Introduction

The demand for blood is growing faster than the donation rates. In fact, the demand is growing at a rate of 7% per year, while the donation rates are only rising at a rate of 2.5% per year¹. With this gap, there has been a push for more research into alternative solutions to blood transfusion. Artificial blood is one of the most promising alternatives because it can achieve all the same goals as traditional blood transfusion. This includes increasing the oxygenation of tissue, removing CO2 from the body, and restoring volume to the blood in order to prevent capillary collapse².

There are many advantages of using artificial blood over real blood. Artificial blood does not need to be refrigerated, whereas real blood does. It also has a longer storage life, of up to three years, in comparison with real blood’s storage life of 42 days. There is also a lack of blood type in artificial blood, so there needs to be no delay in blood transfusion¹. These facts provide substantial reasons for artificial blood to be considered for emergency medicine and military medicine alone.

The two leading types of artificial blood are Hemoglobin Based Oxygen Carriers (HBOCs) and Perfluorochemical Emulsions (PFCs). HBOC is made by refining Hemoglobin from humans or animals. Its molecular size is enlarged by either polymerizing molecules together or conjugating it to a larger molecule³. PFCs are particles that are emulsified in salt and water to keep them in solution⁴.

Blood rheology, the study of blood flow, consists of three important areas: the deformability of RBCs (red blood cells), the mechanical fragility of RBCs, and the viscosity of the medium. Deformability, the degree of shape change an RBC can go through under an applied force, affects blood flow in large and microcirculatory blood flow. Mechanical fragility is how susceptible RBCs are to mechanical stress, which can lead to hemolysis. Blood viscosity is the resistance to flow, which is critical in having proper circulation. While there has been extensive research done on the oxygen carrying capability of artificial blood products, there is still little known about their effects on blood rheology²³⁴. Therefore, the objective of this study was to investigate how the addition of PFC or HBOC solutions to whole blood affects the rheological parameters of blood. Success criteria for this study include obtaining reproducible measurements, as in results with standard error of mean below ±5%.

Methods

Samples were prepared by removing the buffy coat from the blood and resuspending the washed RBCs in plasma at 30% hematocrit and replacing 10% of the plasma with either PFC, HBOC, or saline (control).

Deformability was tested by taking 10 µL of each RBC suspension and adding it to a polyvinylpyrrolidone solution. The suspensions were exposed to shear stresses of 100, 500, and 1000 s⁻¹ in a Linkam Shearing Stage. Multiple (200) images of cells were obtained at each shear rate for each sample. The images were analyzed using ImageJ, a measuring software, and an elongation index (EI) was calculated at each shear rate using the formula

\[
EI = \frac{(a-b)}{(a+b)}
\]

where \(a\) is the major axis and \(b\) is the minor axis of the RBC [trials=14, samples per trial=3, cells analyzed per sample=200]..

Mechanical fragility was tested by rocking 3 mL of each suspension at 18 cycles/minute and ±17⁰ for one hour and then analyzing the amount of hemolysis using a Spectronic Genesys 5ª. A mechanical fragility index (MFI) was calculated for each sample using the formula

\[
MFI = \frac{H_{bexp} - H_{bcont}}{H_{bw,bl} - H_{bcont}} \times 100
\]

where \(H_{bexp}\) and \(H_{bcont}\) is the free Hb of the rocked and control sample, respectively, and \(H_{bw,bl}\) is the total Hb of the suspension \([n=12]\). In order to calculate the total hemolysis, equations were experimentally and mathematically found relating the absorbance of both OxyHb and MetHb, based on the equations proposed by Winterbourne⁵.

Viscosity was tested using a Brookfield Viscometer. Preparations were measured at shear rates of 50 s⁻¹ to 400 s⁻¹ \([n=12]\). Data was analyzed using an ANOVA with the Tukey post-hoc test. Alpha was set to 0.05 and \(p\) was considered significant if it was above 0.05.
**Results**

The results for deformability, as shown in Figure 1, showed no significant difference (p<0.05) between the EI for PFC or HBOC and the control at 100, 500, or 1000 s⁻¹.

![Elongation Index vs. Shear Rate](image)

Figure 1. Mean Elongation Index of PFC, HBOC, and Control groups. Standard error bars are presented.

For mechanical fragility, there was a statistically significant (p<0.05) decrease in MFI between PFC and the control, and a statistically non-significant (p>0.05) decrease in MFI between HBOC and the control. The results for viscosity were only analyzed for asymptotic viscosity (400 s⁻¹). They showed a statistically significant (p<0.05) decrease in viscosity between PFC and the control, due to a slightly lower hematocrit in suspension, and a statistically significant (p<0.05) increase in viscosity between HBOC and the control, due to the higher medium viscosity of HBOC in comparison to plasma or saline.

**Discussion**

This study successfully found any significant differences in the parameters of deformability, mechanical fragility, and viscosity. However, the values for the viscosity of PFC did not correlate well with previous studies, which stated that PFC had an equal or slightly higher viscosity than its control group at high viscosities. It is important to note that the high standard error for HBOC, higher than ±5%, in the mechanical fragility test is due to the need to dilute the HBOC solution before reading it on the spectrophotometer in order to get readings in range.

The fact that the spectrophotometer reads HBOC particles as free Hb meant that the difference in recordable free Hb is relatively smaller, so a slight mistake in dilution would cause a big difference in free Hb calculated. This could be remedied with better dilution techniques.

In summary, it was found that PFC and HBOC did not change the deformability of cells, meaning that RBC’s ability to reach tissues is not affected. PFC and HBOC lowered the mechanical fragility of RBCs, meaning cells are less susceptible to hemolysis. HBOC increased asymptotic viscosity, meaning that blood circulation is slightly hindered. PFC slightly lowered asymptotic viscosity.

**Conclusion**

These findings suggest that PFC and HBOC artificial blood products do not have negative effects on the rheological properties of whole blood or on the mechanical characteristics of RBCs.

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**References**