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ENGINEERING

NOVEL-RESULT

The effect of hemicylindrical disruptors on the cell free layer thickness in animal blood flows inside microchannels

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Abstract

Blood-side resistance to oxygen transport in extracorporeal membrane blood oxygenators (MBO) depends on fluid mechanics governing the laminar flow in very narrow channels, particularly the hemodynamics controlling the cell free layer (CFL) built-up at solid/blood interfaces. The CFL thickness constitutes a barrier to oxygen transport from the membrane towards the erythrocytes. Interposing hemicylindrical CFL disruptors in animal blood flows inside rectangular microchannels, surrogate systems of MBO mimicking their hemodynamics, proved to be effective in reducing (*ca.* 20%) such thickness (desirable for MBO to increase oxygen transport rates to the erythrocytes). The blockage ratio (non-dimensional measure of the disruptor penetration into the flow) increase is also effective in reducing CFL thickness (*ca.* 10–20%), but at the cost of risking clot formation (undesirable for MBO) for disruptors with penetration lengths larger than their radius, due to large residence times of erythrocytes inside a low-velocity CFL formed at the disruptor/wall edge.

Keywords: Microfluidics; Blood flows *in vitro*; Cell free layer built-up; Plasma layer disruptors

1. Introduction

MBO replace temporarily shunted lungs in cardiopulmonary bypass, being a mature technology intensively used worldwide during heart surgery. Yet, relatively low oxygen transfer rates to the erythrocytes (RBC) are still a shortcoming (Matsuda & Sakai, 2007), making of mass transfer enhancement a path for MBO technical/medical progresses (Lim et al., 2006; Yeager & Roy, 2017). The CFL built up at the membrane/blood interface, typical of *in vitro* laminar blood flows (Popel & Johnson, 2005), is a major resistance to the oxygen transport to the erythrocytes. Although recognized that the blood-side resistance to oxygen transport is directly dependent on the blood flow characteristics in very narrow channels (characteristic lengths typical of microfluidics), little attention has been given to this subject, particularly on how interposition of disruptors at the membrane surface acts as potential reducer of CFL thickness (Lim et al., 2006; Yeager & Roy, 2017), which will constitute the main focus of the present work.

2. Objective

Effects on CFL thickness of interposing hemicylindrical disruptors within dog or horse blood flows inside rectangular microchannels, surrogate of MBO mimicking their hemodynamics (Yeager & Roy, 2017), are quantified. Different disruptor geometries, as displayed in Figures 1 (c,d), are placed at one wall for

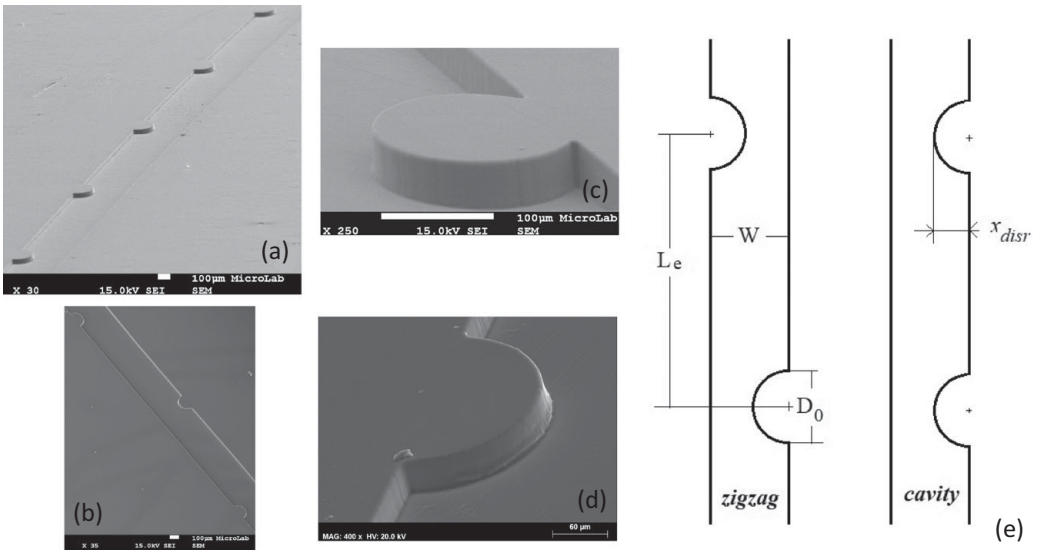

Figure 1. Illustrative SEM images of some microchannels and sketch of the cavity and zigzag disruptors arrangements: (a) SEM image of cavity microchannel (×30); (b) SEM image of zigzag microchannel (×35); (c) SEM image of CFL disruptor with the largest penetration length (×250); (d) SEM image of CFL disruptor with the smallest penetration length (×400); (e) top view sketches of the used zigzag and cavity microchannel arrangements and corresponding dimensions (D_0 – disruptor diameter; W – microchannel width; L_e – distance between disruptors; x_{disr} – disruptor penetration length.
Note – Visual inspection of magnified images acquired both with an optical microscope (objective ×40) and SEM (up to ×500) allowed for the assessment of the good quality of the microchannels (walls smoothness and good definition and perpendicular level of the walls).

cavity-flow, or alternately at opposing walls for zigzag-flow – see Figures 1 (a,b), with arrangements shown in Figure 1 (e).

3. Methods

Microchannels were manufactured with biocompatible PDMS, frequently adopted for *in vitro* blood flow studies (Haeberle & Zengerle, 2007; Ng et al., 2002), by photolithography (mould) and soft-lithography (microdevices).

Blood-flow images, acquired with a high-speed/high-resolution CMOS camera (Optronics-CR600x2) connected to a microscope (Sampaio et al., 2015), were digitally post-processed with MATLAB techniques to accurately determine the CFL thickness: walls were located with histogram threshold (Kim et al., 2009; Otsu, 1979) combined with Sobel/Prewitt filters; RBC/plasma interface identified with multi-threshold clustering (Sampaio et al., 2015).

Calibrating the used syringe pump (blood-flow generator), Nexus-5000-Chemyx, yielded as maximum flowrate uncertainty 6.0%. Blood, kept at 4 °C, was heated in a thermostatic bath (25 °C) before experiments, and waved gently for 1 minute (distributing the RBC and preventing sedimentation). Differently aged dog/horse blood viscosity was measured in a rotary viscometer DV-II + Pro.

Microchannels geometry was characterized by SEM images (Figure 1), testifying their surface smoothness (Silva et al., 2009), perpendicularity and disruptors good definition. Several similar images allowed comparing the actual height and width of all used microchannels with the designed ones (maximum error: 4%). Key parameters are displayed in Table 1.

4. Results and discussion

Different aged blood viscosity results, horse (hematocrit, $H_t \approx 40\%$) and dog ($H_t \approx 46\%$), displayed in Figure 2, evidence its negligible variation with blood age. Therefore, experiments performed up to 1 week after blood collection appear not to affect CFL results.

Table 1. Actual height and width (assessed from SEM images) of the manufactured microchannels. Dimensions are defined in Figure 1 (e). *R*: reference microchannel (no disruptors); *Ci*: cavity-flow type; *Zi*: zigzag-flow type.

Microchannel designation	Actual height [μm]	Actual width W [μm]	Design diameter D_0 of disruptor [μm]	Design distance between disruptors L_e [m]	D_0/W	L_e/W	Blockage ratio [x_{disr}/W]
R	41.5	394.9	–	–	–	–	–
C1	38.6	399.6	200	1,600	0.5	4	0.25
C2	39.3	399.6	260	1,600	0.65	4	0.325
C3	38.7	402.0	140	1,600	0.35	4	0.175
C4	38.6	397.9	200	1,600	0.5	4	0.35
C5	38.7	399.6	200	1,600	0.5	4	0.45
C6	39.3	399.0	200	1,200	0.5	3	0.25
C7	38.7	397.9	200	2000	0.5	5	0.25
Z1	39.7	405.3	200	1,600	0.5	4	0.25
Z2	40.7	415.8	260	1,600	0.65	4	0.325
Z3	38.6	422.1	140	1,600	0.35	4	0.175
Z4	40.5	419.8	200	1,600	0.5	4	0.35
Z5	38.6	409.6	200	1,600	0.5	4	0.45
Z6	40.9	415.8	200	1,200	0.5	3	0.25
Z7	38.8	414.0	200	2000	0.5	5	0.25

Note – design size values were: height = 40 μm; width = 400 μm.

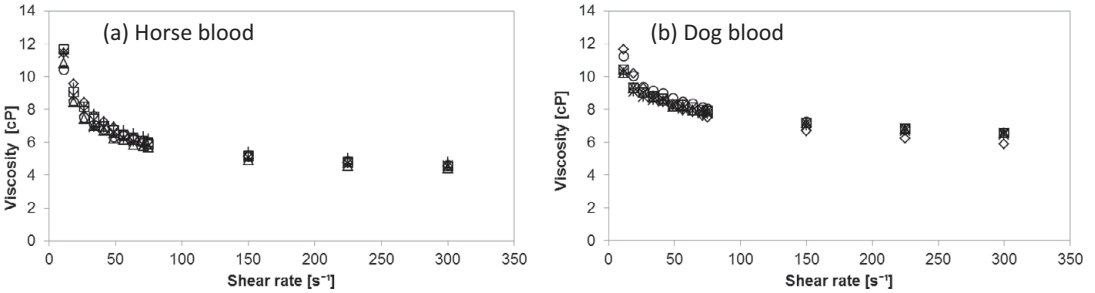

Figure 2. Effect of aging on the viscosity of blood of different animals [◇ 3 hours after collecting blood; □ 10 hours after collecting blood; △ 24 hours after collecting blood; × 48 hours after collecting blood; ∗ 3 days after collecting blood; ○ 7 days after collecting blood; + 15 days after collecting blood]: (a) horse blood (H_t≈40%, at 25 °C); (b) dog blood (H_t≈46%, at 25 °C).

Figures 3 (a-d) show CFL thicknesses for horse and dog blood flows for different flowrates (microchannels R, C1 and Z1). Figures 3 (a,b) evidence the systematic and more pronounced CFL thickness increase with flowrate in microchannel R. Disruptors interposition reduces always the CFL thickness (*ca.* 20%), which is desirable for MBO. However, comparing dog and horse blood flows, CFL thickness is *ca.* 6% smaller for the former: its larger hematocrit renders smaller the space available at the central region for RBC to migrate. Figures 3 (c,d) evidence the spatial periodic character of blood flows after some disruptors for the zigzag arrangement: CFL thickness keeps practically invariable between the 13th and 14th disruptors. This might suggest the zigzag arrangement as preferable.

CFL variation with the diameter was inconclusive (microchannels C1, C2, and C3) or incipient (microchannels Z1, Z2 and Z3). Moreover, CFL thickness kept unchanged with disruptors positioning (microchannels C1, C6, C7, Z1, Z6 and Z7).

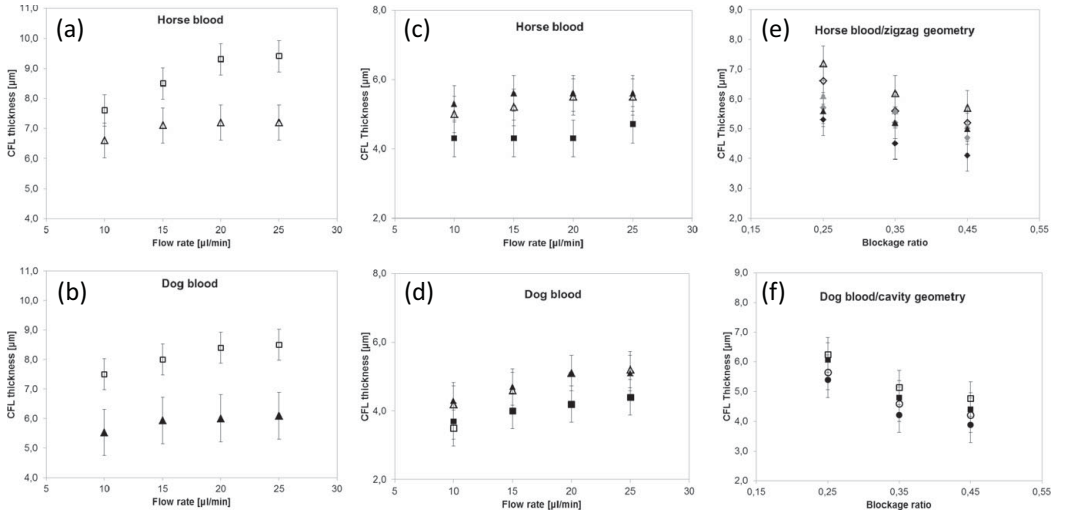

Figure 3. Thickness of CFL as function of the volumetric flowrate and blockage ratio (non-dimensional degree of the disruptor penetration into the flow). (a,b) Horse and dog blood flows [□ microchannel R (free of CFL disruptors); △ zigzag-flow type microchannel Z1; ▲ cavity-flow type microchannel C1]; CFL measured at the wall region opposing the 1st disruptor in microchannels Z1 and C1; (c,d) CFL thickness after some disruptors for horse and dog blood flows in microchannel Z1; CFL measured [□ right above 13th disruptor; ■ right above 14th disruptor; △ wall opposite to the 13th disruptor; ▲ wall opposite to the 14th disruptor]; (e) Thickness of CFL at the wall opposing the disruptor as function of the blockage ratio for horse blood flows in zigzag-flow type microchannels Z1, Z4 and Z5 [◇ 10 μl/min, 1st disruptor; ◆ 10 μl/min, 2nd disruptor; ◆ 10 μl/min, 14th disruptor; △ 25 μl/min, 1st disruptor; ▲ 25 μl/min, 2nd disruptor; ▲ 25 μl/min, 14th disruptor]; (f) Thickness of CFL at the wall opposing the disruptor as function of the blockage ratio for dog blood flows in cavity-flow type microchannels C1, C4 and C5 [○ 10 μl/min, 1st disruptor; ● 10 μl/min, 5th disruptor; □ 25 μl/min, 1st disruptor; ■ 25 μl/min, 5th disruptor]. Note – For the cavity arrangements, CFL exhibited a non-linear increase of its thickness with the flowrate at the downstream disruptors region.

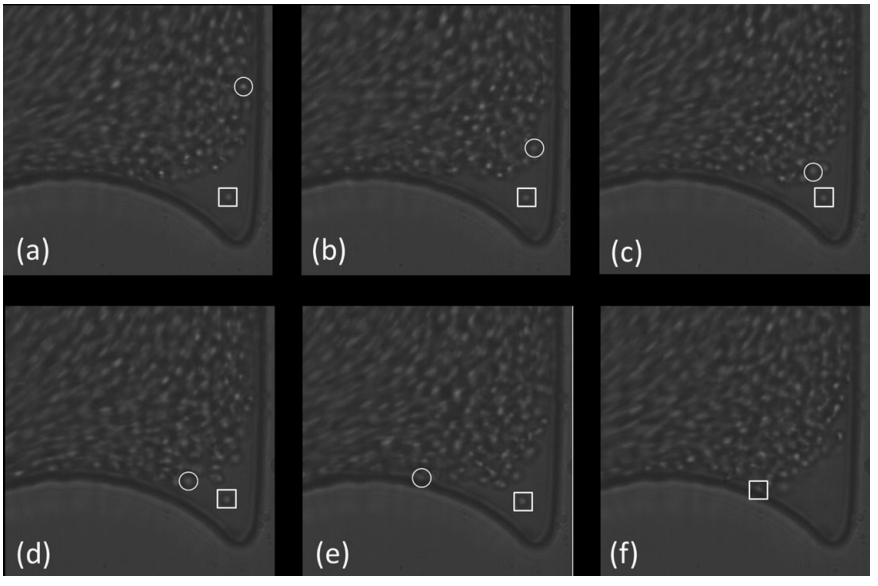

Figure 4. Illustration, for a blood flow around a disruptor with its penetration length into the flow x_{disr} larger than its radius $D_0/2$, of the large residence time of a RBC inside the CFL upstream and close to the disruptor edge (highlighted with a circle marker) in comparison with that of another RBC at the plasma/erythrocytes interface (highlighted with a square marker). Flow images at that region for different instants: (a) t = 0 s; (b) t = 0.01 s; (c) t = 0.02 s; (d) t = 0.03 s; (e) t = 0.04 s; (f) t = 0.26 s. Note – These images, extracted from hundreds of the sequential images acquired with a frame rate of 2000 fps (0.5 ms between consecutive frames), exhibit a partial top view of a disruptor with the flow coming from the top to the bottom of each image. There was no experimental evidence of a similar phenomenon for blood flow around disruptors with penetration lengths x_{disr} smaller than or equal to their radius $D_0/2$.

Figures 3 (e,f) reveal a systematic CFL thickness reduction with the blockage ratio increase (microchannels C1, C4, C5, Z1, Z4 and Z5): between 10–20% for dog blood in cavity-type flows; and 10–15% for horse blood in zigzag-type flows.

Images in Figure 4 suggest a limit for the blockage ratio: when x_{disr} is greater than $D_0/2$, a CFL localized at the disruptor/wall edge, with low velocities and large residence times, may potentiate clot formation (Completo et al., 2014), which is not desirable for MBO: erythrocytes inside such CFL require much more time (*ca.*$\times$6.5) to flow away from it than those outside it.

6. Conclusion

Interposition of disruptors in animal blood microchannel flows revealed effective in reducing CFL thickness (*ca.* 20%), suggesting zigzag arrangement as slightly better, a feature desirable for MBO as it promotes larger oxygen transport rates to the erythrocytes. Variation of the disruptors diameter and positioning yielded no relevant CFL thickness improvements. Blockage ratio (non-dimensional measure of the disruptor penetration into the flow) proved to be quite effective in reducing CFL thickness (*ca.* 10–20%). However, this comes at the cost of risking clot formation (undesirable for MBO) for disruptors with a penetration length greater than its radius.

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Author contributions. Prof. Viriato Semiao and Prof. Goncalo Silva conceived and designed the study. The MSc students Duarte Dias and Duarte Sampaio manufactured the microchannels, conducted experiments and data gathering and gave support to data analysis. Prof. Viriato Semiao, with support of Prof. Goncalo Silva, wrote the article.

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Data availability statement. Readers can contact the authors if they want access to such materials.

Conflict of interest. The authors have no conflicts of interest to declare.

Supplementary Materials. To view supplementary material for this article, please visit http://dx.doi.org/10.1017/exp.2020.60.

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Peer Reviews

Reviewing editor: Prof. Maria Norberta De Pinho
Universidade de Lisboa Instituto Superior Tecnico, Lisboa, Portugal, 1049-001

This article has been accepted because it is deemed to be scientifically sound, has the correct controls, has appropriate methodology and is statistically valid, and has been sent for additional statistical evaluation and met required revisions.

doi:10.1017/exp.2020.60.pr1

Review 1: The effect of hemicylindrical disruptors on the cell free layer thickness in animal blood flows inside microchannels

Reviewer: Prof. Margit Gföhler

Date of review: 25 September 2020

Conflict of interest statement. Reviewer declares none

Comments to the Author: The manuscript addresses a very important and timely topic. The presented experimental results are very interesting and will be valuable for further work on the improvement of mass transfer in blood oxygenators.

Specific comments:

Introduction:

- First sentence: use 'temporarily' instead of 'interim'.
- Line 7: not sure 'fluid mechanics' is the right expression here, as this basically refers to the science. Maybe replace by 'flow characteristics of the blood flow...', or similar

Methods:

- Please explain how exactly the thickness of the CFL layer was determined.
- Last sentence of methods: was the actual height taken at one specific location or at more points? How big is the variation within one channel? The error is meant between set and real height/width?
- Was wall smoothness measured? Or how do you testify from the SEM images?

Results and Discussion:

- Last sentence on page 2: the sentence is not very clear, please re-formulate.
- Figure 4: How were the locations of individual RBCs and their residence times determined in the flow images? Please explain.
- Page 5, last sentence of first paragraph: have the same visualizations as in Fig. 4 been made for other penetration lengths or how do the authors predict that the risk of clot formation only occurs for penetration length greater than radius? Please explain.

Score Card

Presentation

Is the article written in clear and proper English? (30%)	4/5
Is the data presented in the most useful manner? (40%)	5/5
Does the paper cite relevant and related articles appropriately? (30%)	5/5

Context

Does the title suitably represent the article? (25%)	5/5
Does the abstract correctly embody the content of the article? (25%)	5/5
Does the introduction give appropriate context? (25%)	5/5
Is the objective of the experiment clearly defined? (25%)	5/5

Analysis

Does the discussion adequately interpret the results presented? (40%)	4/5
Is the conclusion consistent with the results and discussion? (40%)	5/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	4/5

doi:10.1017/exp.2020.60.pr2

Review 2: The effect of hemicylindrical disruptors on the cell free layer thickness in animal blood flows inside microchannels

Reviewer: Prof. Miguel Minhalma

Date of review: 26 October 2020

Conflict of interest statement. Reviewer declares none

Comments to the Author: The paper presents important results obtained in a surrogate MBO system used to study the effect of disruptors in microchannels. The paper is in general clear and well written but there are some phases that should be rewritten: i) pag.2 – "… ,as illustrated in figures 1(a,b) and 1(c,d) for cavity- and zigzag-flow types, respectively, …", because fig b corresponds to a zigzag type; ii) "Figures 3 (a,b) evidence the systematic CFL thickness increase with flowrate in microchannel R", in fact there is an increase for all types of microchannels, but it is more pronounced in the R type; iii) ":its larger hematocrit renders space available at the flow central region for RBC to migrate smaller", this phase is confusing and should be clearer.

In page 3, the authors suggest that zigzag arrangement is preferable, but do not show or refer the results obtained for the cavity arrangement.

The last paragraph of the results should state clearly that the "dead volume" cavity in the disruptor only exists in the systems where xdisr is greater than D0/2 and that is shown in fig. 4.

Figure 4 caption should refer that it is a partial top view of a disruptor with the flow coming from the top to the bottom of the image. The authors should also refer (maybe in the introduction) that the flow inside the microchannels is always laminar and that the CFL layer dimensions are not related to the limit layer.

Score Card

Presentation

4.3 /5

Is the article written in clear and proper English? (30%)	4/5
Is the data presented in the most useful manner? (40%)	4/5
Does the paper cite relevant and related articles appropriately? (30%)	5/5

Context

4.8 /5

Does the title suitably represent the article? (25%)	5/5
Does the abstract correctly embody the content of the article? (25%)	5/5
Does the introduction give appropriate context? (25%)	4/5
Is the objective of the experiment clearly defined? (25%)	5/5

Analysis

Does the discussion adequately interpret the results presented? (40%)	4/5
Is the conclusion consistent with the results and discussion? (40%)	5/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	5/5