COMPARISON OF HEMOLYSIS IN PROTOTYPE LOW-STRESS AND STANDARD HYPODERMIC NEEDLES

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INTRODUCTION

Hemolysis describes the rupturing of red cell membranes resulting in release of hemoglobin into the plasma. Plasma-free hemoglobin in clinical blood tests interferes with the assays of a number of metabolites, electrolytes and enzymes [1]. Consequentially, assay results are inaccurate and blood draws must be repeated. Repeated blood draws waste time and resources in a clinical environment and can be a risk to the patient's health if the assay results are needed in an emergency situation.

Hemolysis caused by hypodermic needles may lead to complications in hemodialysis procedures. Hemolysis can result in symptoms of nausea and abdominal or back pain occurring typically in the last hour of the dialysis session [2].

Blood transfusions in infants are associated with risk of hemolysis due to the small size of needles required for delivery of blood to pediatric patients [3-5]. A high level of hemolysis can result in a considerable threat to the health of the infant.

Lysis of red blood cells can result from high stresses present in blood flow. In the case of a hypodermic needle, a portion of the blood is drawn around the sharp edge downstream and diametrically opposite the tissue-piercing point. It was hypothesized that the stresses imposed on the red blood cells in the vicinity of this edge may be the source of significant hemolysis. For applications such as those above, a needle geometry modified to reduce flow-induced stresses may decrease the incidence of complications due to hemolysis. This research consists of a comparative study of hemolysis between two 16 gauge needle groups with different entrance geometries.

METHODS AND MATERIALS

The control group consists of three standard 1.5" length, 16 gauge hypodermic needles, whereas the test group consists of three 1.5" length, 16 gauge needles modified using an electrical discharge machining process, with which a 0.012" radius was machined into the downstream inside edge of the elliptical profile of the needle opening to create a rounded entrance (Figs. 1 and 2). This feature was designed to decrease flow-induced stress in the blood as it flows around the edge.

Three units of donated forty-one day old packed red blood cells were obtained and diluted using a 0.9 percent hypotonic saline solution to a hematocrit concentration of 40 to 45 percent.

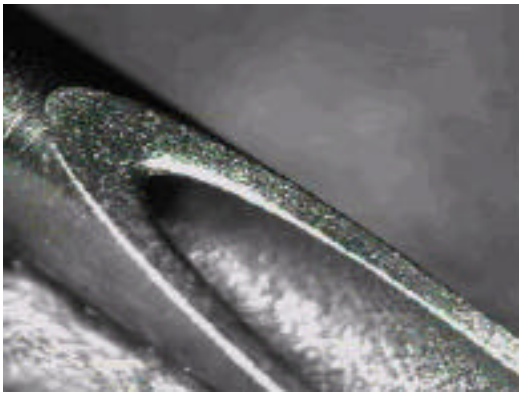

Figure 1. Standard needle entrance geometry

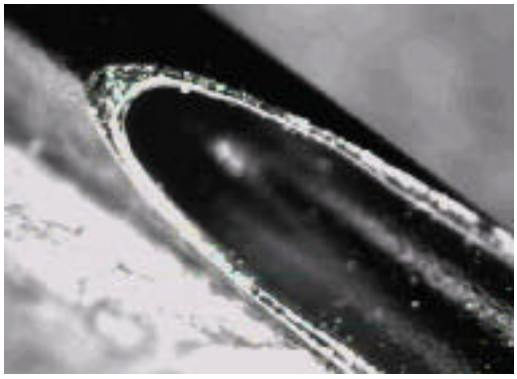

Figure 2. Modified needle entrance geometry

This blood was sheared by forcing it through the needle by means of a pressure difference. A schematic of the experimental setup is shown in Fig. 3. In order to create pressure differences large enough to reliably cause significant hemolysis, a pressure chamber was built to surround a syringe containing blood. The pressure inside the chamber was regulated by valves connected to the building air supply. A trigger engaged the plunger of the syringe to prevent the pressure from forcing the blood out of the syringe until the trigger was released.

Release was accomplished by withdrawing the trigger with an electrical solenoid. The trigger mechanism allowed convenient operation of the experiment and provided quick and repeatable release of the plunger. Blood exiting the needle was captured in a test tube for analysis.

Two needle orientations were tested. The normal orientation, with the female Luer hub of the needle attached to the male Luer tip of the syringe in the usual way, simulated blood infusion. The reverse orientation (Fig. 3), with the tip of the needle extending into the body of the syringe, simulated venipuncture blood draws.

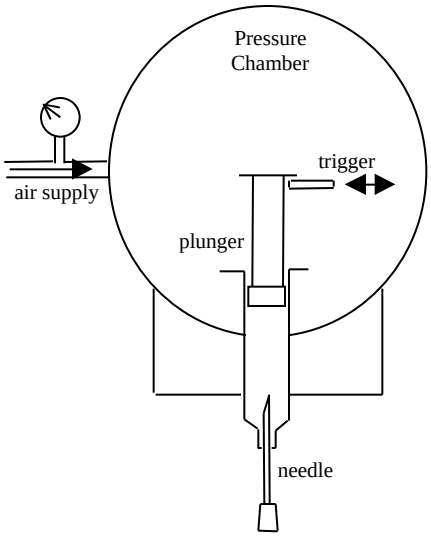

Figure 3. Experiment setup with a needle in the reverse orientation, simulating a venipuncture blood draw.

Experiments were performed with variations in needle type (standard and modified), needle orientation (reverse and normal), and driving pressure. The experimental level of pressure difference was determined by preselecting three values that provided little/no hemolysis, moderate hemolysis, and large amounts of hemolysis.

For hemolysis measurement, plasma-free hemoglobin was quantified by a spectrophotometric method (1). The blood samples, collected in 5ml glass tubes, were centrifuged at 1200g for 20 minutes at 4ºC. The plasma was then pipetted from the tube into a microcuvette which was placed into the Beckman spectrophotometer (model DU-640) previously calibrated with control plasma prepared from the same unit of blood as the experimental samples.

Hemolysis (plasma free hemoglobin, mg/dl) was determined from the weighted difference in absorbance determined at three different wavelengths:

$$Hemolysis = \left[A_1 - A_3 - \left(A_2 - A_3\right)\left(\frac{\lambda_1 - \lambda_2}{\lambda_3 - \lambda_2}\right) \right] 176 \qquad (1)$$

where A_1 is the absorbance at wavelength λ_1 = 576.5 nm, A_2 is the absorbance at wavelength λ_2 = 596.0 nm, A_3 is the absorbance at wavelength λ_3 = 560.0 nm, and 176 is an empirical factor. Percent hemolysis was calculated from measurements of plasma free hemoglobin in graded reference samples osmotically hemolyzed in distilled water.

RESULTS AND DISCUSSION

For each of the three standard and modified needles, a unique run combination was completed once per unit of donor blood in randomized order. The completed database includes data from three separate units of donor blood, yielding 101 total samples. The data was analyzed using standard analysis of variance techniques.

The average hemolysis levels in the standard needle in the reverse orientation were –0.003%, 0.116% and 1.288% at the low, moderate and high driving pressures, respectively. (Negative hemolysis may be indicative of a small offset calibration error.) Lower hemolysis was anticipated in the modified needles for flow in the reverse orientation due to the bell-mouthed entrance, but not in the forward direction, since a rounded exit would not be expected to significantly affect the stresses in the exiting jet. At the lowest driving pressure, the resulting hemolysis in the reverse orientation was 694% less in the modified needle than in the standard needle (ANOVA p = 0.074). (This reduction is exaggerated by normalization by the small hemolysis occurring in the standard needle.) At the moderate driving pressure, hemolysis in the reverse orientation was 51% less in the modified needle (ANOVA p = 0.089). At the high driving pressure, hemolysis was 26% less in the modified needle (ANOVA p = 0.065).

In the normal needle orientation at the lowest driving pressure, the resulting hemolysis was less in the modified needle than in the standard needle, but the p-value (0.428) was not significant. At the moderate driving pressure, hemolysis was greater in the modified needle, but the p-value (0.169) was not significant. At the high driving pressure, hemolysis was greater in the modified needle, but again the p-value (0.286) was not significant.

CONCLUSIONS

These preliminary data suggest that the modified entrance profile of the prototype needles may indeed reduce fluid stresses in the intended direction of flow. The p-values resulting from comparison of needle groups in the reverse direction are all below .010 with just nine samples per group for each pressure level. While the current results are promising, a larger sample population may drive the p-value to greater statistical significance.

The p-values comparing the two needle groups in the normal orientation suggest that fluid stresses are not significantly different for infusion through the standard and modified needles.

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