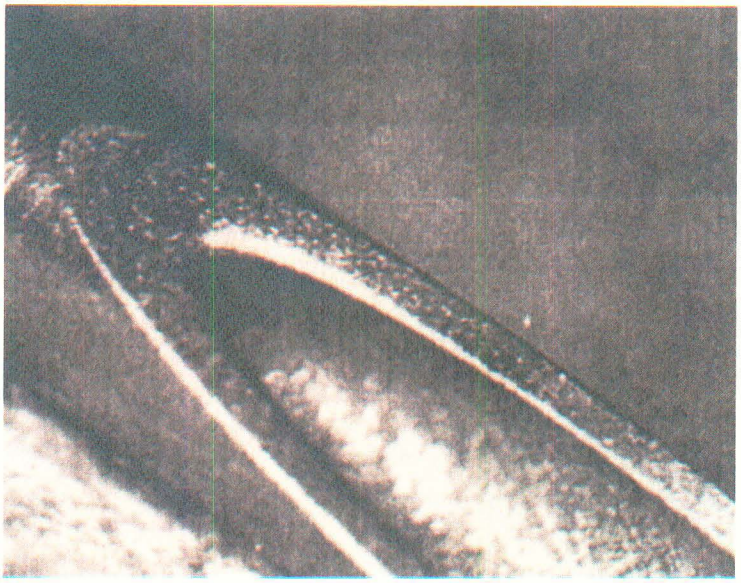

Figure 5. Standard needle

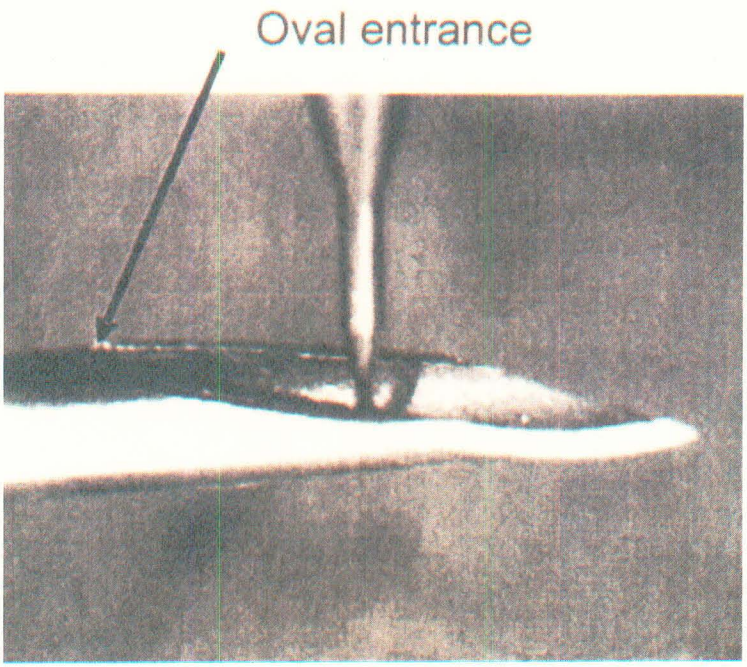

Figure 6. Modified needle without oval shape

II. LITERATURE REVIEW

Many experiments have been conducted to study the different factors effecting hemolysis including stress, time of exposure to stress, cell-cell interactions and cell-surface interactions [35]. Shapiro & Williams studied the effect of low shear stress on blood in a double gap viscometer [35]. They concluded from their experiments (low stress) that stress is not the only factor responsible for hemolysis and in their case the hemolysis was due to surface interactions (cell-cell and cell-surface). They also found that hemolysis is an increasing function of time when the stress is constant [35].

Leverett and many other researchers performed experiments in a rotational viscometer [27]. They found that there is a threshold shearing stress (1500 dyn/cm^2 in their case) above which the hemolysis is only due to shearing stress and time of exposure to stress, and all the remaining factors such as cell-cell interactions, cell-surface interactions, mixing of sheared and unsheared layers and viscous heating were negligible. They also concluded that in low-stress regions, cell damage is low and is due to surface interactions and in high stress regions, cell damage is high and is purely based on the high shear stress and time of exposure [27].

Leverett et al. came to a conclusion that when stresses is above 1500dyn/cm^2, the cell damage is purely due to shear stress and all the other factors like cell-cell and cell-surface interactions had no significant role [7]. Many other experiments in which the blood cells were subjected to low stresses showed that cell damage is directly proportional to the surface-to-volume ratio [35].

From experiments, many other scientists obtained different values of threshold stresses. The threshold stress depends on the magnitude of stress and the time of exposure [27]. Rooney (1970) conducted experiments with pulsating gas bubble immersed in blood and Williams et al. (1970) using oscillating wire obtained a threshold value of 5600 dyn/cm^2. The times of exposure were 10^{-3} sec and 10^{-4} sec respectively for both the experiments. Keshvaiah (1970) and Blackshear (1971) obtained the threshold stress value of 4500 dyn/cm^2 for ordinary capillaries and 7000 dyn/cm^2 for capillaries with smooth and tapered entrance. They conducted their experiments with canine blood flow through capillaries and the time of exposure was 10^{-2} sec. Forkstrom (1969) and Blackshear (1970) performed experiments with jets of blood and jets of other liquids into blood and they obtained the threshold stress value as high as 40000 dyn/cm^2 when exposed for 10^{-5} sec [27]. The magnitude of stress and the time of exposure calculated from various experiments are tabulated below in Table 1.

SUMMARY OF EFFECT OF SHEAR STRESS ON HEMOLYSIS

Type of exposure	Order of magnitude of exposure time	Threshold level of damage	References and comments
	sec	*dynes/cm²*	
Turbulent jet	10^{-5}	40,000	Forstrom (1969) and Blackshear (1971)
Oscillating wire	10^{-4}	5600	Williams et al. (1970) (human and canine)
Oscillating bubble	10^{-3}	4500	Rooney (1970) (human and canine)
Capillary flow	10^{-2}	5000	Bacher and Williams (1970) (bovine blood)
Capillary flow	10^{-2}	4500–7000	Keshaviah (1970) and Blackshear (1971) (canine blood)
Concentric cylinder	10^{2}	1500	This work
Concentric cylinder, maximum stress, 600 dynes/cm²	10^{2}–10^{3}	Relatively little hemolysis per unit time	Shapiro and Williams (1970) (surface effects dominate)
Concentric cylinder, maximum stress, 250 dynes/cm²	10^{3}	Relatively little hemolysis per unit time	Knapp and Yarborough (1969) (surface effects dominate)
Concentric cylinder, maximum stress, 600 dynes/cm²	10^{3}	Relatively little hemolysis per unit time	Steinbach (1970) and Blackshear (1971) (surface effects dominate)

Table 1. Red blood cell damage by shear stress [27]

Mechanical hemolysis has been studied in blood with flows in different devices such as concentric viscometer, cone & plate viscometer, capillary tube. In all of these devices, the surface effects have a role in the hemolysis. This makes it difficult to study the effect of shear stress alone. Experiments were conducted to reduce the surface effects where pulsating gas bubble and oscillating wire were used to hemolyze blood [26].

Blackshear defined three classes of mechanical hemolysis:

(i) surface induced- where the surface interactions have a significant role in the hemolysis.

(ii) in-bulk medium stress (1000 – 2500 dyn/cm^2)

(iii) in-bulk high stress (order of 40000 dyn/cm^2)

The hemolysis of the second type takes a longer time of exposure whereas the hemolysis of the third type is more spontaneous (Fig. 7).

Sutera and Mehrjardi conducted experiments in a concentric cylinder viscometer, applying stress in increments. They studied the cell shape after fixing the cells with gluteraldehyde while the viscometer was rotating. To observe the cells, a 1-2 ml sample was centrifuged and then washed with distilled water. A drop of distilled water was placed on a 12mm dia glass cover slip placed on a scanning electron microscope (SEM) stub. Small amount of fixed cells were added and finally coated with chromium in a vacuum evaporator. They concluded that the flow in their case (stress 100-4500 dyn/cm^2) was turbulent. They also checked the reversibility of the deformation. They found that the red cells that were subjected to low shear could regain their original shape [26].

Hemolysis in needles was also found to depend on the driving pressure. Eurerius and Smith performed experiments while varying the driving pressure and found that there was an increase in hemolysis with increase in driving pressure and age of blood [3]. Blood

was forced through needles of 18, 22 and 26G at driving pressures of 100, 200 and 300 mm HG. A fenwal bag system was used to deliver the above mentioned pressure [3].

Hemolysis was found to depend on the age of the blood. Wilcox, et al. conducted experiments on blood using a constant-rate syringe delivery pump for 25G needle and found that the hemolysis was greater in the samples that were stored for 9 days compared to the samples stored 2 days [2].

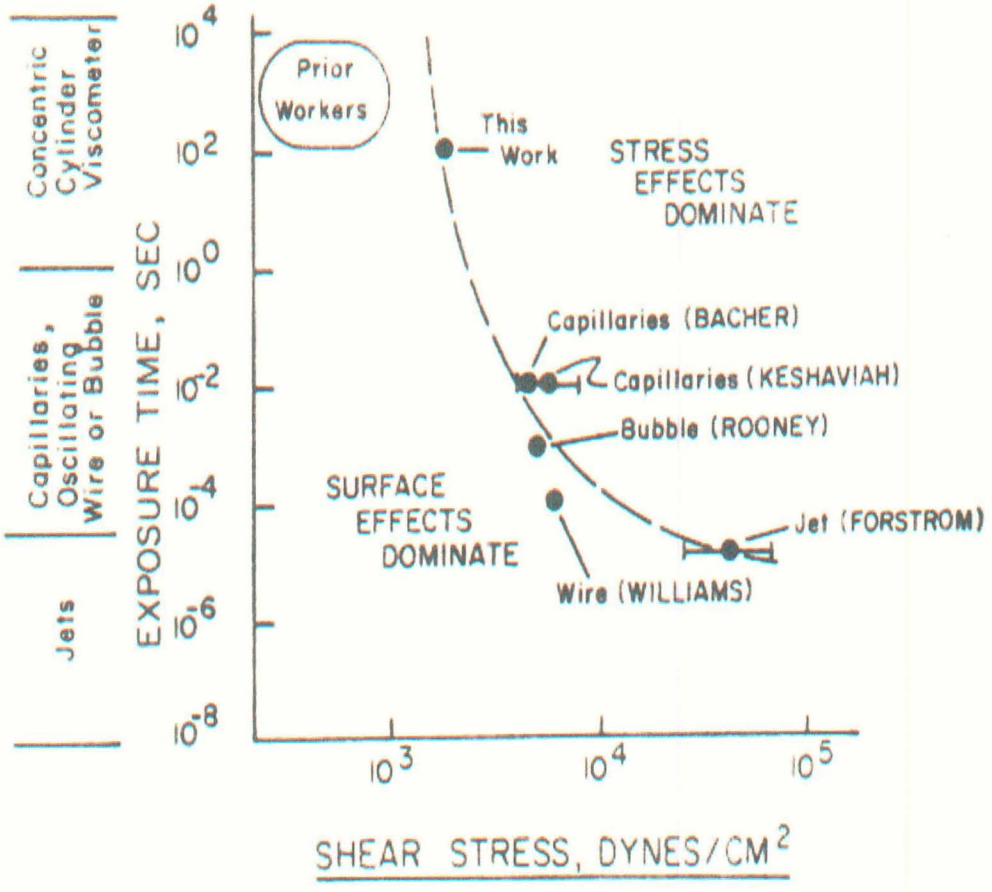

Figure 7. Effect of exposure time on the threshold shear stress [27,32]

Calkins and others studied the effects of dilution, pressure and flow rate on hemolysis [24]. They conducted experiments and found that the hemolysis was less in samples, which were diluted compared to the undiluted ones. They found that high pressure, more dilution and short tubing will cause increase in flow rate that in turn increases the hemolysis [24].

Laugel and Beissinger performed experiments to study hemolysis under low-stress conditions in a capillary tube. They concluded from their results that blood damage increases very fast in the first few seconds and increases slowly afterwards. They also concluded that the RBC's must travel a minimum capillary length before any hemolysis occured and that the cell damage depended on two factors: time of shearing and shearing intensity level [33].

Yang and Lin conducted experiments with a minimodule dialyser and studied the flow conditions by changing the flow rates and blood volume. They found that hemolysis increased with increase in blood flow velocity and decreased with increase in blood volume [29].

Yasuda and other researchers performed experiments on a rotational viscometer to study the effects of pressure and shear stress on hemolysis. They found that there was no influence of pressure and temperature on hemolysis and there was significant hemolysis when the shearing rate was 1500 /sec or more applied for 120 minutes [5,7] (Fig. 8).

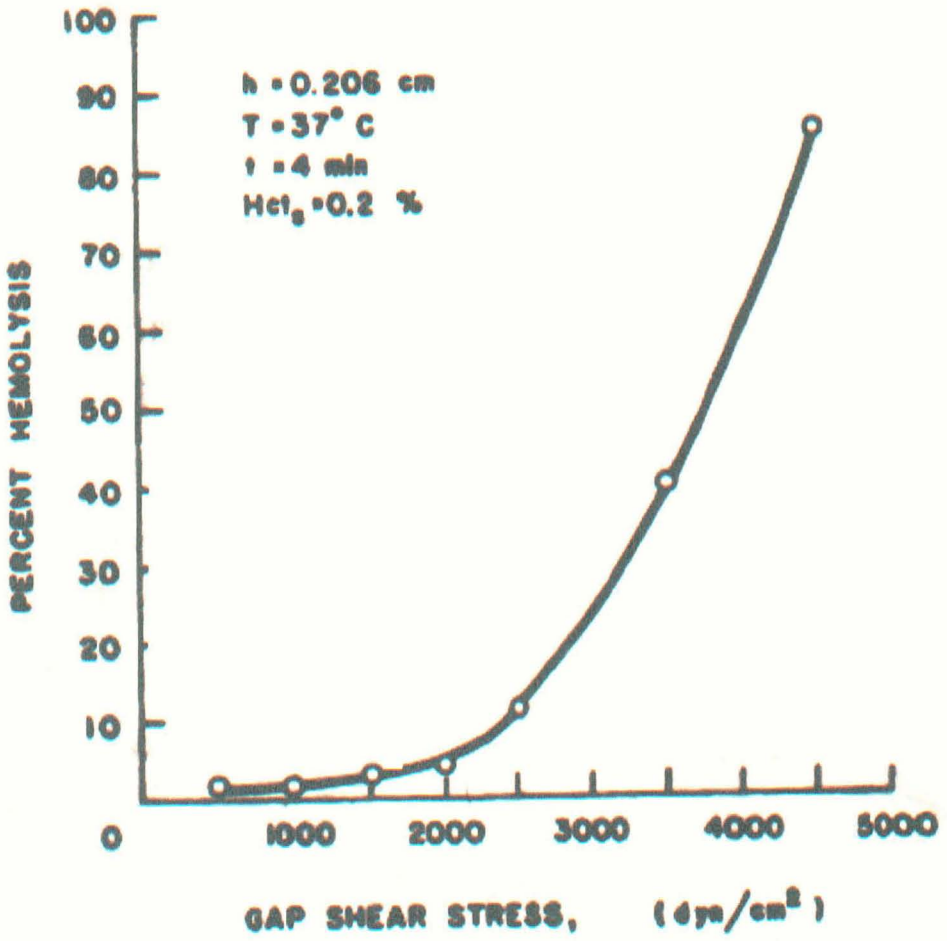

Figure 8. %Hemolysis as a function of applied stress [26]

In summary, hemolysis in needles can be attributed primarily to stress and time of exposure to stress, and secondly to cell-surface interactions and age of blood. In this thesis experiment there were two sets of needles (16G & 20G) and each set comprised three modified (rounded or beveled entrance - test needles) and three standard needles (control needles). Experiments were performed with the needle in both normal and reverse orientation with three different driving pressures (explained in detail in later sections).

III. EFFECT OF SHEARING STRESS ON BLOOD CELLS

Hemolysis is known to depend mainly on two reasons, high shear stress acting on the blood cells and the time of exposure to high shear stress. The flow velocities in the needle are maximum at the center of the needle and minimum at the needle walls. Blood when acted upon by high shear stress damages the red blood cell membrane and the stress values at the needle entrance are almost three times higher compared to the stresses at the needle outflow [19].

The blood cells pass through the needle tip and they spend only a fraction of a second near the tip and still it causes damage to the blood cell. From this it can be understood that the stresses acting on the blood cell near the tip are lot more than the stresses acting inside the needle near the wall. The shear stress increases with increase in blood flow [22,19].

When stress acts on the red blood cell, the cell deforms and regains its shape if the stress applied is low. There is a certain value of the stress (3500 dyne/cm^2 in this case) [24] above which the cell can't regain its original shape after deformation. When the applied stress acting on the red blood cell is gradually increasing, the red blood cell becomes ellipsoidal in shape and then gradually into dumbbell shape (Fig. 9) and finally they separate into two parts (Fig. 10-11).

Figure 9. One of the cells is stretched into a dumbbell shape [26]

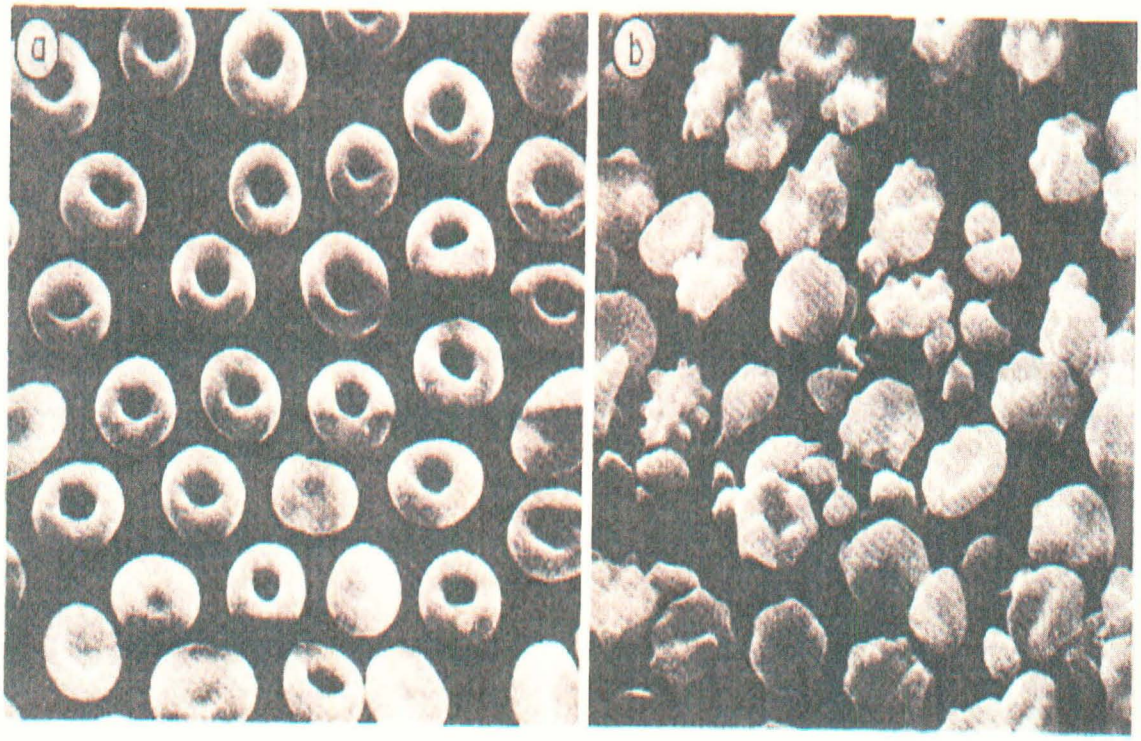

Figure 10. Human red blood cells sheared for 4min at (a) 2000dynes/cm2 (b) 3500dynes/cm2 and then recovered. (a) completely recovered its original shape [26]

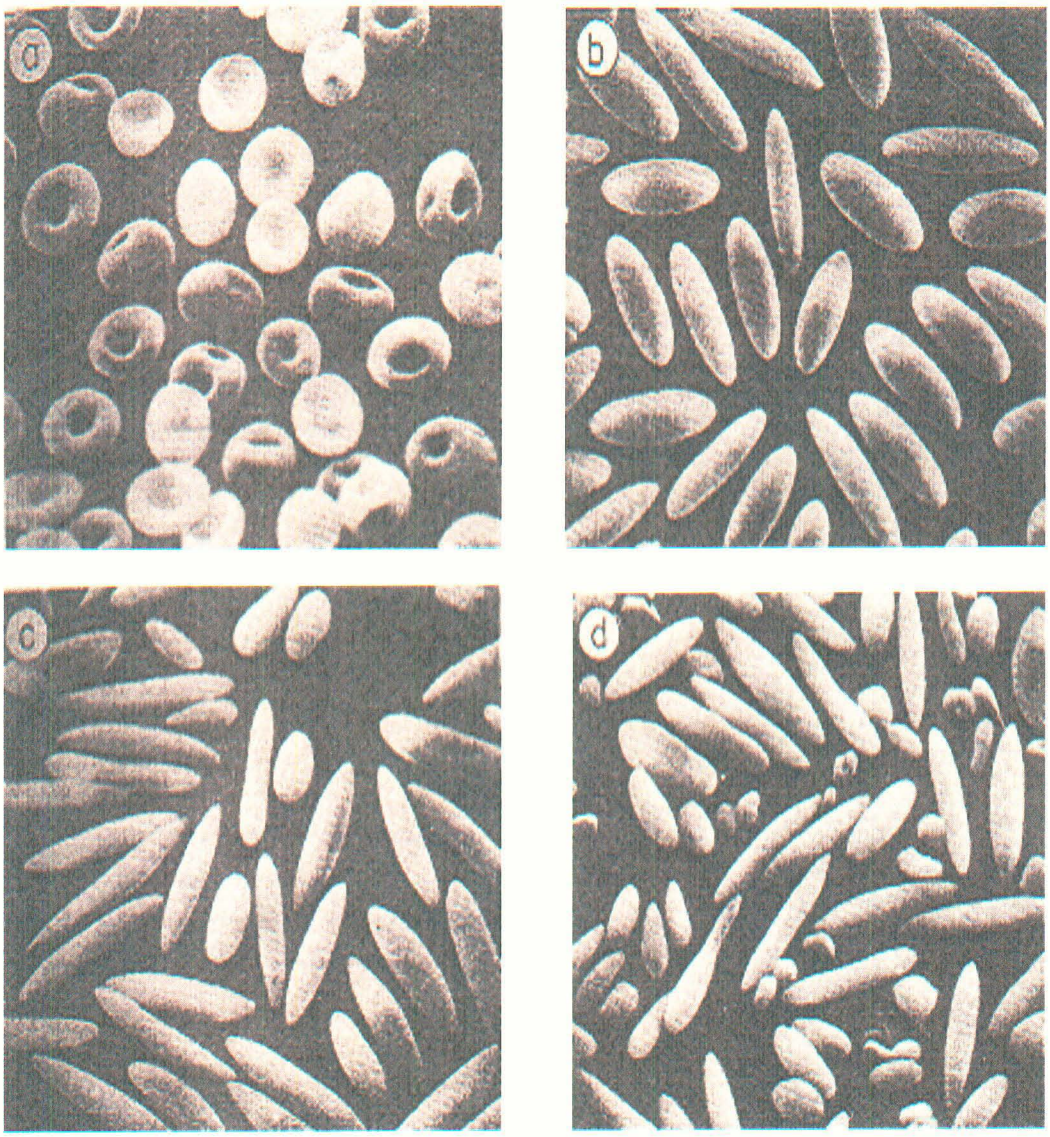

Figure 11. Human red cells fixed after sheared at (a)100dynes/cm2 (b) 2000dynes/cm2 (c) 3500dynes/cm2 (d) 4500 dynes/cm2 [26]

IV. METHODS AND MATERIALS

Experiments were performed with both 16G and 20G needles with the modified and standard entrance geometries and results were compared. Two sets of the 16G needles are manufactured. (i) beveled entrance by micro-milling (ii) rounded entrance by electric discharge machining (EDM). The 16G and 20G needles used in the experiment were 1 ½" in length, all the experimental conditions are tabulated in Table 2. The pressure driving the flow through the needle bore was reduced compared to the chamber pressure due to the friction of the plunger against the bore of the syringe, viscous losses of the blood flowing in the syringe and hub, and inertia of the blood in the syring and needle. The driving pressure was calculated by the model in Sharp & Mohammad (1998).

Needle Size	Needle Shape	Manufacturing Technique	Chamber Pressure (psi)	Driving Pressure (psi)
16G	Rounded	EDM	15	5.5
			30	15.5
			45	24.4
16G	Beveled	Micro-milling	15	5.5
			30	15.5
			45	24.4
20G	Rounded	EDM	20	12.2
			35	22.4
			50	31.7

Table 2. Detailed experimental conditions with needle size, shape, manufacturing technique and pressures.

Electric discharge machining (EDM), also known as spark machining or spark eroding, is a non-traditional machining method primarily used for hard materials. This method involves a series of rapidly recurring electric arc discharges between an electrode, which is the cutting tool and the workpiece. The cutting tool is made to travel along the desired path very close to the work piece without actually touching it resulting in the formation of series of micro-craters on the workpiece. The removed particles are washed away by the dielectric fluid. There are primarily two types of EDM machines, ram EDM and wire-cut EDM. The method used in manufacturing the needles for this experiment is wire EDM. Wire-cut EDM has the ability to machine very intricate and complicated shapes and uses water as its dielectric. The limitation of using EDM method is that it can be applied only to electrically conductive materials. Micro-milling is similar to the more conventional machining process, except on a small scale. The needles with rounded entrance are difficult to manufacture using the micro-milling process, therefore EDM technique was used for this and micro-milling was used only for making the beveled needles.

Three different pressure values were selected that provided little or no hemolysis, moderate hemolysis and high hemolysis for all the needle sizes. There are some steady flow losses in the syringe and also by the fluid and syringe plunger inertia that account for the pressure difference in the chamber pressure and the pressure across the needle [14]. Therefore the actual driving pressure across the needle is less than the chamber pressure, they were calculated for the two sets of pressures and are tabulated in Table 3.

Blood when drawn by pulling the plunger of the syringe, pressure created by vacuum is much lower than the lowest pressure that we operate. There might not be significant difference in hemolysis for the modified and standard needles at such low pressure. In order to see hemolysis, much higher driving force is used. To apply a controlled pressure to force blood through the needles, a pressure chamber was designed that used a four-inch cast iron flanged tee (Fig. 12). This airtight chamber was connected to a supply of compressed air. Two valves and a pressure gauge were provided to adjust the pressure to the desired value before each test.

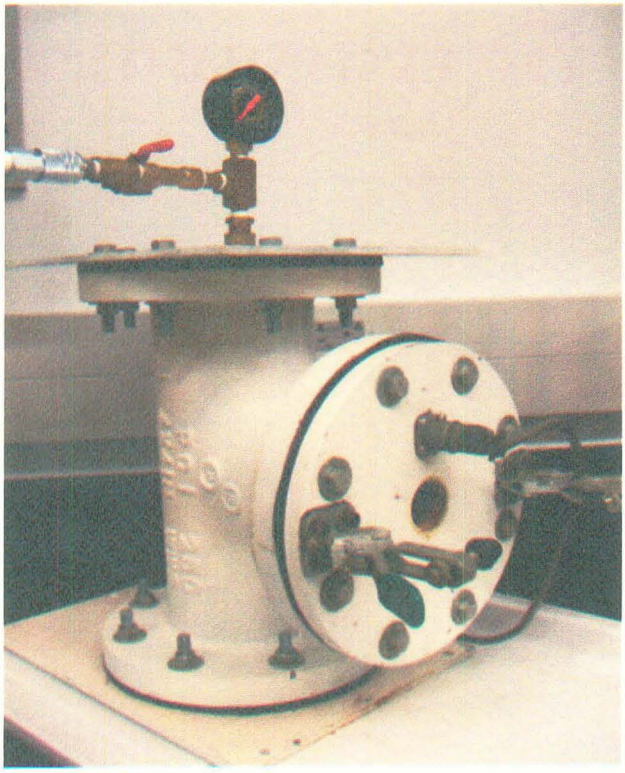

Figure 12. Fixture that holds the syringe

Located inside the chamber was a mechanism with a solenoid that served to hold the plunger of the syringe in place and release the plunger when the solenoid was engaged (Fig. 14).

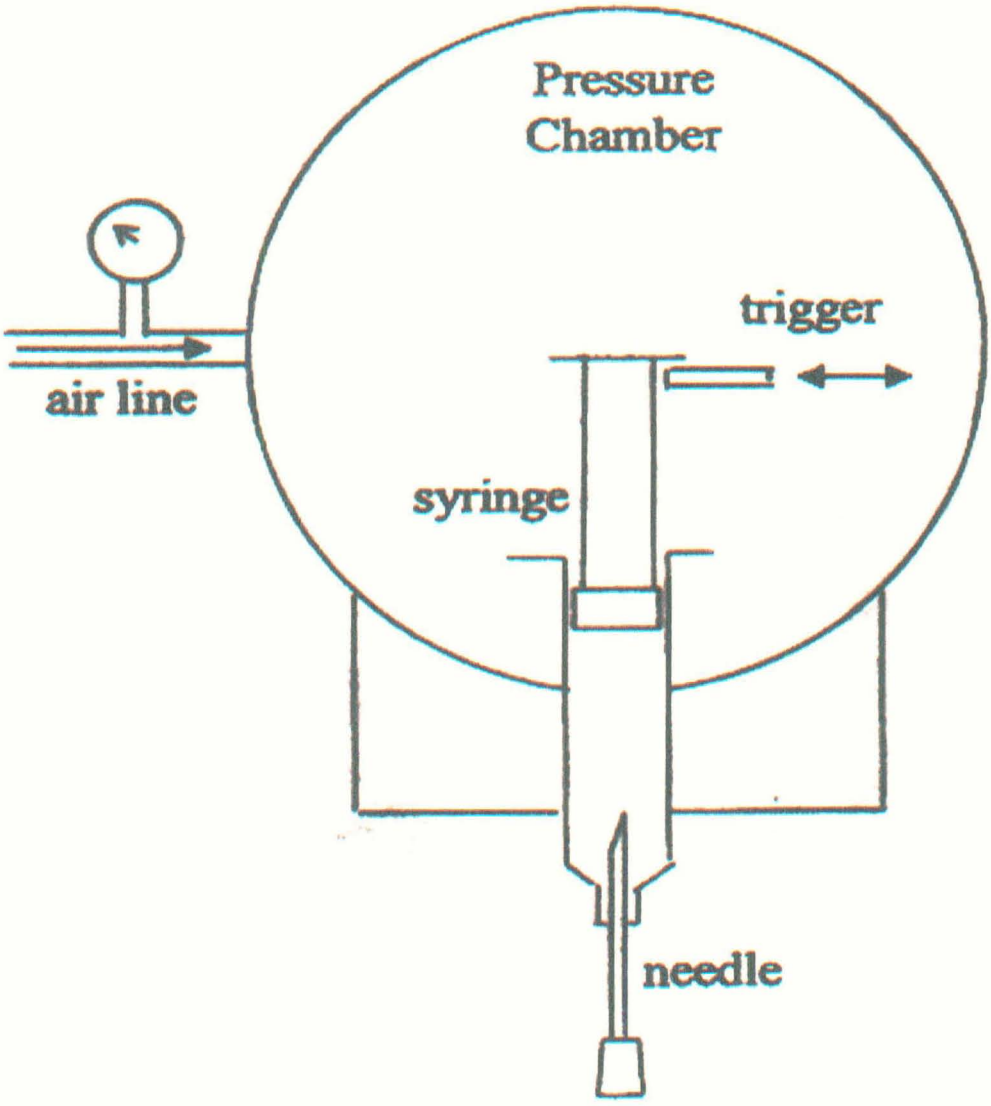

Figure 13. Schematic of the experimental setup with needle in the reverse orientation [14]

When the plunger was released, the pressure inside the chamber forced the blood in the syringe through the needle. A syringe adapter facilitated an air-tight seal between the syringe and the chamber, and hold-down clamps allowed quick attachment of the assembly to the chamber.

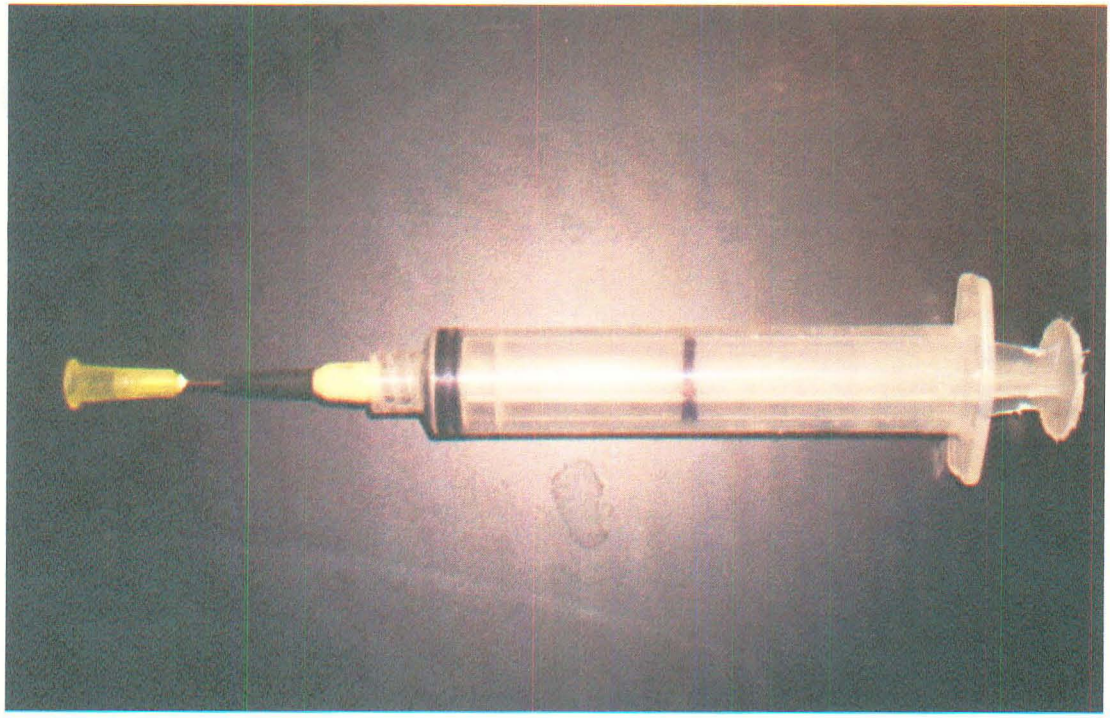

Figure 14.Needle attached in the reverse orientation

Figure 15.Needle attached in the normal orientation

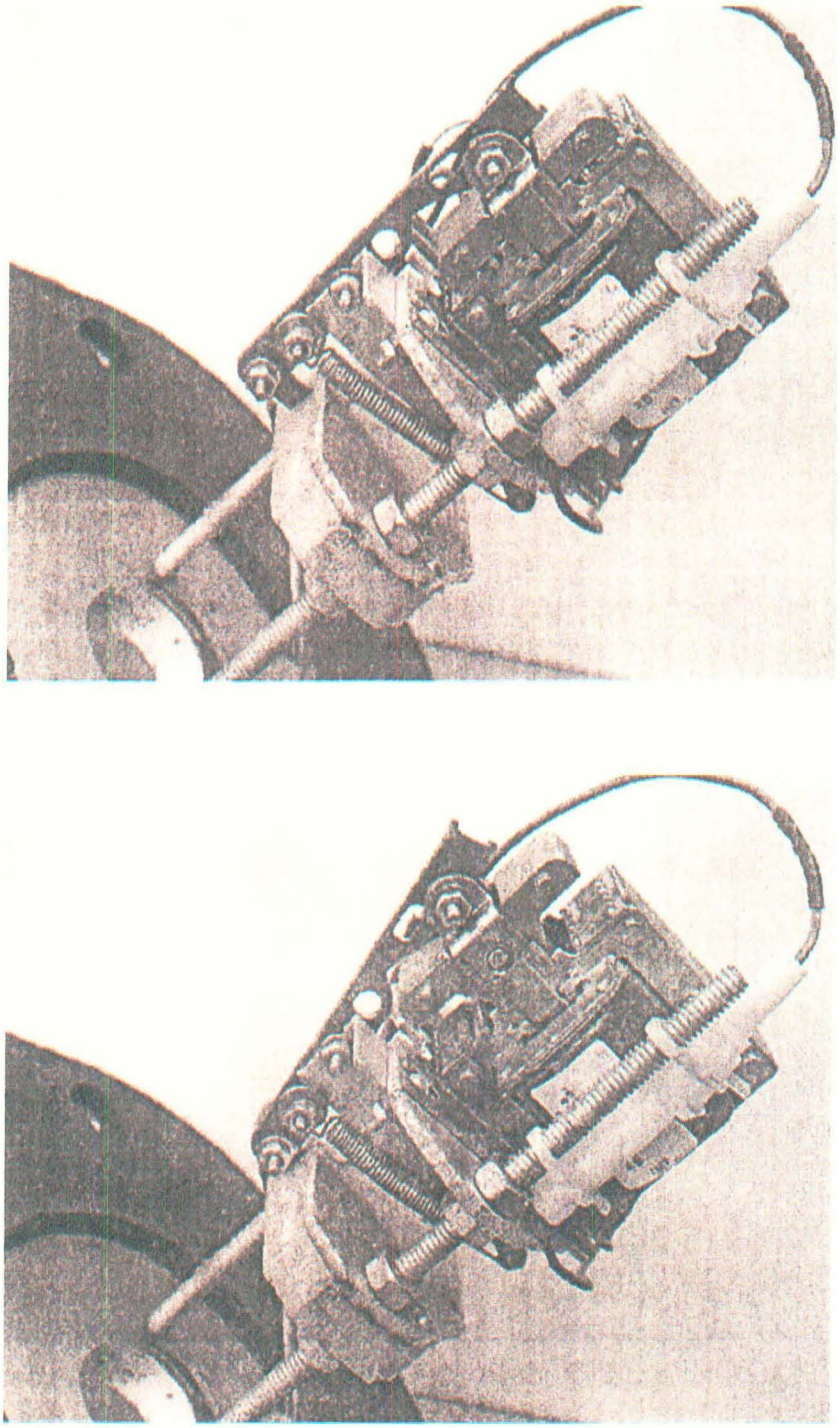

Figure 16.Solenoid in (a) engaged position (plunger unlocked) (b) disengaged position (plunger locked)

V. EXPERIMENTAL PROCEDURE

Each array of experiments required approximately 300ml of expired banked blood. These units of blood were acquired from the University of Louisville blood bank. The following procedure was followed to measure the hemolysis in needle flow:

The blood sample was first tested for its hematocrit level. Two samples were drawn in micro capillary tubes and then centrifuged for 10 minutes in an AUTOCRIT ULTRA 3 centrifuge (Fig. 15). During the centrifugation process, the red blood cells that are higher in density compared to plasma, settle to the bottom of the capillary tube. The hematocrit level was then measured as the ratio of the length of the column of red blood cells to the length of the column of whole blood.

Before the experiment with each blood sample, the hematocrit of the sample was adjusted if necessary by adding saline to make the hematocrit value between 40%-45%. Saline solution is added to the blood instead of water because the addition of water to blood will result in the rupturing of red blood cells. An isotonic solution of NaCl and water was used so that the RBC's are in equilibrium with the solution and the cells are not ruptured or destroyed.

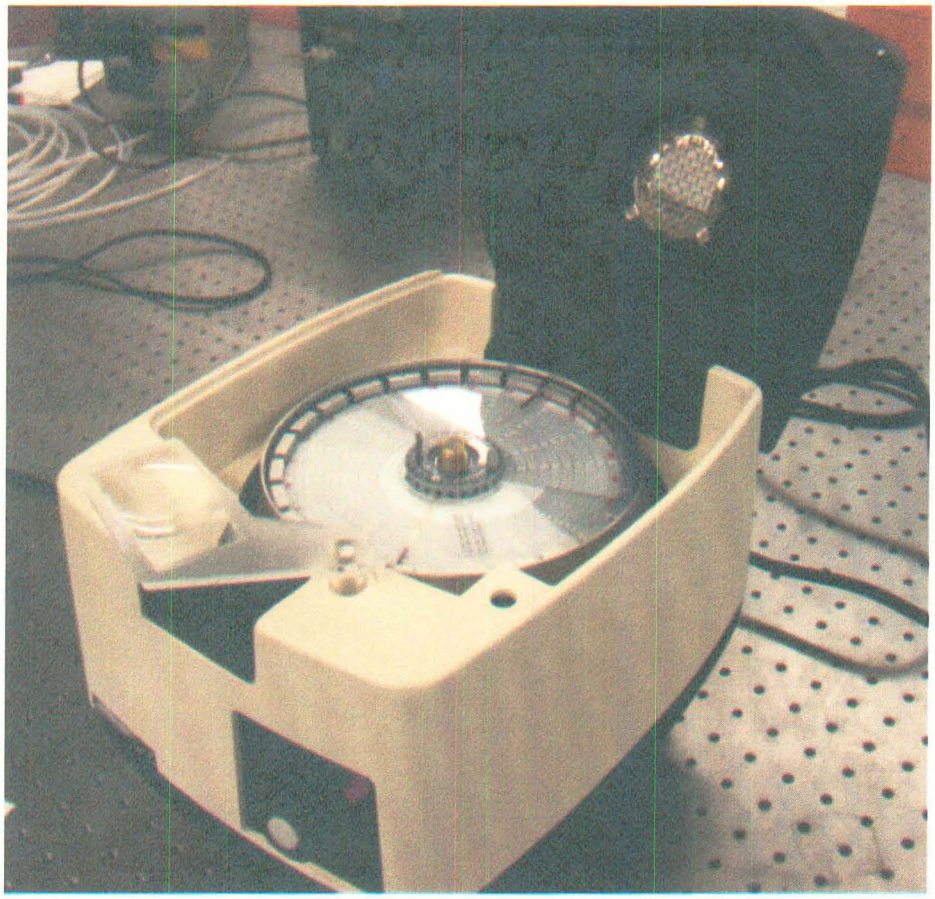

Figure 17. AUTOCRIT ULTRA3 - Centrifuge to measure the hematocrit

The necessary dilution to adjust hematocrit was calculated by the following method.

Assume the initial volume of the blood = 300 ml

Let the measured hematocrit = X ml

Volume of 0.9 % Saline to be added =Y ml

$300 * X = (300+Y) * 0.45$ ..... (i)

From (i), we can calculate the volume of saline needed to add to the whole blood to make it 45% hematocrit.

Two more samples of blood were then taken and centrifuged again for 10 minutes and the hematocrit was determined. The above procedure was performed until the hematocrit level was around 40% - 45%.

For each of the cases in the main array of experiments, 3-5 ml of blood was drawn into the syringe. The proper needle was then attached to it and the assembly was placed in the fixture. The two hold-down clamps on the fixture were used to secure the syringe before applying pressure. The solenoid was disengaged, which allowed the trigger bar to slide under the plunger by spring action. The valves were used to adjust the chamber pressure and when the desired pressure was attained, the solenoid was engaged releasing the plunger. The blood flowing out of the needle was collected in a test tube. This process was repeated until all the combinations of pressures and needle orientations were completed with the control and test needles. The tests were conducted with two different orientations of the needle. (i) normal orientation (ii) reverse orientation. For the reverse

orientation, the needle was fixed to the syringe using a fiber optic cable boot (Fig. 16). The order of the tests was randomized.

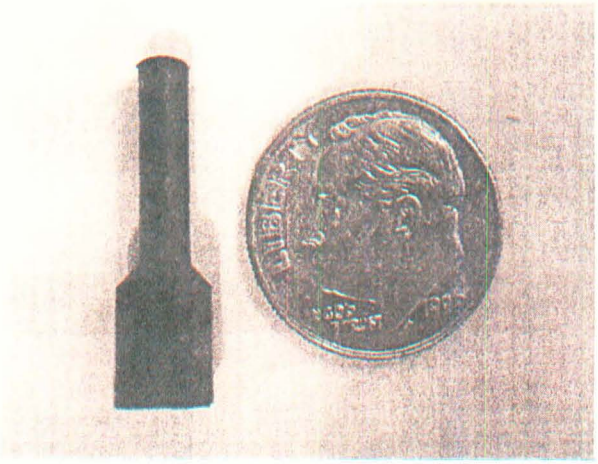

Figure 18. Fiber optic cable boot

Reference samples were also prepared by adding whole blood into distilled water based on Table 3.

Hemolysis (%)	Water (ml)	Whole Blood (ml)
10	4.500	0.500
5	4.750	0.250
2	4.900	0.100
Hemolysis (%)	**Water (ml)**	**10% Hemolysed (ml)**
1	4.500	0.500
0.5	4.750	0.250
0.2	4.900	0.100
0.1	4.950	0.050
0.05	4.975	0.025
Hemolysis (%)	**Water (ml)**	**2% Hemolysed (ml)**
0.02	4.950	0.050
0.01	4.975	0.025

Table 3. Table used for preparing reference samples

The sample test tubes along with the reference samples were centrifuged for twenty minutes at 2400rpm speed at 4^0C in a Beckman TJ-6 centrifuge (Fig. 17). After the blood samples were centrifuged, the red blood cells settle down due to the high density compared to the plasma (Fig. 18-19).

Figure 19. Beckman TJ-6 centrifuge

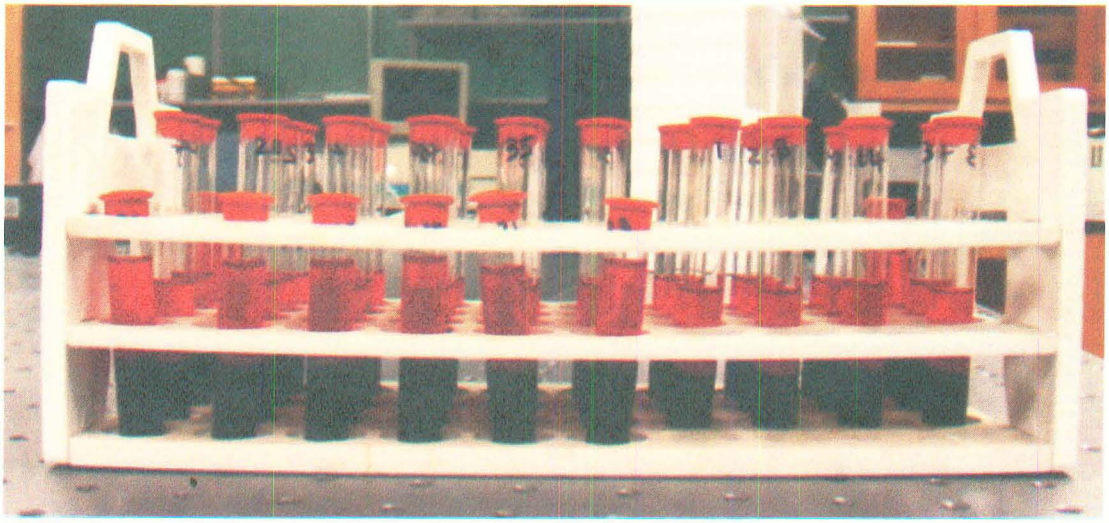

Figure 20. Test specimens after centrifugation

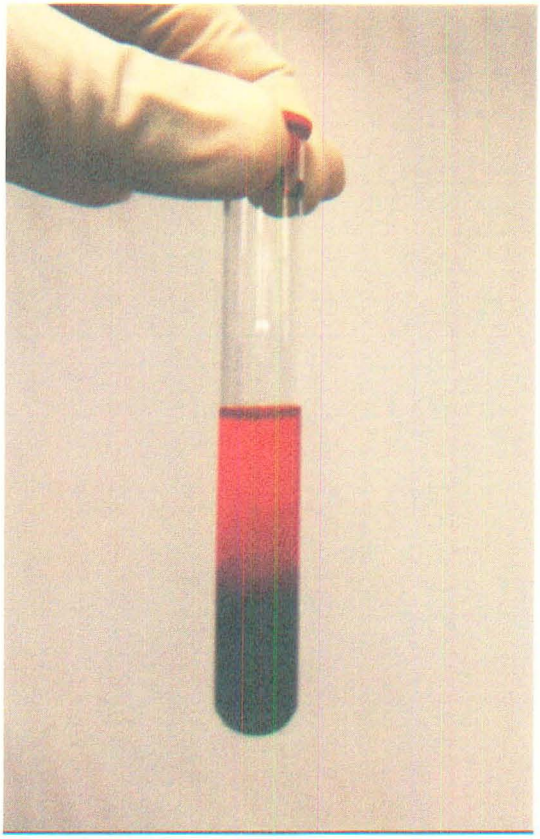

Figure 21. RBC's settle at the bottom after the centrifugation process

Plasma at the top of the sample test tubes was carefully pippetted so that there is no cell structure floating in it. This plasma was pippetted into a micro cuvette and then placed in a 6-cell holder (Fig. 20) inside a Beckman DU-650 Spectrophotometer (Fig. 21), to measure hemolysis.

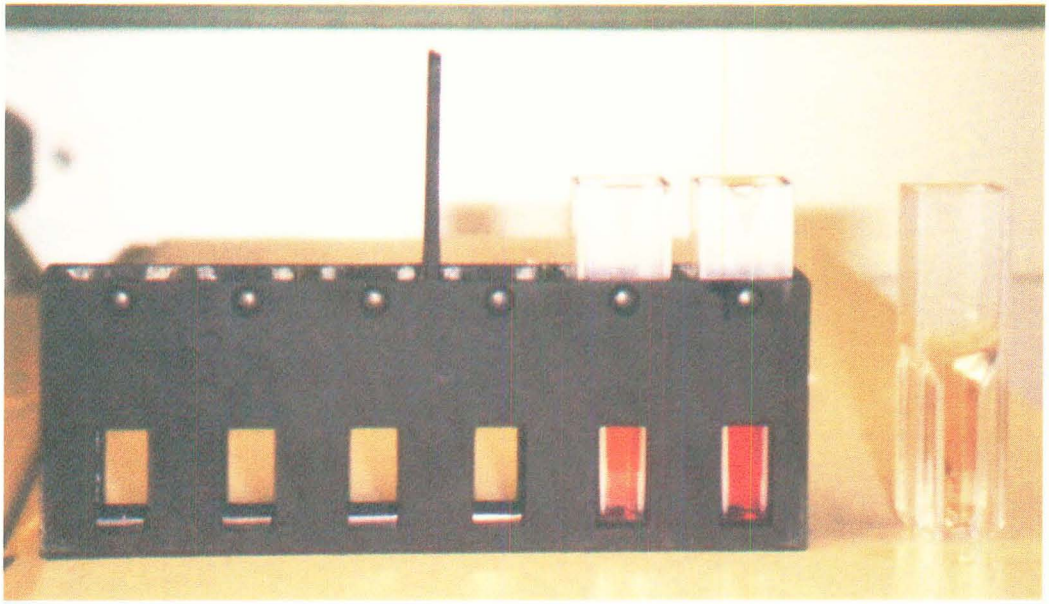

Figure 22. Cuvette placed in the 6-cell holder

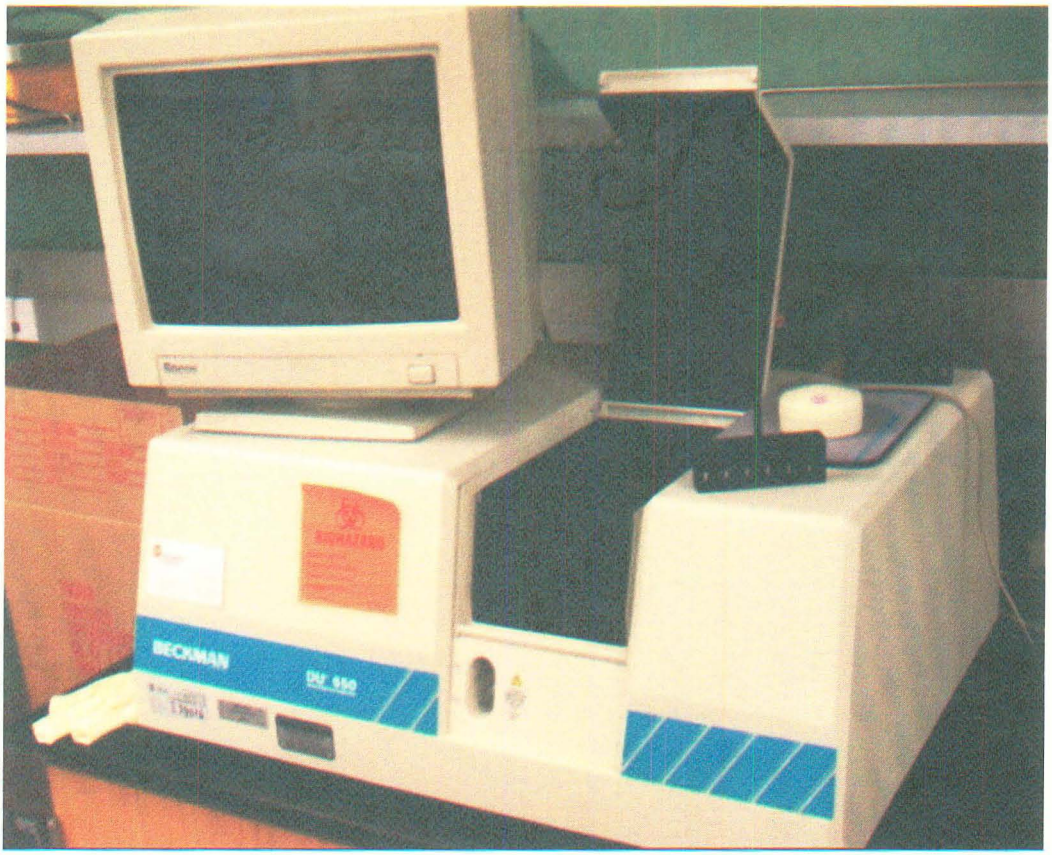

Figure 23. Beckman 650 Series Spectrophotometer

Spectrophotometer works on the principle of absorbance of wavelengths. Light is passed through the sample and is absorbed on the other end by a photodetector. Wavelengths are set based on the absorption spectrum of sample being tested. Three fixed wavelengths were used in this experiment.

After getting all the values from the spectrophotometer, hemolysis was calculated using the equation below:

$$\textbf{PFHB (Plasma-free hemoglobin)} = \left[A_1 - A_3 - (A_2 - A_3)\left(\frac{\lambda_1 - \lambda_2}{\lambda_3 - \lambda_2}\right) \right] 126 \quad \text{mg/dl} \; [12,14]$$

Where A_1 = absorbance at wavelength λ_1 = 576.5 nm

A_2 = absorbance at wavelength λ_2 = 596.0 nm

A_3 = absorbance at wavelength λ_3 = 560 nm

126 is an empirical factor

To calibrate the hemolysis values more precisely, the reference samples with known percent hemolysis were tested in the spectrophotometer. The PFHB values obtained by the spectrophotometric method were plotted versus known % hemolysis (example in Fig. 22). A common characteristic of the spectrophotometric method is that for high hemolysis, the curve becomes non-linear and inaccurate. To avoid this problem, a plot like Fig. 22 was produced for each unit of blood. The upper limit of linearity (usually 5% hemolysis) was observed and a linear curve fit was obtained for the points up to that limit. Samples with measured PFHB higher than the linear limit were diluted with saline to reduce the PFHB below the limit and remeasured with the spectrophotometer. The remeasured PFHB was then adjusted by the dilution fraction to obtain the PFHB of the original sample.

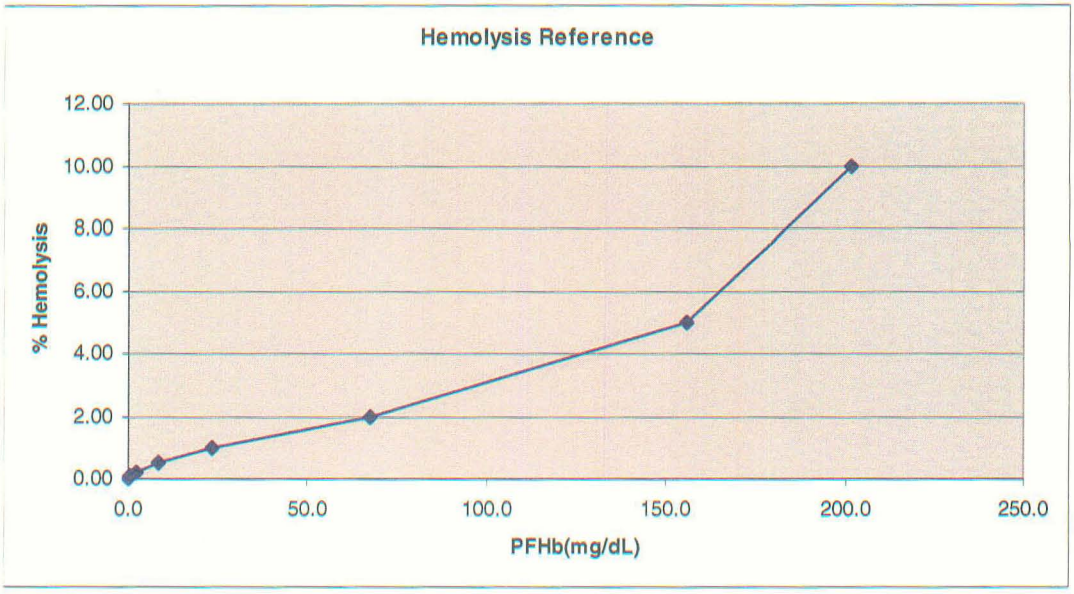

Figure 24. A typical graph plotted from an experiment depicting the hemolysis values to lie in the linear range

VI. RESULTS

This chapter presents the results. Experiments were conducted with the needle oriented in two different directions (normal & reverse) at three pressures of 15psi, 30psi and 45psi for the two types of 16G needles and 20psi, 35psi, 50psi for the 20G needles and the results are discussed separately.

The results for both the reverse and normal orientation of the 16G (rounded & beveled) and 20G rounded needles are summarized in Tables 4-6. The numbers in the tables represent the average percentage of hemolysis, standard deviation and ANOVA p-value for each control and test needle at the three different specified pressures calculated from the data in Appendices I-VI. A few values in the appendices were left blank, as some samples were not operated at correct pressures and in some cases, the blood sample unit was not sufficient to run all combinations of pressures.

20G Needle

The 20G needles with rounded entrance produced less hemolysis than the 20G standard needles for all pressures in reverse orientation (p-values less than about 0.05), but differences were not significant in normal orientation (Table 4).

REVERSE ORIENTATION						
	20psi		35psi		50psi	
	Standard	Rounded	Standard	Rounded	Standard	Rounded
AVG % HEMOLYSIS	-0.004	-0.062	0.417	0.153	2.364	1.006
STD DEV	0.102	0.124	0.283	0.168	0.933	0.610
P-VALUE	0.053		4.6 E-08		1 E-08	
NORMAL ORIENTATION						
	Standard	Rounded	Standard	Rounded	Standard	Rounded
AVG % HEMOLYSIS	-0.025	-0.013	0.182	0.148	1.108	1.264
STD DEV	0.113	0.085	0.109	0.170	0.529	0.770
P-VALUE	0.665		0.357		0.365	

Table 4. Average percent hemolysis, standard deviation and p-values for the 20G needles in normal and reverse orientation

16G NEEDLE (MICRO-MILLED)

There was no reduction in hemolysis with the 16G needles with beveled entrance. P-values for the beveled needles in both normal and reverse orientations for all pressures were 0.09 or more (Table 5).

REVERSE ORIENTATION						
	15psi		30psi		45psi	
	Standard	Beveled	Standard	Beveled	Standard	Beveled
AVG % HEMOLYSIS	0.021	0.045	0.378	0.279	1.485	1.387
STD DEV	0.214	0.243	0.338	0.151	0.415	0.499
P-VALUE	0.704		0.172		0.444	
NORMAL ORIENTATION						
	Standard	Beveled	Standard	Beveled	Standard	Beveled
AVG % HEMOLYSIS	0.023	0.007	0.280	0.348	1.171	1.352
STD DEV	0.240	0.238	0.207	0.274	0.341	0.413
P-VALUE	0.810		0.309		0.090	

Table 5. Average percent hemolysis, standard deviation and p-values for the 16G needles (micro-milled) in normal and reverse orientation

16G NEEDLE (EDM)

Hemolysis values were significantly reduced for the 16G needles with rounded entrance in the reverse orientation at all pressures and there was no significant difference in the normal orientation. P-values for the reverse orientation at all pressures were less than 0.05 and p-values for the normal orientation were not (Table 6).

REVERSE ORIENTATION						
	15psi		30psi		45psi	
	Standard	Rounded	Standard	Rounded	Standard	Rounded
AVG % HEMOLYSIS	-0.004	-0.017	0.232	0.143	1.368	1.090
STD DEV	0.015	0.022	0.120	0.095	0.277	0.303
P-VALUE	0.017		0.004		0.001	
NORMAL ORIENTATION						
	Standard	Rounded	Standard	Rounded	Standard	Rounded
AVG % HEMOLYSIS	-0.003	-0.013	0.207	0.298	1.047	1.124
STD DEV	0.032	0.022	0.136	0.206	0.260	0.258
P-VALUE	0.176		0.063		0.282	

Table 6. Average percent hemolysis, standard deviation and p-values for the 20G (rounded & beveled) needles (EDM) in normal and reverse orientation

The average percent hemolysis, standard deviation and the p-values for the 20G and 16G needles with rounded entrance for the reverse orientations are plotted in Fig. 25-26.

Fig. 25 is plotted with the average percent hemolysis against three pressures 15psi, 30psi and 45psi for the 16G needles with rounded entrance in the reverse direction. Hemolysis was significantly reduced at all pressures.

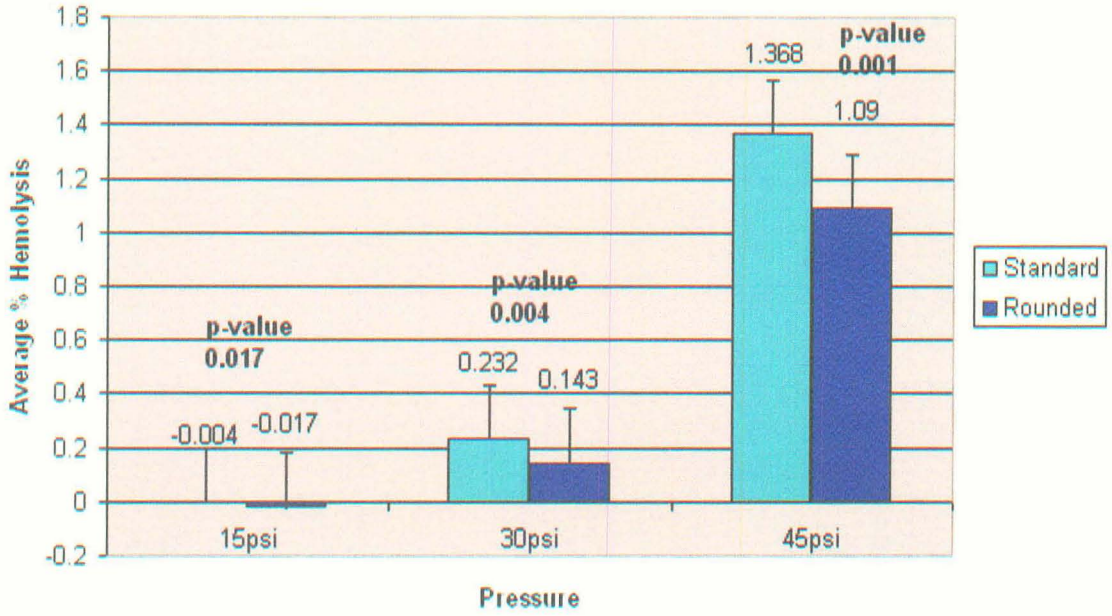

Figure 25. Graph with average percent hemolysis versus pressure for the 16G needles with rounded entrance in the reverse orientation

Fig 26 is plotted with the average percentage hemolysis against three pressures 20psi, 35psi and 50psi for the 20G rounded needles in the reverse. Hemolysis was significantly reduced at all pressures.

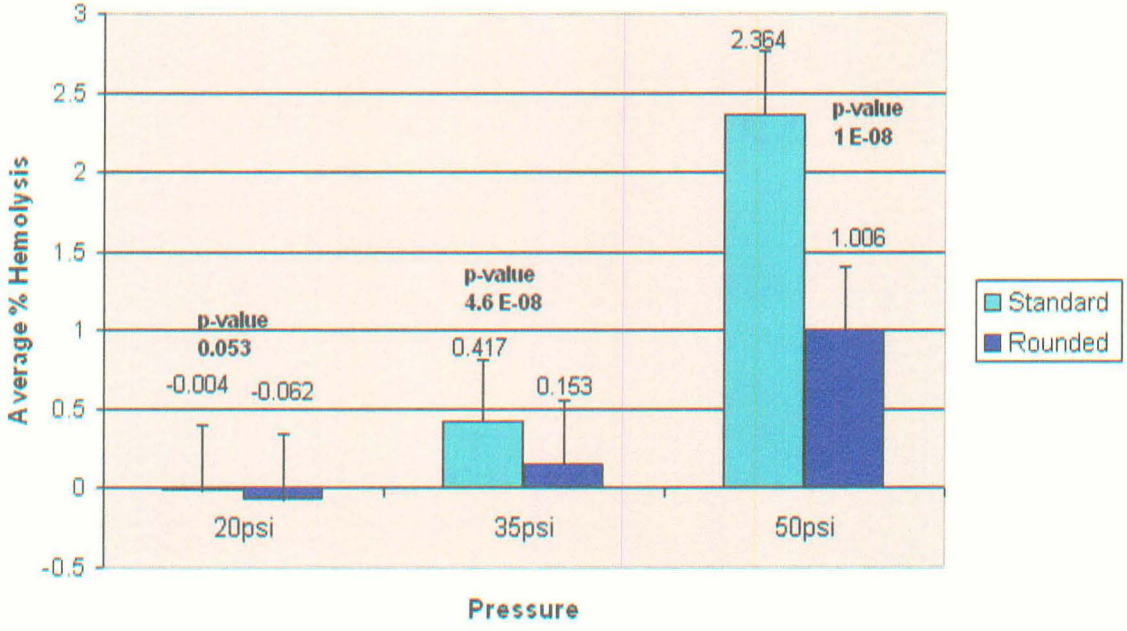

Figure 26. Graph with average percent hemolysis versus pressure for the 20G EDM needles in the reverse orientation

VII. DISCUSSION

This chapter explains the results and contains related discussion. 16G and 20G needles with rounded entrance significantly reduced hemolysis in the reverse direction and 16G needle with beveled entrance showed no significant improvement in reducing hemolysis.

The main reason that the needles with rounded and beveled entrances were expected to reduce hemolysis is that these entrance geometries are known to reduce pressure drop and fluid stresses for flow into such entrances in conventional piping systems, with the rounded entrance producing the greater effect. This expectation was confirmed by the 16G and 20G rounded needles in reverse orientation. In addition, rounding and beveling has little effect on pressure drop and fluid stresses when the flow is exiting these geometries in conventional piping systems, thus no difference in hemolysis was expected for normal orientation. Again, the 16G and 20G rounded needles demonstrated this expected behavior in the normal orientation. The 16G beveled needles also showed no significant differences in hemolysis in the normal orientation. Thus there is one important result to be explained - why the beveled entrance did not reduce hemolysis in the reverse direction.

Firstly, red cells have inherently nonlinear response to fluid stresses. While minor releases of hemoglobin may occur due to high stress of short duration when self-healing pores are created in the membrane, the major component of hemoglobin loss is associated with fragmentation of the cell at a combination of high stress and higher duration. Stress or duration of stress greater than the threshold to cause membrane failure does not result in greater hemoglobin release from cells that have already failed. Therefore, reduction of fluid stresses may not be effective in reducing hemolysis until stress is decreased below the threshold for membrane failure. It is possible, then, that beveling the entrance of the needle does not decrease stress enough to reduce hemolysis. Second, the rounded and beveled needles were manufactured by difference methods. 16G and 20G needles with rounded entrance were manufactured by wire EDM method and the 16G needles with beveled entrance were manufactured by micro-milling method. In the wire EDM method, the machined surface tends to exhibit small pits caused by electrochemical erosion, but is overall relatively smooth. On the other hand, micro-milling method can leave burrs and rough edges, depending on the speed, depth and direction of the cut and the condition of the cutting edge. While the micro-milled needles were inspected carefully and found to be free of burrs, microscopic rough edges might have remained, causing localized high stresses and obscuring the potential reduction in hemolysis in the reverse direction. Perhaps most telling, however, is that a CFD-based hemolysis simulation (Chen 2006) predicted a reduction in hemolysis for the rounded entrance, but not for the beveled entrance. This simulation showed reduced stresses for the beveled needle, but not small enough to reduce hemolysis.

Negative values of hemolysis were obtained from some cases, which is clearly not possible. The negative values were very small and indicate the level of accuracy of the measurements and the calibration method.

Considerable variability was found among the units of blood. Each unit can have different composition of blood plasma and RBC's, which will give different values of hemolysis for the same experimental conditions. Different units may have different volumes of blood captured from the experiment, which in turn can have different percent of hemolysis. There can also be slight variations in the pressures applied during the experiment, which may occur while setting the chamber pressure. This difference in pressure changes the velocity of flow and gives different values of hemolysis.

Red cells increase in vulnerability to stress with increasing age. Blood used in the experiments differed in the age from 41 to 48 days. However, the greatest change is susceptibility to hemolysis occurs in the first few days, and it was found that the difference in hemolysis among samples with ages 3-14 days (mean 11 days) versus 16-39 days (mean 29 days) was less than 5% [56]. Therefore, the age effect is likely not important in these experiments.

VIII. CONCLUSION & RECOMMENDATIONS FOR FUTURE WORK

In conclusion, hemolysis in 20G and 16G with rounded entrances was significantly reduced for reverse orientation compared to the standard needles, but no relation could be found between the standard and beveled 16G (micro-milled) needles, nor among any of the groups for normal orientation.

Observations regarding the experimental apparatus include that, after several runs of the experiments, the plunger of the syringe sometimes released before attaining the desired pressure. This occurred due to the plunger being worn out and the problem was rectified by replacing with a new plunger. It was also found that all moving parts inside the fixture should be lubricated.

All the experiments in this thesis were performed with expired blood samples (age greater than 40 days). Since the important application for the new needles is in drawing or transfusing fresh blood, experiments with fresh human samples should eventually be performed on the final needle design. Prior to these final in vitro experiments, however, research should be done on how to manufacture these needles in a more economical way, and the needles with entrance designs with promising compromises between potential for hemolysis reduction and cost of production should be tried. Cold forming or cold forging

is one method that should be tried to manufacture needles. In this method, metal is placed within a die and a punch is pressed to cold form the part. This method is faster than EDM, produces little or no waste of material and also consumes less energy. Etching, which can be used on metals, semiconductor materials and even glass using acids, bases or other chemicals, preferentially attacks projecting edges, and could produce a rounded entrance in needles. The sharp skin-piercing tip would need to be protected during this process, or formed in a subsequent operation. Etching has the advantage that, in contrast to the individual operations necessary for EDM and cold forming, large numbers of needles could be processed in the same etching bath.

REFERENCES

1. Alfredo J. Herrera, James Corless: Blood transfusions: Effect of speed of infusion and of needle gauge on hemolysis. Journal of Pediatrics 1981, 99(5); 757-758.
2. G. J. Wilcox, A. Barnes, H. Modanlou: Does Transfusion using a syringe infusion pump and small-gauge needle cause hemolysis?. Transfusion 1981, 21(6); 750-751.
3. Stephen Eurenius, Robert M. Smith: Hemolysis in blood infused under pressure. Anesthesiology 1973, 39(6); 650-651.
4. Paula Tanabe, Demetrious N. Kyriacou, Franklyn Garland: Factors affecting the risk of blood bank specimen hemolysis. Academic Emergency Medicine 2003, 10(8); 897-900.
5. Toshitaka Yasuda, Akio Funakubo, Fujio Miyawaki, Tsuyoshi Kawamura, Tsuyoshi Higami, Yashuhiro Fukui: Influence of static pressure and shear rate on hemolysis of red blood cells. ASAIO JOURNAL 2001, 47; 351-353.
6. Toshitaka Yasuda, Kenji Shimokasa, Akio Funakubo, Tetsuya Higami, Tsuyoshi Kawamura, Yasuhiro Fukui: An investigation of blood flow behavior and hemolysis in artificial organs. ASAIO J 2000, 46; 527-531.
7. Toshitaka Yasuda, Akio Funakubo, Yasuhiro Fukui: An investigation of blood damage induced by static pressure during shear-rate conditions. Artificial Organs 2002, 26(1); 27-31.

8. Jeffrey L. Angel, William F. O'Brien, Robert A. Knuppel, Melinda B. Warren, German F. Leparc: Infusion of packed erythrocytes: An In Vitro Study of Hemolysis. Obstetrics & Gynecology 1987, 69; 948-950.
9. Kennedy, Collen, Angermuller, Sarah, King, Robin, Noviello, Sheri, Walker, Joann, Warden, Joanie, Vang, Susan: A comparision of hemolysis rates using intravenous catheters versus venipuncture tubes for obtaining blood samples. Journal of Emergency Nursing 1996, 22(6); 566-569.
10. Zbylut J. Twardowski, Jimmy D. Haynie, Harold L. Moore: Blood flow, Negative pressure, and hemolysis during hemodialysis. Home Hemodial Int 1999, Vol3; 45-50.
11. H. L. Goldsmith: The effects of flow and fluid mechanical stress on red cells and platelets. Trans. Amer. Soc. Artif. Int. Organs 1974, Vol XX; 21-26.
12. M. Keith Sharp, S. Fazal Mohammad: Hemolysis in needleless connectors for phlebotomy. ASAIO J 2003, 49(1); 128-130.
13. M. Keith Sharp, S. Fazal Mohammad: Hemolysis in clinical blood samples: The role of flow in needles and catheters. Bioengineering Conference ASME 2001, 50; 523-524.
14. M. Keith Sharp, S. Fazal Mohammad: Scaling of hemolysis in needles and catheters. Annals of Biomedical Engineering 1998, 26; 788-797.
15. Justin D. Volz, M. Keith Sharp: Comparision of hemolysis in prototype low-stress and standard hypodermic needles. 2003 Summer Bioengineering Conference.

16. M. Dhaene, B. Gulbis, N. Lietaer, N. Gammar, C. Thayse, H. A Ooms, J. L. Vanherweghem: red blood cell destruction in single-needle dialysis. Clinical Nephrology 1989, 31(6); 327-331.

17. Grant, Marian Sue: The effect of blood drawing techniques and equipment on the hemolysis of ED laboratory blood samples. Journal of Emergency Nursing 2003, Volume 29(2); 116-121.

18. Kelly J. Burch, Stephanie J. Phelps. Thomas D. Constance: Effect of an infusion device on the integrity of whole blood and packed red blood cells. American Society of Hospital Pharmacists 1991, 48; 92-97.

19. Dirk De Wachter, Pascal Verdonck: Numerical calculation of hemolysis levels in peripheral hemodialysis cannulas. Artificial Organs 2002, 26(7); 576-582.

20. Sharon J. Northup: Hemocompatibility: Not all devices are created equal, 1997.

21. Stephen J. Sweet, Sharon Mc Carthy, Richard Steingart, Tina Callahan: Hemolytic reactions mechanically induced by kinked hemodialysis lines. American Journal of Kidney Diseases 1996, 27(2); 262-266.

22. Dirk S. De Wachter, Pascal R. Verdonck, Ronny F. Verhoeven, Remi O. Hombrouckx: Red cell injury assessed in a numerical model of a peripheral dialysis needle. ASAIO J 1996, 42; M524-M529.

23. M. C. Yang, C. C. Lin: Influence of design of the hemodialyzer inlet chamber on red blood cell damage during hemodialysis. ASAIO J 2001, 47; 92-96.

24. Jerry M. Calkins, Robert W. Vaughan, Randall C. Cork, John Barberii, Cleamond Eskelson: Effects of dilution, Pressure and Apparatus on Hemolysis and Flow

Rate in Transfusion of Packed Erythrocytes. Anesthesia and Analgesia 1982, 61(9); 776-780.

25. M-C. Yang, C. C. Lin: Influence of design of the hemodialyzer inlet chamber on red blood cell damage during hemolysis. ASAIO Journal 2001, 47(1); 92-96.
26. S. P. Sutera, M.H. Mehrjardi: Deformation and fragmentation of human red blood cells in turbulent shear flow. Biophysics Journal 1975, 15(1); 1-10.
27. L. B. Leverett, J. D. Hellums, C. P. Alfery, E. C. Lynch: Red blood cell damage by shear stress. Biophysics Journal 1972, 12(3); 257-273.
28. Mizuno T, Tsukiya T, Taenaka Y, Tatsumi E, Nishinaka T, Ahnishi H, Oshikawa M, Sato K, Shioya K, TakewaY, Takano H: Ultrastructural alterations in red blood cell membranes exposed to shear stress. ASAIO J 2002, 48(6); 668-670.
29. Ming-Chien Yang, Chi-Chang Lin: In Vitro Characterization of the occurrence of hemolysis during extracorporeal blood circulation using a mini hemodialyzer. ASAIO Journal 2000, 46(3); 293-297.
30. Krishna K. Yeleswarapu, James F. Antaki, Marina V. Kameneva, Kumbakonam R. Rajagopal: A mathematical model for shear induced hemolysis. Artificial Organs 1995, 19(7); 576-582.
31. Marina V. Kameneva, Brandon M. Repko, Ellen F Krasik, Brett C. Perricelli, Harvey S. Borovetz: Polyethylene glycol additives reduce hemolysis in red blood cell suspensions exposed to mechanical stress. ASAIO Journal 2003, 49; 537-542.
32. Masaaki Tamagawa, Teruaki Akamatsu, Keijiroh Saitoh: Prediction of hemolysis in turbulent shear orifice flow. Artificial Organs 1996, 20(6); 553-559.

33. J. F Laugel, R. L. Beissinger: Low Stress Shear-induced hemolysis in capillary flow. Tranasactions America Society of Artificial Internal Organs 1983, 29; 158-162.

34. Reinhard Paul, Jorn Apel, Sebastian Klaus, Frank Schugner, Peter Schwindke, Helmut Reul: Shear stress related blood damage in laminar coquette flow. Artificial Organs 2003, 27; 517-529.

35. Stephen I. Shapiro, Michael C. Williams: Hemolysis in simple shear flows. American Institute of Chemical Engineers 1970, 16(4); 575-580.

36. Herbert Silver: Blood , Blood components & derivatives in transfusion therapy.

37. Symposium on Circulation & Homeostasis (5- 9 Oct 1953).

38. Greenwalt & Jamieson: Formation & Destruction of Blood cells.

39. Derek Ogston: The Physiology of Homeostasis.

40. Dorothy S. Dittmer: Blood & other Body fluids

41. Prankerd: The Red Cell

42. Sunil Unnikrishnan, Thanh N. Huynh, B.C. Brott, Y. Ito, C. H. Cheng, A. M. Shih, M. Allon, Andreas S. Anayiotos; Turbulent Flow Evaluation of the Venous Needle during Hemodialysis. Journal of Biomechanical Engineering 2005, 127(7); 1141-1146.

43. R. N. Vaishnav, D. J. Patel, H. B. Atabek, M. D. Deshpande, F. Plowman, J. Vassoughi; Determination of the Local Erosion of the Canine Endothelium using a Jet Impingement Method. Journal of Biomechanical Engineering 1983, 106; 77-83.

44. R. J. Lutz, J. N. Cannon, K. B. Bischoff, R. L. Dedrick, R. K. Stiles, D. L. Fry; Wall Shear Stress Distribution in a Model Canine Artery during Steady Flow. Circulation Research 1977, 41; 391-399.

45. A. W. Clowes; Pathologic Intimal Hyperplasia as a Response to Vascular Injury and Reconstruction. Vascular Surgery 1994, 285-295.

46. C. F. Dewey, S. R. Bussolari, M. A. Gimbrone; The Dynamic Response of Vascular Endothelial Cells to Fluid Shear Stress. Journal of Biomechanical Engineering 1981, 103; 177-185.

47. P. F. Davies, A. Remuzzi, E. J. Gordon, C. F. Dewey, M. Gimbrone; Turbulent Fluid Shear Stress induces Vascular Endothelial cells to Fluid Shear Stress. Proc. Natl. Acad. Sci. U.S.A. 83; 2114–2117.

48. S. H. Swedberg, G. B. Brown, R. Sigley, T. N. Wight, D. Gordon, S. C. Nicholls; Intimal Fibromuscular Hyperplasia at the Venous Anastomosis of PTFE Grafts in Hemodialysis patients. Circulation 1989, 80; 1726-1736.

49. M. F. Fillinger, E. R. Reinitz, R. A. Schwartz, D. E. Resetarits, A. M. Paskanik, D. Bruch, C. E. Bredenberg; Graft Geometry and Venous Intimal-medical Hyperplasia in Arteriovenous Loop Grafts. Journal of Vascular Surgery 1990, 11(4); 556-566.

50. R. M. Nerem, J. F. Cornhill; The role of Fluid Mechanics in Atherogenesis. Journal of Biomechanical Engineering 1980, 102; 181-189.

51. M. Ojha; In Vitro studies on Anastomotic Intimal Hyperplasia. ASAIO JOURNAL 1997, 43; 242.

52. C. K. Zarins, D. P. Giddens, B. K. Bharadvaj, V. S. Sottiurai, R. Mabon, S. Glagov; Carotid Bifurcation Atherosclerosis. Circulation Research 1983, 54; 502-514.

53. H. S. Bassiouny, S. White, S. Glagov, E. Choi, D. P. Giddens, C. K. Zarins; Anastomotic Intimal Hyperplasia: Mechanical Injury or Flow induced. Journal of Vascular Surgery 1992, 15; 708-717.

54. R. Pfeffer, P. Ganatos, A. Nir, S. Weimbaum; Diffusion of Micromolecules across the arterial Wall in the presence of Multiple Endothelial Injuries. Journal of Biomechanical Engineering 1981, 103; 197-203.

55. D. L. Fry; Acute Vascular Endothelial Changes Associated with Increased Blood Velocity Gradients. Circulation Research 1968, 22; 165-197.

56. Regina Frelich, Martin H. Ellis; The effect of external pressure, catheter gauge, and storage time on Hemolysis in RBC transfusion 2001, 41; 799-802.

57. Yansheng Chen; Flow-induced hemolysis, PhD thesis, Department of Mechanical Engineering, University of Louisville, 2006.

APPENDIX I

The below table (Table A1) shows hemolysis values of different specimens obtained from the spectrophotometer for the 20G needle in the reverse orientation.

		% Hemolysis, Reverse Orientation					
		20 psi		35 psi		50 psi	
	Needle	Current needle	Rounded needle	Current needle	Rounded needle	Current needle	Rounded needle
E1	#1	-0.29	-0.423	0.381	0.271	3.96	1.842
	#2	-0.24	-0.257	0.974	0.124	3.978	2.44
	#3	-0.12	-0.397	0.565	-0.048	3.926	2.229
E2	#1	0.007	-0.237	0.575	-0.064	3.698	1.577
	#2	-0.242	-0.22	0.552	0.333	2.798	1.905
	#3	0.144	-0.185	0.51	0.026	2.559	1.142
E3	#1	-0.019	-0.058	0.632	0.279	2.416	1.174
	#2	-0.011	0.025	0.708	0.311	2.962	1.15
	#3	-0.06	0.062	0.72	0.261	2.771	1.431
E4	#1	0.016	-0.021	0.298	0.091	1.643	0.75
	#2	-0.012	-0.019	0.556	0.058	1.942	0.787
	#3	0.012	0.016	0.548	0.696	2.79	1.226
E5	#1	0.018	-0.008	0.583	0.356	2.451	1.065
	#2	0.042	0.021	0.478	0.41	2.31	0.951
	#3	0.071	-0.017	0.504	0.167	2.635	1.339
E6	#1	-0.009	-0.041	0.002	-0.006	0.889	0.452
	#2	-0.036	-0.007	0.037	0.014	0.747	0.211
	#3	-0.03	-0.035	0.026	-0.023	0.941	0.24
E7	#1	0.099	-0.017	0.049	0.085	0.842	0.37
	#2	0.019	-0.018	-0.005	0.031	1.648	0.141
	#3	0.085	-0.042	0.022	0.092	1.288	0.112
E8	#1	0.004	-0.025	0.08	-0.006	1.194	0.427
	#2	0.014	-0.011	0.14	-0.012	3.199	0.179
	#3	-0.022	-0.01	0.013	-0.012	1.49	0.564
E9	#1	0.072	0.016	0.68	0.197	2.605	1.092
	#2	0.078	0.001	0.774	0.267	2.772	1.359
	#3	0.049	0.023	0.712	0.146	2.665	1.193
E10	#1	0.065	-0.002	0.35	0.165	2.45	0.9
	#2	0.092	0.032	0.454	0.208	2.625	0.937
	#3	0.077	-0.003	0.604	0.178	2.716	0.998

Table A1: Summary of all the experiments for the 20G needle in reverse orientation

APPENDIX II

Table A2 shows hemolysis values of different specimens obtained from the spectrophotometer for several experiments with the 20G needle in the normal orientation.

		% Hemolysis, Normal Orientation					
		20 psi		35 psi		50 psi	
	Needle	Current needle	Rounded needle	Current needle	Rounded needle	Current needle	Rounded needle
E1	#1	-0.29	0.112	0.387	0.855	2.588	2.779
	#2	-0.4	-0.112	0.136	0.244	2.139	2.417
	#3	0.02	-0.283	0.13	-0.002	1.455	1.893
E2	#1	-0.052	-0.017	0.159	-0.067	1.598	1.646
	#2	-0.228	-0.276	0.36	0.053	1.123	3.521
	#3	-0.202	-0.076	0.101	0.055	0.786	0.921
E3	#1	0.001	0.103	0.306	0.24	1.359	2.092
	#2	0.065	0.006	0.209	0.347	1.376	1.73
	#3	0.051	0.001	0.218	0.127	1.073	1.143
E4	#1	0.025	0.004	0.287	0.142	1.437	1.489
	#2	-0.026	0.022	0.132	0.194	0.958	1.432
	#3	-0.019	0.062	0.154	0.043	0.58	1.128
E5	#1	0.043	0.046	0.205	0.252	1.514	1.601
	#2	0.131	0.004	0.251	0.182	1.332	1.256
	#3	-0.007	0.037	0.362	0.277	1.067	1.016
E6	#1	-0.027	-0.062	0.035	0.046	0.438	0.402
	#2	0.021	-0.031	0.006	-0.01	0.559	0.789
	#3	-0.025	-0.049	-0.008	0.016	0.342	0.394
E7	#1	0.002	0.008	0.016	0.048	0.313	0.175
	#2	0.038	-0.009	0.076	0.081	0.612	0.837
	#3	0.003	0.027	0.129	0.058	0.678	0.891
E8	#1	-0.008	0.021	0.125	0.031	0.889	0.175
	#2	-0.025	-0.027	0.254	0.052	1.365	0.722
	#3	-0.009	0.009	0.028	0.021	0.432	0.172
E9	#1	0.025	0.012	0.236	0.209	1.511	1.331
	#2	0.108	0.025	0.209	0.262	1.375	1.426
	#3	0.031	0.026	0.201	0.155	1.327	0.985
E10	#1	0.012	0.002	0.327	0.259	1.242	1.691
	#2	0.006	-0.001	0.204	0.176	1.257	1.235
	#3	0.009	0.015	0.22	0.083	0.524	0.624

Table A2: Summary of all the experiments for the 20G needle in normal orientation

APPENDIX III

Table A3 shows hemolysis values from several runs of experiments with standard and modified needles obtained from the spectrophotometer for the 16G micro-milled needle in reverse orientation.

		% Hemolysis, Reverse Orientation					
		15 psi		30 psi		45 psi	
	needle	Current needle	Beveled needle	Current needle	Beveled needle	Current needle	Beveled needle
E1	#1	-0.03	-0.485	0.019	0.143	2.678	2.746
	#2	-0.55	-0.180	-0.068	0.039		1.807
	#3	-0.37	-0.579	0.218	0.195	1.993	2.387
E2	#1	0.011	0.057	0.197	0.198	1.630	1.576
	#2	0.042	-0.006	1.377	0.194	1.148	1.397
	#3	0.005	0.043	1.308	0.117	1.607	0.184
E3	#1	0.003	-0.013	0.225	0.185	1.001	0.993
	#2	0.245	-0.012	0.120	0.291	0.792	0.940
	#3	0.087	0.000	-0.003	0.241	1.034	0.953
E4	#1	-0.078	0.145	0.305	0.223	1.893	1.557
	#2	0.009	-0.076	0.158	0.216	1.691	1.942
	#3	-0.088	0.039	0.282	0.331	1.892	1.546
E5	#1	0.437	0.598	0.701	0.530	1.053	0.990
	#2	0.497	0.382	0.582	0.520	1.091	1.037
	#3	0.340	0.575	0.630	0.660	1.073	1.075
E6	#1	-0.068	0.014	0.634	0.367	1.923	1.526
	#2	0.145	0.008	0.261	0.140	1.771	1.554
	#3	-0.113	0.205	0.517	0.375	1.830	1.836
E7	#1	-0.052	0.139	0.464	0.344	1.493	1.321
	#2	0.063	-0.025	0.214	0.254	1.311	1.476
	#3	-0.085	0.102	0.441	0.361	1.468	1.323
E8	#1	-0.056	0.068	0.311	0.192	1.360	1.221
	#2	0.072	-0.030	0.180	0.168	1.134	1.168
	#3	-0.091	0.160	0.328	0.196	1.353	1.135
E8	#1	0.094	0.048	0.275	0.579	1.544	1.579
	#2	-0.093	0.084	0.287	0.332	1.218	1.567
	#3	0.192	-0.053	0.244	0.157	1.625	1.021

Table A3: Summary of all the experiments for the 16G (micro-milled) needle in reverse orientation

APPENDIX IV

Table A4 shows hemolysis values from the spectrophotometer for various specimen samples of the 16G micro-milled needle in normal orientation.

		% Hemolysis, Normal Orientation					
		15 psi		30 psi		45 psi	
	needle	Current needle	Bevelled needle	Current needle	Bevelled needle	Current needle	Bevelled needle
E1	#1	-0.394	-0.515	-0.186	0.347	1.816	2.527
	#2	-0.406	-0.335	0.126	-0.159		2.105
	#3	-0.575	-0.593	0.041	-0.094	0.931	1.706
E2	#1	0.012	-0.013	0.097	0.278	0.205	1.559
	#2	0.016	0.261	0.249	1.326	0.943	1.628
	#3	0.022	-0.013	0.168	0.185	0.801	0.281
E3	#1	-0.003	-0.032	0.124	0.274	0.869	0.917
	#2	0.032	0.015	0.151	0.213	0.937	0.917
	#3	0.002	0.052	0.117	0.165	0.901	1.003
E4	#1	-0.073	-0.100	0.141	0.334	1.381	1.871
	#2	-0.008	-0.013	0.355	0.211	1.611	1.450
	#3	0.067	-0.080	0.376	0.278	1.166	1.444
E5	#1	0.358	0.358	0.609	0.665	1.028	1.098
	#2	0.489	0.491	0.611	0.646	1.059	1.026
	#3	0.578	0.390	0.789	0.649	0.943	1.030
E6	#1	-0.084	-0.024	0.286	0.374	1.405	1.859
	#2	0.065	0.038	0.249	0.348	1.706	1.414
	#3	0.185	-0.052	0.406	0.429	1.158	1.191
E7	#1	-0.084	-0.072	0.396	0.538	1.292	1.558
	#2	0.082	0.046	0.416	0.327	1.481	1.359
	#3	0.144	-0.059	0.402	0.368	1.163	1.392
E8	#1	-0.063	0.136	0.223	0.347	1.123	1.356
	#2	0.080	0.002	0.170	0.268	1.270	1.143
	#3	0.150	-0.034	0.284	0.189	0.913	1.257
E8	#1	0.167	0.190	0.388	0.498	1.378	1.225
	#2	-0.055	-0.094	0.510	0.170	1.540	1.521
	#3	-0.089	0.240	0.052	0.210	1.421	1.424

Table A4 : Summary of all the experiments for the 16G (micro-milled) needle in normal orientation

APPENDIX V

Below (Table A5) is the summarized table of hemolysis values of standard and modified needles for the 16G EDM needle in the reverse orientation.

		% Hemolysis, Reverse Orientation					
		15 psi		30 psi		45 psi	
	Needle	Current needle	Rounded needle	Current needle	Rounded needle	Current needle	Rounded needle
E1	#1		-0.065	0.326	0.037	1.578	1.004
	#2	0.004	-0.052		0.048	0.908	1.297
	#3		-0.073	0.055	-0.006	1.409	0.788
E2	#1	-0.008	-0.022	0.094	0.137	0.895	1.210
	#2	0.016	0.007	0.135	0.050	0.886	0.534
	#3	0.016	-0.008	0.082	0.060	1.775	0.682
E3	#1	-0.011	-0.020	0.077	0.077	1.692	1.666
	#2	-0.016	0.004	0.114	0.058	1.134	0.649
	#3	-0.026	-0.017	0.046	0.050	1.314	0.707
E4	#1	0.015	-0.011	0.438	0.223	1.455	1.508
	#2	0.005	-0.004	0.309	0.338	1.526	1.363
	#3	0.009	-0.014	0.350	0.318	1.535	1.382
E5	#1	-0.012	0.001	0.214	0.355	1.283	1.118
	#2	0.014	-0.018	0.251	0.219	1.355	1.063
	#3	0.002	-0.014	0.262	0.206	1.331	1.310
E6	#1	0.007	0.013	0.198	0.145	1.671	0.657
	#2	-0.003	0.004	0.297	0.162	1.748	1.234
	#3	-0.011	-0.002	0.229	0.230	1.801	1.219
E7	#1	-0.014	0.020	0.299	0.138	1.222	0.995
	#2	-0.014	-0.023	0.220	0.139	1.203	0.986
	#3	-0.030	-0.033	0.559	0.123	1.184	1.169
E8	#1	-0.013	-0.038	0.209	0.027	1.626	1.671
	#2	-0.031	-0.029	0.262	0.129	1.268	1.238
	#3	-0.021	-0.014	0.262	0.140	1.725	1.085
E9	#1	0.008	-0.003	0.268	0.187	1.185	0.921
	#2	0.004	-0.021	0.166	0.126	1.051	1.134
	#3	0.015	-0.020	0.302	0.142	1.180	0.835

Table A5 : Summary of all the experiments for the 16G (EDM) needle in reverse orientation

APPENDIX VI

Table A6 shows hemolysis values from several runs of experiments with standard and modified needles obtained from the spectrophotometer for the 16G EDM needle in the normal orientation.

		% Hemolysis, Normal Orientation					
		15 psi		30 psi		45 psi	
	Needle	Current needle	Rounded needle	Current needle	Rounded needle	Current needle	Rounded needle
E1	#1		-0.064	0.061	0.134		0.986
	#2	-0.053	-0.040		0.314	1.285	1.524
	#3		-0.070	0.051	1.047	0.528	0.944
E2	#1	0.039	-0.012	0.077	0.167	0.871	1.003
	#2	0.006	0.004	0.093	0.160	1.152	1.262
	#3	0.088	0.001	0.057	0.118	0.970	0.719
E3	#1	-0.094	-0.012	0.054	0.081	0.785	0.923
	#2	-0.004	-0.015	0.206	0.123	1.188	1.342
	#3	-0.016	0.001	0.051	0.040	0.584	0.877
E4	#1	0.031	0.008	0.238	0.319	1.517	1.446
	#2	0.000	-0.007	0.475	0.664	1.540	1.539
	#3	0.009	-0.012	0.230	0.495	1.151	1.474
E5	#1	-0.010	-0.002	0.489	0.347	0.809	1.198
	#2	0.002	0.002	0.435	0.510	1.045	1.288
	#3	-0.004	-0.013	0.273	0.259	1.141	1.260
E6	#1	-0.011	0.001	0.360	0.263	1.374	0.883
	#2	0.009	-0.008	0.413	0.398	1.011	1.581
	#3	0.001	-0.019	0.149	0.247	0.876	0.808
E7	#1	-0.020	-0.033	0.160	0.258	0.979	0.805
	#2	0.002	0.025	0.218	0.435	1.148	1.084
	#3	-0.012	-0.020	0.148	0.253	1.046	0.775
E8	#1	-0.020	-0.039	0.258	0.186	1.079	1.149
	#2	-0.028	-0.034	0.307	0.235	1.484	1.338
	#3	-0.014	-0.017	0.125	0.260	1.035	1.136
E9	#1	0.010	0.012	0.145	0.298	0.901	1.133
	#2	0.001	0.006	0.192	0.282	0.999	1.018
	#3	0.006	-0.010	0.112	0.153	0.729	0.865

Table A6: Summary of all the experiments for the 16G (EDM) needle in normal orientation

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