On the use of polymeric additives to promote plasma separation from whole blood in open microchannels

E. Lee[1], J. Berthier[2,3], P. Laurent[2,3], F. Navarro[2,3], M. Cubizolles[2,3*]

[1] Department of Chemical Engineering, MIT, Cambridge, MA 02139
[2]University Grenoble Alpes F-38000 Grenoble,
[3] Department of Biotechnology, CEA, LETI, MINATEC Campus, F-38054, Grenoble, France.
France
*Corresponding author: myriam.cubizolles@cea.fr

ABSTRACT

Polymers additives in whole blood are known to modify blood physical properties. For example, IgG, IgM and Dextran molecules accelerate the aggregation of re-suspended RBCs in Ringer solutions.

In this work, we first investigate the effect of polymers such as Dextran, PEG (polyethylene glycol) and PVP (polyvinyl propylene) on the aggregation of RBCs directly in whole blood. We find that Dextran does not promote the aggregation in whole blood (in contrast to re-suspended RBCs in Ringer solutions), which PEG and PVP achieve.

In a second step, we make use of the aggregative properties of PEG and PVP to boost plasma separation from whole blood in open microfluidic channels. The approach is based on an enhanced sedimentation rate of RBCs aggregated by the polymers.

Keywords: Point-of-Care (POC), Whole blood, RBC aggregation, Dextran, PVP, PEG.

1. INTRODUCTION

Blood plasma contains a wide variety of analytes useful for diagnostic purposes, but sample preparation of plasma for Point-of-Care (POC) use continues to be a challenge, despite advances in other lab-on-a-chip technologies [1,2]. At the present time, no passive solution exists that fits the POC requirements such as no dilution, duration inferior to 10 min, volumes larger than 5 µl in free space (not in a fiber matrix). For example, the interesting system of Wang et al. requires dilution with PBS [3], and the plasma is trapped in a cellulose matrix in the paper device from Yang et al. [4].

It is known that antibodies such as IgG and IgM provoke the aggregation of RBCs, but they are dependent of the blood type, and may contaminate the plasma [5]. In this work, we explore a novel approach consisting in incorporating polymers additives in whole blood. It is expected that a judicious choice of polymers facilitates the aggregation of RBCs and consequently their sedimentation. It has been shown that washed red blood cells sediment quicker if mixed with dextran of various sizes in a Ringer solution [6].

In this work, we first investigate the effect of polymers such as Dextran, PEG (polyethylene glycol) and PVP (polyvinyl propylene) on the aggregation of RBCs in vertical microtubes. We find that Dextran does not promote the aggregation in whole blood (at the opposite of re-suspended RBCs in Ringer solutions), while PEG and PVP achieve thus goal.

In a second step, we make use of the aggregative properties of PEG and PVP to boost plasma separation from whole blood in horizontal open microfluidic channels. The advancing front of the capillary flow enriches progressively in plasma due to the enhanced sedimentation of the RBCs aggregates.

2. MATERIALS AND METHODS

Uncoated capillary tubes were obtained from Drummond Scientific Company (USA). Type A whole human blood in ethylene diamine tetraacetic acid (EDTA) was obtained from the blood bank (EFS, Etablissement Français du

TechConnect Briefs 2017, TechConnect.org, ISBN 978-0-9988782-0-1

Sang). Polyethylene glycol (PEG) was purchased from Sigma-Aldrich, and polyvinylpyrrolidone (vinylpyrrolidone-acetate copolymer, PVP VA 64F) was obtained from BASF (Germany).

Testing the efficacy of sedimentation reagents PEG and PVP VA 64F were dissolved in pure ethanol at concentrations of 40mg/mL and 50mg/mL, respectively, and were both diluted to various concentrations. Polymer-ethanol solutions were then added to capillary tubes cut in half with scissors, and were placed under vacuum at room temperature for 4 days to dry**.** Whole blood was then added to the dried capillary tubes, as well as untreated capillary tubes for controls, within 10 minutes. Tubes were left to stand upright in modeling clay for 90 minutes at room temperature. Pictures were then taken with the Canon EOS600D camera.

Polymethyl methacrylate (PMMA) chips and cyclic olefin copolymer (COC) chips were milled using the Datron Milling Machine (Datron, Germany). The design was created using DraftSight (Dassault Systèmes, France), and is shown in figure 1. Chips were then treated with plasma O_2 for 10 minutes at 200W such that the contact angle between the chip and water was inferior to 30°, and 150µL of whole blood was added within 5 hours after treatment. Pictures were taken every 30 seconds with the Canon EOS600D for about 40 minutes.

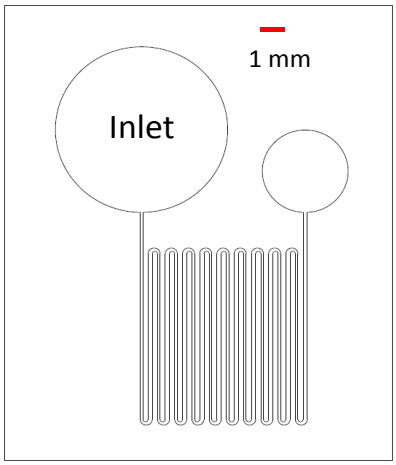

Fig.1. View of the open channel.

3. VERTICAL SEDIMENTATION-TUBES

We use vertical capillary tubes of 500 µm diameter (hematocrit tubes)—simply immobilized in modeling clay—to determine the sedimentation rate of whole blood alone and whole blood with different concentration of polymers.

The plasma:total volume of blood ratios of whole blood left to sediment inside the capillary tubes of various coatings were measured using the ImageJ Line Measure function. Figure 1 shows the differences in sedimentation for capillary tubes with and without adjunction of Dextran (70 and 200 kDa). It is observed that Dextran does not promote sedimentation, as was the case if added in Ringer solutions (RBCs resuspended in PBS).

Figure 2 shows the effect of PEG, and PVP VA 64F on blood sedimentation as compared to untreated capillary tube controls. Sedimentation is clearly accelerated by the addition of these polymers.

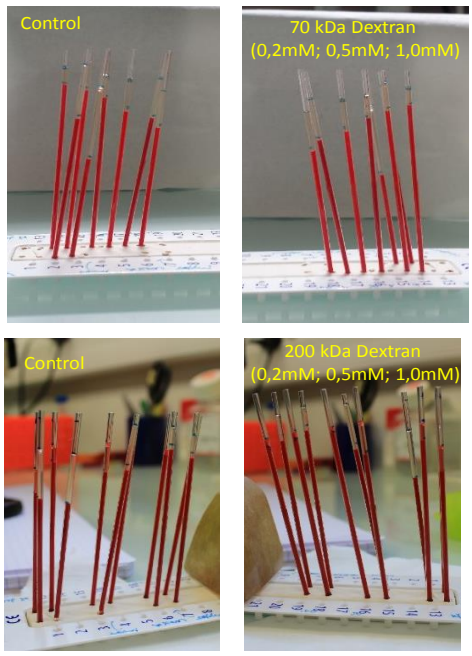

Fig.1. Sedimentation of whole blood: Dextran does not promote sedimentation. Top: comparison of sedimentation times between whole blood (control) and whole blood with 70 kDa Dextran at different concentrations; bottom: same as before with 200 kDa Dextran molecules.

PEG and PVP demonstrate comparable effects on red blood cell sedimentation (Fig. 2).

In conclusion, it is found that the presence of Dextran molecules (70 and 200 kDa) does not increase the sedimentation rate of RBCs in whole blood, unlike the reported observation of Dextran added to RBCs re-suspended in Ringer solution.

On the other hand, PEG and PVP used in the same way decrease considerably the sedimentation time of the RBCs by aggregating the RBCs together. High packing of RBCs

in the bottom of the tube is obtained: the ratio of plasma to total volume of blood approaches 0.45 in about 30 min.

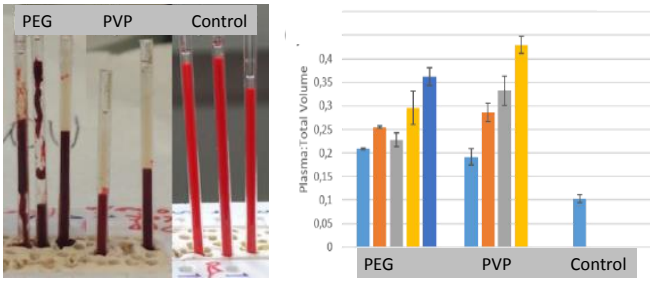

Fig.2. Sedimentation of whole blood in 10 min: comparison between control (no additives) and PEG and PVP solutions. Left: capillary tubes coated with (left) 64,4mg/mL PEG, (middle) 50mg/mL PVP VA 64F, (right) nothing; right: PEG concentrations: 2.5, 5, 10, 20, 40 mg/L; PVP concentrations: 5, 12.5, 25 and 50 mg/L: Addition of PEG or PVP increase considerably the sedimentation rate.

In the literature, the physics of aggregation of RBCs by polymers is the subject of discussions. It has been reported that it is the size, not the nature, of the polymer that is the dominant factor for aggregation [7,8]. A molecular weigth of 200 kDa approximately is cited by Armstrong et al. [7], and a hydrodynamic radius of 5 nm by Baskurt et al. [8]. On the other hand, Mosbah et al. [9] correlate the sedimentation rate to the deformability of the RBCs in presence of the polymer.

4. OPEN CAPILLARY CHANNELS

We now use open rectangular U-grooves to transport whole blood. The device is 45 cm long, 1 mm deep and 300 µm wide. Whole blood flows in the channel driven by the action of the capillary forces [10]. The velocity of motion is low due to high viscosity of whole blood [11].

During the flow, RBCs progressively sediment and a plug of plasma forms. After approximately 20 minutes in both the COC and PMMA chips (Figs. 3(a) and 3(c)), a small plasma plug forms at the tip of the flow (underlined by a black square in the figure). The plug continues to grow in size while the rest of the blood containing red blood cells flows down the channel, resulting in a sizeable plasma plug after about 40 minutes.

When PVP (or PEG) is added to the blood, sedimentation is boosted, as demonstrated before in vertical sedimentation-tubes. The plasma plug forms earlier, in 20 to 30 minutes approximately.

Figure 4 shows the formation of plasma plugs in microfluidic chips after letting whole blood flow, and figure 5 presents a detailed view of the plug (in the case of PVP, after 30 min). It is to be noted that plasma comprises about half of the volume of whole blood. The yield in plasma obtained with this open microchannel system is reasonably high: in the case of PVP, after 30 min, the volume of free plasma is approximately 15 µl, while the total volume of blood is 70 µl, and the total volume of blood plasma approximately 35 µl. Hence the efficiency of the extraction is of the order of 35% in 30 min.

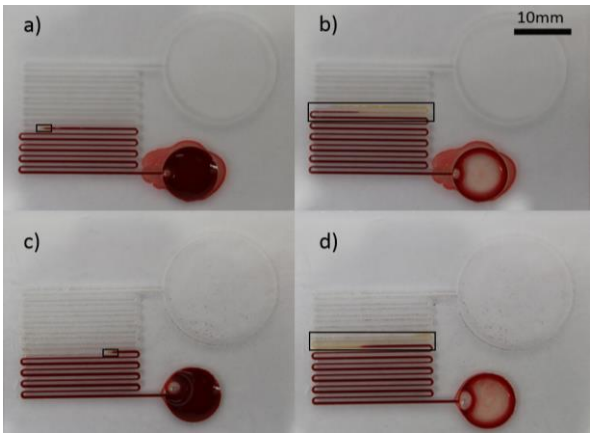

Fig.3. Formation of plasma plugs in microfluidic chips. (a) Formation of small plasma plug 22 minutes after addition of whole blood to COC microfluidic chip treated with plasma O_2 and (b) the plasma plug 41 minutes after addition of whole blood. (c) Formation of plasma plug after 23 minutes in PMMA microfluidic chip treated with plasma O_2 and (d) the plug after 43 minutes.

CONCLUSION

It has been shown that coatings of polymer such as PEG and principally PVP VA 64F noticeably increase the sedimentation rate of whole blood. Dissolution of these polymers triggers the formation of aggregates and accelerates the sedimentation rate of the RBCs contained in the blood, liberating blood plasma.

Plasma plugs have been demonstrated to occur in open microfluidic channels made of PMMA and treated with plasma O_2, and coated with PEG or PVP.

Future points of interest include revealing the mechanism by which PEG and PVP coatings promote sedimentation of whole blood, verification that the plasma maintains fidelity and does not change in composition as a result of mixing with PVP or PEG, and improvement of the

TechConnect Briefs 2017, TechConnect.org, ISBN 978-0-9988782-0-1

microfluidic system such that the plasma yield is increased in a shorter period of time.

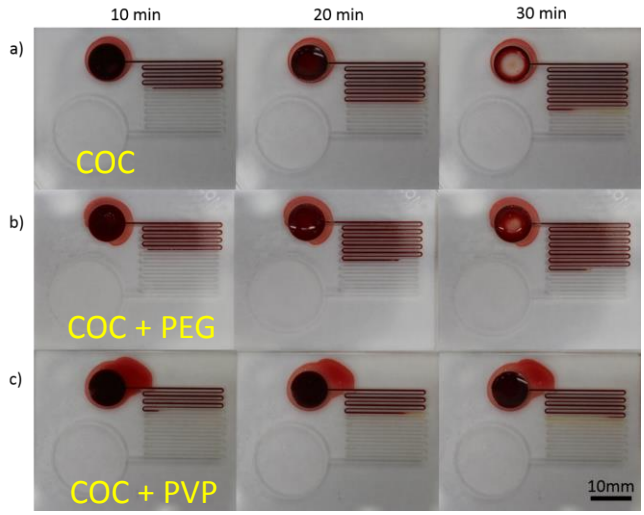

Fig.4. Promotion of plasma plug formation in microfluidic chips coated with polymer. Whole blood flowing through COC chip (a) without coating, (b) coated with PEG, and (c) coated with PVP VA 64F after 10 minutes, 20 minutes, and 30 minutes since blood was initially added to the input reservoir.

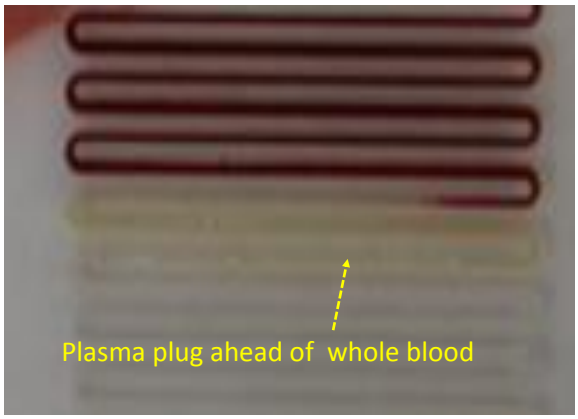

Fig.5. Details of the plasma plug in the open rectangular channel. The volume of plasma is approximately 15 µl.

ACKNOWLEDGEMENTS

This work was performed in the CEA-LETI labs during the internship project GIANT with MIT.

REFERENCES

[1] S. Tripathi, Y.V.B.V. Kumar, A. Prabhakar, S.S. Joshi and A. Agrawal, Passive blood plasma separation at the microscale: a review of design principles and microdevices, J. Micromech. Microeng. **25**, 083001, 2015.
[2] M. Kersaudy-Kerhoas and E. Sollier, Micro-scale blood plasma separation: from acoustophoresis to egg-beaters, Lab Chip **13**(17), 3323-46, 2013.
[3] S. Wang, D. Sarenac, M.H. Chen, S.H.Huang, F.F. Giguel, D.R. Kuritzkes and U. Demirci U, Simple filter microchip for rapid separation of plasma and viruses from whole blood, Int. J. Nanomed. **7**, 5019, 2012.
[4] Xiaoxi Yang, O. Forouzan, T.P. Brown and S.S. Shevkoplyas, Integrated separation of blood plasma from whole blood for microfluidic paper-based analytical devices, Lab Chip, **12**, 274, 2012.
[5] J.P. Cartron, P. Rouger, L'agglutination des globules rouges, Revue Française de Transfusion et Immuno-h6matologie, Tome XXII, **3**, 277, 1979.
[6] Shu Chien, Kung-Ming Jan, Ultrastructural basis of the mechanism of rouleaux formation, Microvascular Research **5**: 155-166, 1973.
[7] J. K. Armstrong, R. B. Wenby, H. J. Meiselman, and T. C. Fisher, The hydrodynamic radii of macromolecules and their effect on red blood cell aggregation, Biophysical Journal **87**, 4259–4270, 2004.
[8] O. Baskurt, B. Neu, H.J. Meiselman, Red blood cell aggregation, CRC Press, 2011.
[9] I.B. Mosbah, R. Franco-Gou, H.B. Abdennebi, R. Hernandez, G. Escolar, D. Saidane, J. Rosello-Catafau, and C. Peralta, Effects of polyethylene glycol and hydroxyethyl starch in University of Wisconsin preservation solution on human red blood cell aggregation and viscosity, Transplantation Proceedings **38**, 1229–1235, 2006.
[10] J. Berthier, K.A. Brakke, E.P. Furlani, I.H. Karampelas, V. Poher, D. Gosselin, M. Cubizolles, P. Pouteau, V-groove Whole blood spontaneous capillary flow in narrow V-groove microchannels, Sensors and Actuators B **206**, 258–267, 2015.
[11] D. Gosselin, M. Huet, M. Cubizolles, D. Chaussy, N. Belgacem, J. Berthier, Capillary flow of whole blood: the Newtonian to non-Newtonian transition, AIMS Biophysics **3**(3), 340-357, 2016.