

# The Effects of Fatty Acid Ethyl Esters on the Mechanical Properties of Red Blood Cell Membranes

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## Abstract

The ability of red blood cells (RBCs) to deform is very important to the oxygen transport capacity within the body. When the delivery of oxygen is disrupted tissues are adversely affected. Fatty acid ethyl esters (FAEEs) are believed to be incorporated into RBC membranes following alcohol consumption. Furthermore, we speculate that this incorporation of FAEE leads to an increased rigidity of the RBC membranes.

This study analyzes the effects of FAEEs on RBC membranes using a microfluidic device, which is designed to measure pressure, drop. RBCs will be exposed to various concentrations of FAEE solutions. These samples will then be compared to normal samples of RBCs. Normal samples refer to RBCs that have not been exposed to FAEE solutions or ethanol. In order to evaluate the impact of FAEEs on RBC membrane rigidity RBC morphology and flow properties through the microfluidic device will be assessed.

## Introduction

The red blood cell (RBC) has a relatively simple structure, in that it does not contain a nucleus and can be described as a membrane which encapsulates hemoglobin. Its primary function is to supply oxygen to tissues within the body. A RBC has an average life span of 120 days during which time it recirculates through the heart about 170,000 times. A typical RBC is approximately 8  $\mu\text{m}$  in diameter and must be able to deform to flow through capillaries as small as 3  $\mu\text{m}$  in diameter.

Alcohol exposure has been shown to increase the order within RBC membranes. This increase in order leads to a decrease in the deformability of the RBC. If the RBC has more difficulty deforming this can lead to a lack of adequate supply of oxygen to the tissues. Chronic exposure to ethanol can cause a RBC to become more prone to hemolysis. Hemolysis of the RBC can lead to an increased concentration of free hemoglobin within the blood stream, which is toxic.

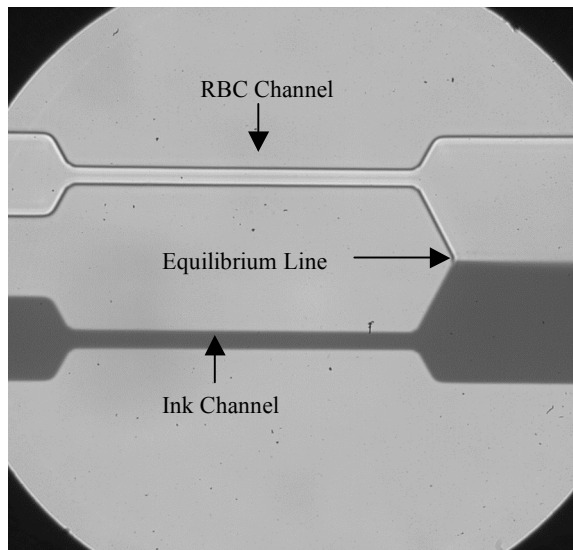
It is now believed that Fatty acid ethyl esters, esterification products of ethanol and fatty acids, incorporate into RBC membranes and thus may account for the observed increase in RBC rigidity. Because of this fact FAEE may prove to be useful as markers for detecting alcohol consumption. FAEE are cytotoxic ethanol metabolites found in tissue, plasma, and blood following alcohol consumption. In a study conducted by Best *et al.* (2003), it was concluded that there were strong correlations between RBC FAEE

levels and blood alcohol concentration. They further concluded that there was an even stronger correlation between plasma FAEE levels and blood alcohol concentration.

### Overview

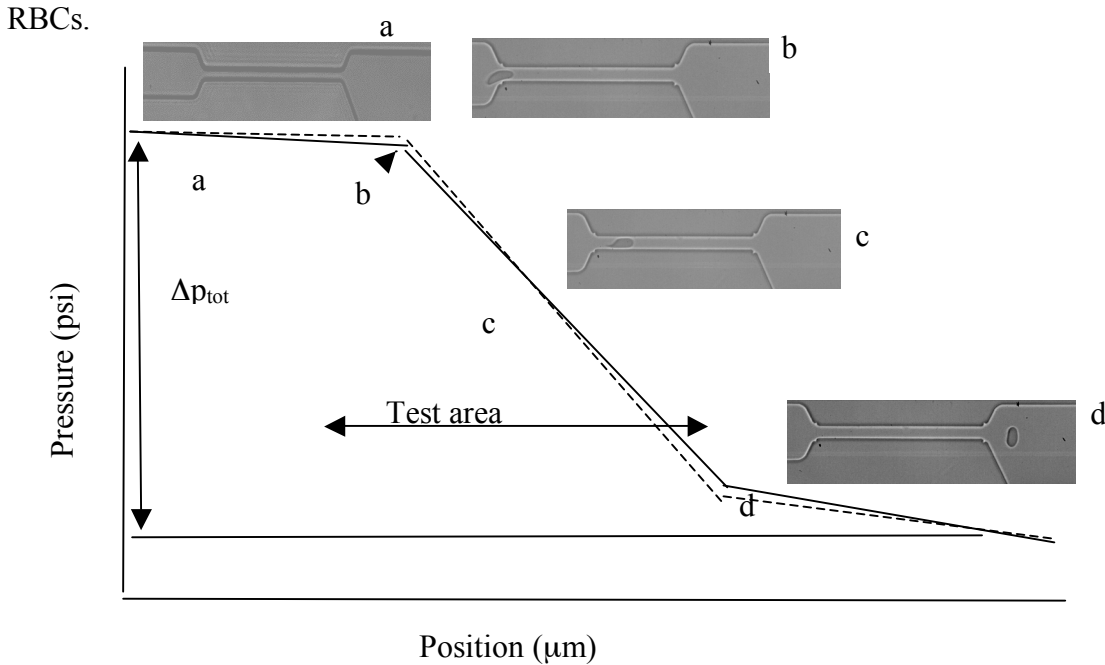
The goal of this project is to determine whether FAEEs, which incorporate into the phospholipid bilayer of RBC membranes, increase the rigidity of the membrane. Microfluidic devices designed for pressure measurements will be employed to test this hypothesis. These devices, which have a cross section of  $5\ \mu\text{m} \times 5\ \mu\text{m}$ , introduce the cells to constriction and provide an environment similar to that of the small capillaries found within the body.

RBCs were isolated from the plasma and other blood cells by centrifugation. The RBCs were washed and resuspended in phosphate buffered saline, and were incubated with varying concentrations of FAEEs (0, 10, 20, 40, and  $50\ \mu\text{M}$ ) or ethanol (16, 22 mM). These concentrations are equivalent to alcohol concentrations typically found in social drinkers and heavy drinkers as well. These test samples are then compared to normal RBCs (0 FAEE). For flow through the microchannel devices an analysis sample is prepared using  $100\ \mu\text{L}$  of prepared blood solution and 1.25 mL of 9% dextran solution.



**Figure 1.** Microfluidic device.

The microfluidic devices are created using soft lithography. Each device consists of two parallel microchannels, with independent input ports, which converge at a common exit chamber. RBCs are passed through one of the microchannels, while filtered ink is simultaneously passed through the other channel. These solutions are driven by a constant flow of nitrogen gas. Upon exiting their respective channels the RBC and ink streams meet and form a distinct interface within the exit chamber. The RBCs are pushed at 5 psi while the pressure of the ink is manipulated until the ink line forms a straight line, designated the equilibrium line, within the exit chamber (see Figure 1). Fluctuations in the ink-line, above and below the equilibrium line, are monitored and are used to measure changes in pressure within the device. This pressure change is a result of the RBCs blocking the channel, which is directly related to the deformation capability of the



**Figure 2.** Pressure profile of device.

Figure 2 is a schematic of the pressure change within the channel as a cell is just about to enter the channel up to the exit of the cell. At point a, the pressure is relatively constant. Point b is the point at which the cell deforms while entering the channel. In region c, the RBC is completely within the channel and deforming towards a steady shape. Region d indicates that the RBC has completely exited the channel. The dashed line indicates the change in pressure drop with cells present while the solid line indicates the change in pressure without the presence of cells. This change is relatively small and therefore causes a minor change in flow rate. The test area refers to the separate microchannel of the device.

The deviation of the ink line from equilibrium, assessed with Matlab software, will provide a quantitative measurement of the RBC deformation by comparison with a calibration curve, a measurement of the change in pressure caused by the cell. The calibration is performed by first determining the equilibrium pressure of the ink when the pressure of the RBC solution is at 5 psi. Once this pressure is determined, the pressure of the RBC solution is manipulated at 0.1 psi increments over a range of 4.5 psi to 5.5 psi. The curve generated from this process is then used to interpret the pressure drop profile generated using MatLab.

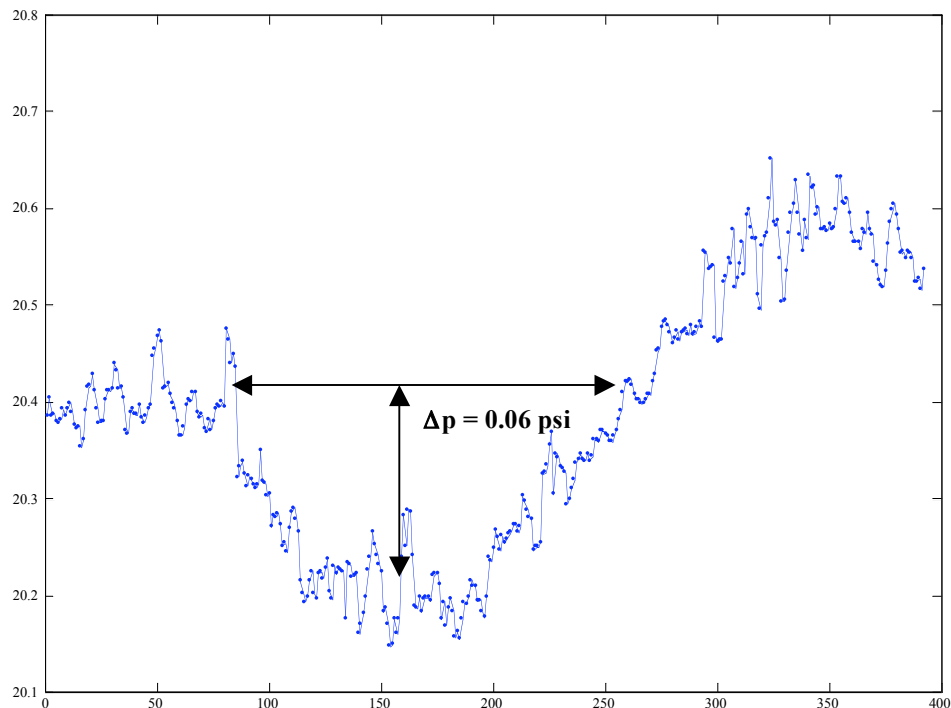
It is known that changes in the mechanical properties of RBC membranes affect the pressure change within the device. Because the movement of the RBCs is so fast, the high-speed Phantom camera is needed to sufficiently monitor the RBCs deformation and flow properties. Also, a magnification of 100x with the oil lens is used to view the cells within the devices.

The rigidity of the cell membrane, or the deformability of the membrane, will also be assessed based on the length the RBC travels before it reaches a static shape within a

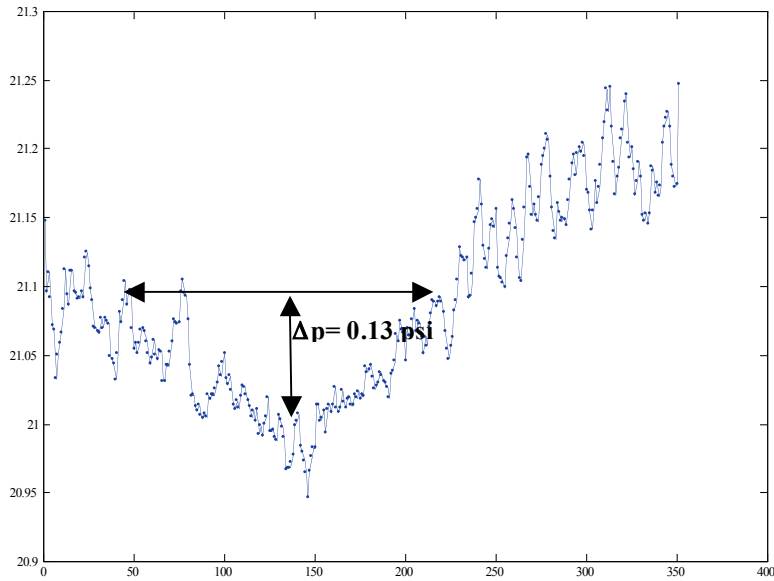
channel. This observation can provide some comparative analysis of the rigidity of the membrane of RBCs at various FAEE concentrations.

## Results

The following graphs (Figures 3 and 4) display the pressure drop in the exit chamber fluid following the entrance of a cell into the microchannel. Using a calibration curve of the device it is found that the cells treated with FAEE cause a pressure drop of 0.13 psi while the normal RBC solutions cause a pressure drop of 0.06 psi. The normal RBC and the FAEE treated RBC analyzed are approximately the same volume. This indicates that the FAEE treated RBCs experienced more resistance when deforming to pass through the channel.

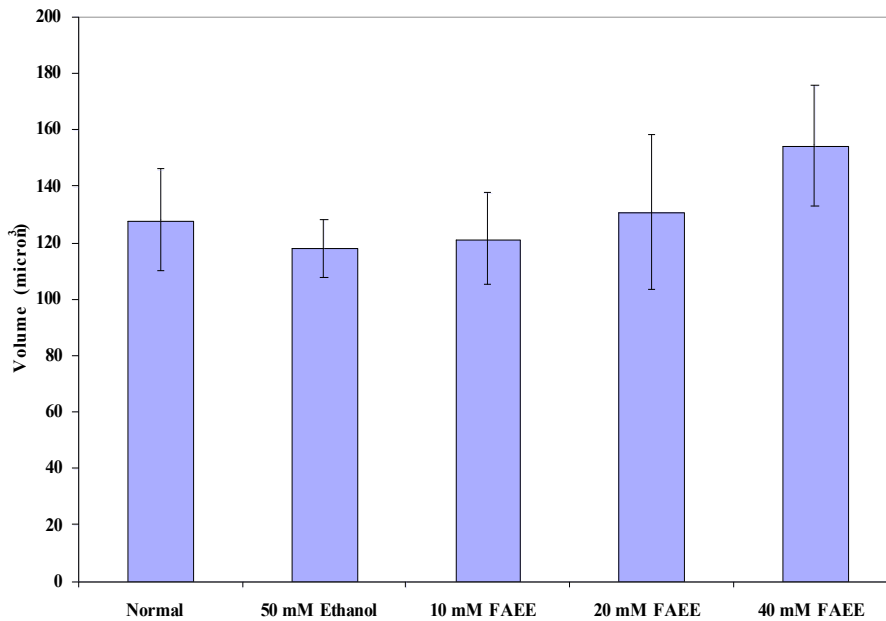


**Figure 3.** Pressure drop profile for a normal RBC.



**Figure 4.** Pressure drop pProfile for 40.

Figure 5 provides the average RBC volume of the various RBC test samples. This graph shows that as the concentration of FAEE increases the volume of the cell increases. This increase in RBC size can be linked to the hypothesis that FAEEs incorporate into the phospholipids bilayer of the cell membrane. Also, this finding is consistent with the observation that alcoholics tend to have larger RBCs.



**Figure 5.** Volume comparison of red blood cells with varying concentrations of FAEE.

## Conclusions and Future Work

Through data analysis there is evidence that FAEE treated cells are larger than normal unmanipulated RBCs. The cells treated with FAEEs also cause a larger pressure drop than the normal cells while flowing through the microfluidic devices. These are indications that FAEEs do in fact result in increased rigidity of the cell membrane and in turn a decrease of the deformability of the cell.

Future plans for this project include further data analysis in order to better quantify observations. A broad evaluation of current findings and instrument limitations will determine whether we have reportable findings acceptable for publication within a scientific journal.

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