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Supporting Information

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On the Improvement of Alveolar-Like Microfluidic Devices for Efficient Blood Oxygenation

*Magdalena Malankowska, Ismael Pellejero, Ignacio Julian, Hoon Suk Rho, Pedro Pinczowski, Roald M. Tiggelaar, Han Gardeniers, Reyes Mallada, and Maria Pilar Pina**

Supporting Information

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M. Malankowska [a,b,c], I. Pellejero [d], I. Julian [a,b], H.S. Rho [c], P. Pinczowski [e], R.M. Tiggelaar [c,f], H. Gardeniers [c], R. Mallada [a,b,g], M.P. Pina [a,b,g,]*

[a]Instituto de Nanociencia y Materiales de Aragón (INMA), Universidad de Zaragoza-CSIC, 50009 Zaragoza, Spain.

[b]Departamento de Ingeniería Química y Tecnología del Medio Ambiente, Universidad de Zaragoza, 50009, Spain.

[c]Mesoscale Chemical Systems, MESA+ Institute, University of Twente, P.O. Box 217, 11 7500 AE Enschede, The Netherlands.

[d]Institute for Advanced Materials, Public University of Navarra, Edif. Jerónimo de Ayanz, Campus Arrosadia, s/n, 31006 Pamplona-Iruña, Spain

[e]Veterinary School of Zaragoza, C/ Miguel Servet 177, 50013 Zaragoza, Spain

[f]MESA+ NanoLab cleanroom, MESA+ Institute, University of Twente, P.O. Box 217, 11 7500 AE Enschede, The Netherlands.

[g]Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, 28029 Madrid, Spain

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1. Microdevice fabrication process

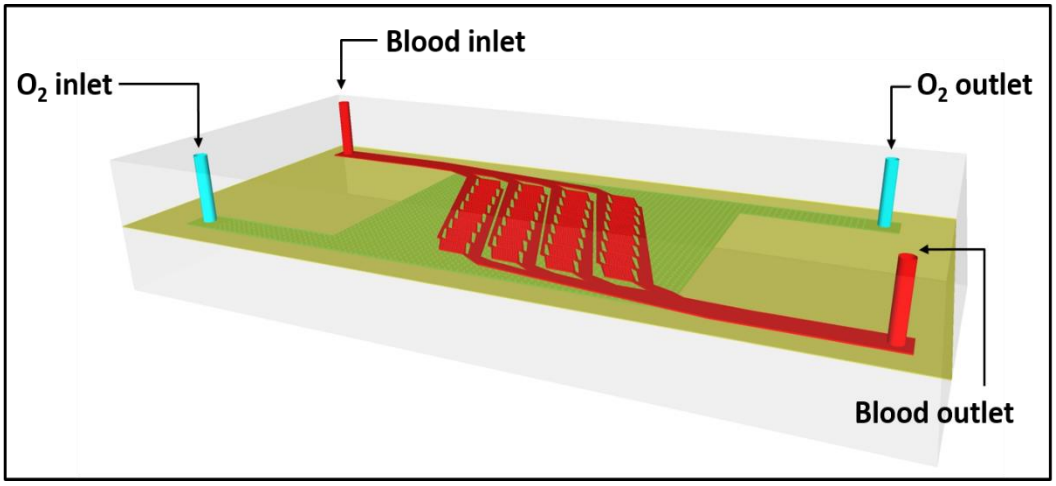

Figure S1 Schematic representation of the AD1 microdevice cross section with the inserted metallic pins that have an angle of 90° with respect to the flow direction in the microfluidic channels.

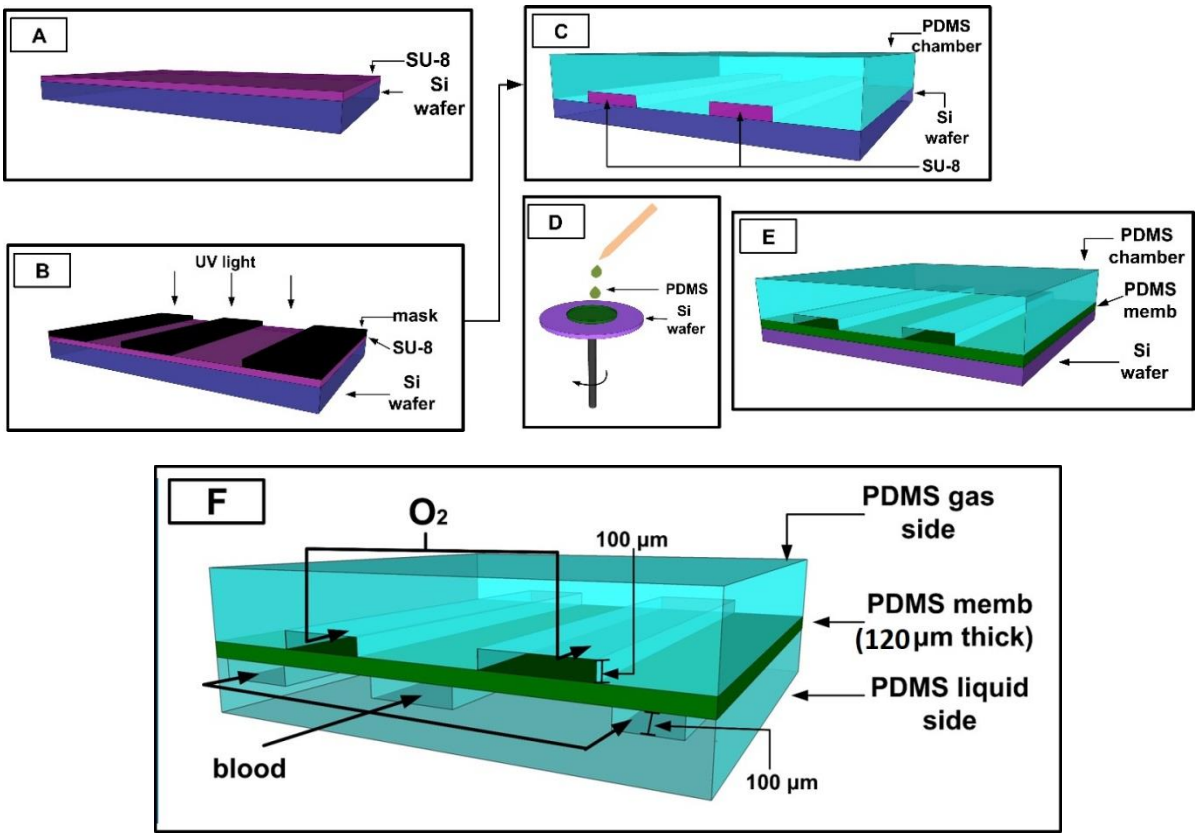

Figure S2 Process outline of the microfluidic chip fabrication. Spin coating of the SU-8 negative photoresist on the silicon wafer (A), mask alignment, UV exposure and SU-8 development (B), Pouring of PDMS on a created mould (C) and peeling off after curing. Membrane preparation by spin coating on a silicon wafer (D) and connecting to the PDMS mould by O_2 plasma procedure (E). After curing in the oven, peeling off the membrane from the chamber of the wafer and connecting to the other PDMS chamber (F).

2. PDMS surface modification and characterization of hemocompatibility

2.1. Pluronic F-68 + O_2 plasma treatment

In artificial lung assist devices, the adhesion of red blood cells is highly undesired to avoid membrane fouling and blood channels blockage. Hydrophilic surfaces are commonly preferred due to less protein unfolding [1, 2]. In this work, the surface of PDMS liquid channels as well as the membrane were modified with Pluronic F-68 (Sigma Aldrich), to make them more hydrophilic following the recipes already published [3, 4]. Briefly, the surface of the PDMS platforms were activated in the oxygen plasma chamber as it was described in the fabrication section. This treatment led to the formation of reactive hydroxyl groups on the surface. Next, Pluronic F-68 was thoroughly mixed with DI water in the concentration of 10 $mg \cdot mL^{-1}$ and introduced into the vascular channels with a syringe. The solution was kept inside the channels for 3 hours and subsequently washed with DI water for approximately 15 min [3, 5]. A schematic representation of the PDMS-Pluronic F-68 condensation reactions on the microdevice surface is illustrated in Figure S3.

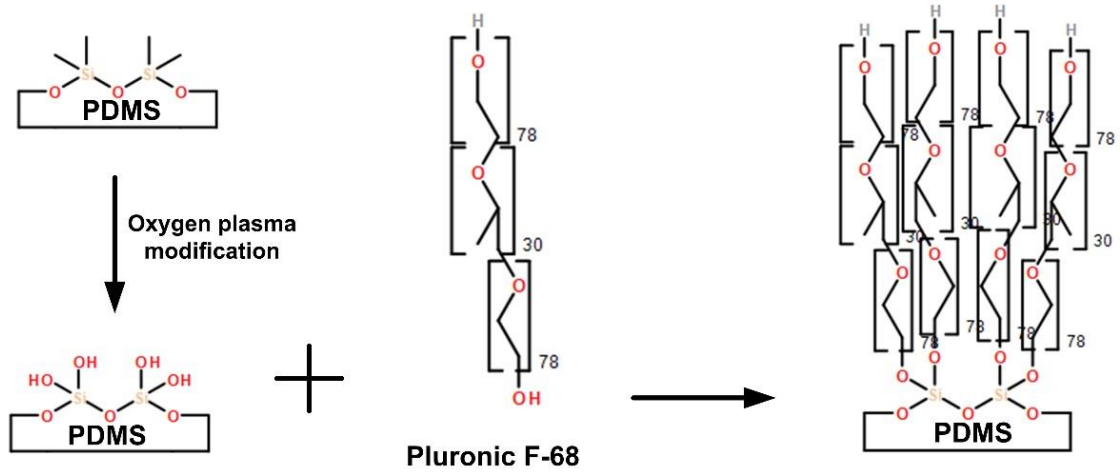

Figure S3 Schematic representation of the proposed PDMS surface modification

2.2. Modified device characterization

The PDMS surface alteration was evaluated by means of: visual inspection and contact angle measurements, adsorption of fibrinogen, and red blood cells viability test after oxygenation in the PDMS chips.

2.2.1. Visual inspection and Contact angle measurement

The static contact angle measurements were carried out with NEURTEK OCA 15EC instrument. The drop of distilled water (approximately 5 μL) was injected on the surface of cleaned untreated PDMS and PDMS modified by O_2 plasma and Pluronic F-68.

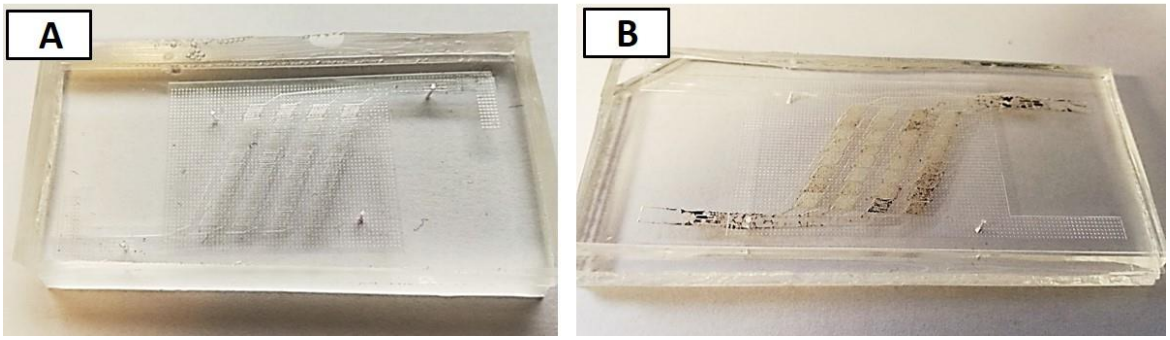

Figure S4 Visual inspection of AD1 type microdevices with: A) surface modification by O_2 plasma and Pluronic coating; and, B) non-modified surface after blood oxygenation experiments

Figure S4 illustrates the comparison of Pluronic modified PDMS surfaces, i.e. hydrophilic character with static contact angle (SCA) = 10˚±3 (Figure S4 A) and unmodified PDMS surfaces, i.e. hydrophobic character with SCA = 125˚±0.6 (Figure S4 B) after blood oxygenation experiments. It is visible that the Pluronic modified surface exhibits significantly less surface-bound clots than the un-coated control. However, the O_2 plasma treatment itself shows a decrease of SCA down to 65˚ ±8 – this effect was stable during 1 day. It was tested that the hydrophilicity that resulted from the combination of O_2 plasma and Pluronic F-68 lasted at least 20 days.

2.2.2. Fibrinogen adsorption measurement

Fibrinogen, a large protein present in blood, has high affinity to be adsorbed on the hydrophobic, positively charged surfaces [6]. Thanks to this feature fibrinogen is commonly used in the measurements of protein adsorption onto different superficies [7]. Firstly, the surface of PDMS was incubated at 37 ^{o}C overnight in PBS to prepare it for further treatment with fibrinogen by maintaining optimal conditions, such as temperature, humidity, oxygen content etc. Next, fibrinogen in the concentration of 0.5 mg/mL was spread on the surface of each sample (1cm x 1cm), and then the samples were incubated in a dark and humid environment for 24 hours at 37 °C. Afterwards, the samples were rinsed with distilled water. The samples were placed in a slide plate with 8 wells for examination under a fluorescence microscope, Leica SP2ADBS. The excitation was with Argon laser λ=488 nm and the

emission was collected in the range λ=494 to 572 nm. All the images are presented at a magnification x63. Finally, in order to clean the polymer surface, the PDMS was washed with DI water during approximately 15 minutes. Fluorescence images were recorded from: A) unmodified PDMS without a protein, i.e. blank experiment, B) unmodified PDMS with protein, i.e. control experiment, C) Pluronic modified PDMS without a protein and D) Pluronic modified PDMS with fibrinogen (Figure S5).

The protein adhesion becomes evident on the unmodified PDMS surface as indicated by the presence of green-fluorescence dots (see Figure S5 B). On the other hand, no proteins are distinguished on PDMS modified by O_2 plasma and Pluronic, in accordance to previous works [1, 2].

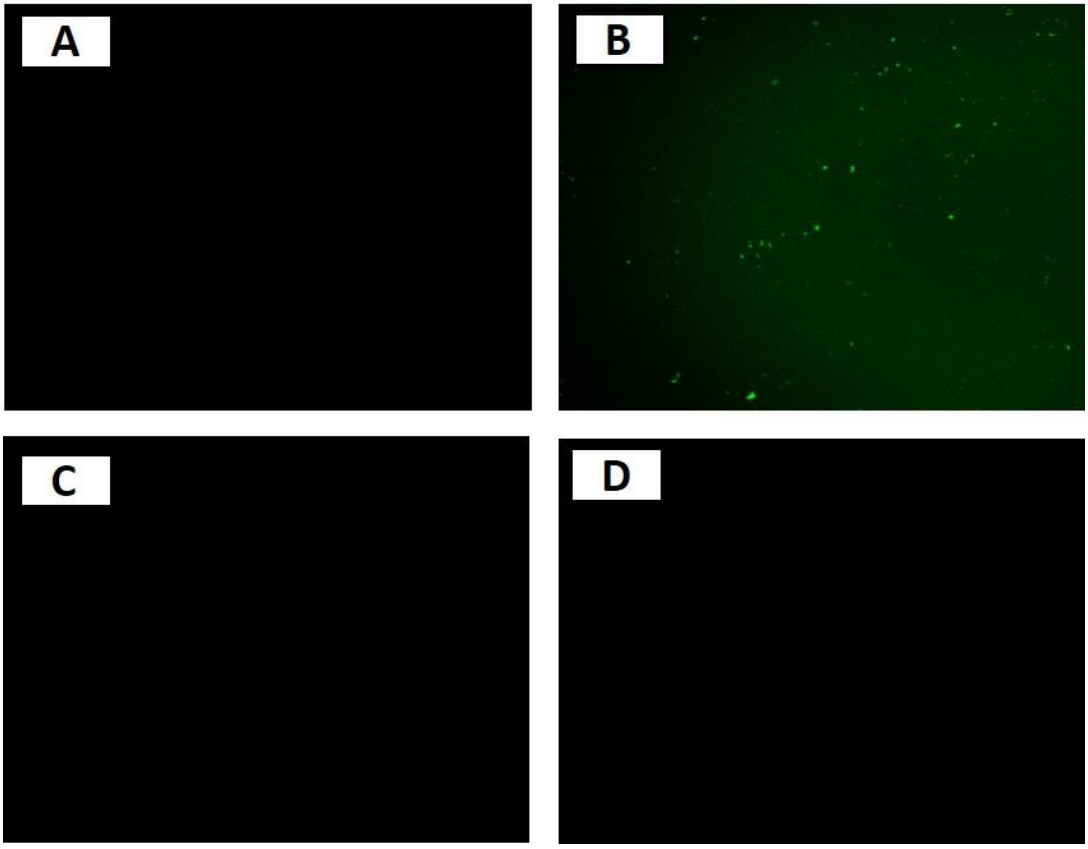

Figure S5 Visual representation of the fibrinogen adhesion. Fluorescence images of A) Untreated PDMS without protein, B) untreated PDMS with protein, C) Modified PDMS without protein and D) Modified PDMS with protein

2.2.3. Red blood cells (RBCs) viability measurement

The viability of RBCs was performed by an observation of the deterioration of the cells under the optical microscope. In order to be able to observe and count the cells and to check

their shape and structure of erythrocytes they had to be first stained. A blood smear was performed on the microscope glass slide and stained with hematologic staining (Differential Quik Stain Kit). The kit consists of three reagents: 1) a fixative for air-dried cell, 2) an eosinophilic to stain the cytoplasm, and 3) a basophilic solution to stain the nucleus. The blood smear was immersed 10 times in each solution and rinsed in water. After it dried, the smear was visualized in an optical microscope to assess the cell morphology. Additionally, the haemoglobin concentration was also evaluated by gasometry (Vet abc Classic, Animal Blood Counter) before and after each oxygenation experiment.

Figure S6 comparatively shows the white and red blood cells distribution, shape and structure before and after oxygenation in modified PDMS microdevices. Slight modification of RBCs represented by darker colour and no uniform outer edge (see Figure S6 B and C) in comparison to the cells before the experiment (Figure S6 A) is qualitatively displayed. In an attempt to quantify the extension of the RBCs deterioration, the concentration of erythrocytes before and after the experiment was measured by gasometry (see Table S1). The registered difference considered negligible when compared to the RSD values, to highlight the stability and functionality of the hydrophilic PDMS coating.

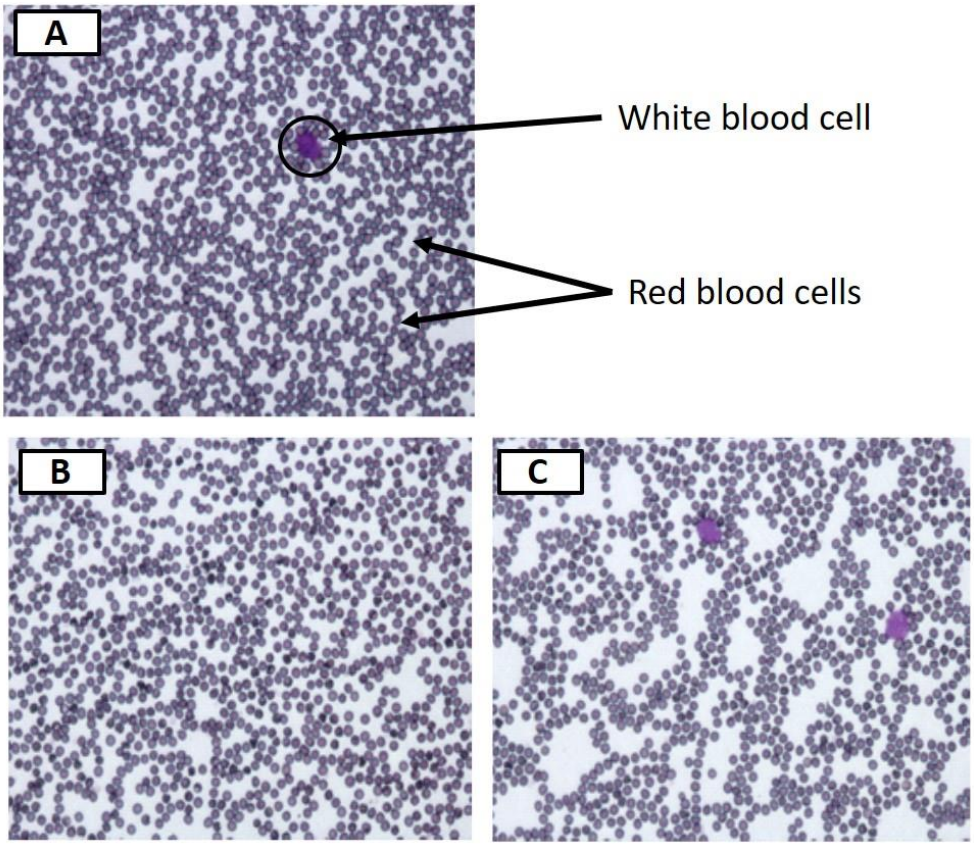

Figure S6 Optical microscope images of blood cells A) before and B), C) after the blood oxygenation experiments in the surface modified PDMS microdevice

Table S1 Gasometry results before and after the oxygenation experiments in the modified PDMS microfluidic device. Three different samples have been analysed for each case

Blood Sample	**Hb conc [g/dL]**	**Erythrocytes [mm^{-3}]**
Before oxygenation in the chip	13.5±0.6	$13.5\pm0.3\cdot10^{6}$
After oxygenation in the chip	13.1±0.7	$12.5\pm0.4\cdot10^{6}$

3. Blood oxygenation experiments

Table S2.A List of the experimental parameters (blood flow rates, PO_2 inlet and SO_2 inlet) and experimental results (oxygenation temperature, PO_2 outlet and SO_2 outlet and O_2 transfer rate).

AD1/Run 1

Q [mL/min]	Temp [ºC]	pH	[Hb] g/mL	*Po2 inlet (mmHg)	*SO2 inlet(%)	*Po2 outlet (mm Hg)	*SO2 oulet(%)	**O2 transfer [mL/min*m2]
0,1	32,9	7,41	0,136	37,7	75,45	81,7	97,53	18,48
0,3	32,6	7,4	0,136	37,7	75,45	71,4	96,19	51,79
0,5	32,5	7,41	0,136	37,7	75,45	64,3	94,71	79,83
0,7	32,4	7,41	0,136	37,7	75,45	57,8	92,67	99,53
1	34	7,43	0,136	37,7	75,45	51,7	89,73	117,58
2	27,5	7,41	0,128	61,2	93,82	97,7	98,62	83,25
3	27,1	7,4	0,128	61,2	93,82	87,5	98,02	106,95
4	26	7,41	0,128	61,2	93,82	86,1	97,91	138,54
5	26	7,42	0,128	61,2	93,82	85,1	97,83	169,51

AD1/Run 2

Q [mL/min]	Temp [ºC]	pH	[Hb] g/mL	*Po2 inlet (mmHg)	*SO2 inlet(%)	*Po2 outlet (mm Hg)	*SO2 oulet(%)	**O2 transfer [mL/min*m2]
0,1	30,4	7,38	0,142	38,9	77,26	153,4	99,68	20,54
0,3	29,5	7,41	0,142	38,9	77,26	104,2	98,88	57,54
0,5	27,5	7,4	0,142	38,9	77,26	101,4	98,77	95,27
0,7	29,7	7,42	0,142	38,9	77,26	77,3	97,04	120,80
1	28,8	7,43	0,142	38,9	77,26	67,6	95,47	157,96
2	27,5	7,39	0,128	47,4	86,76	66,5	95,23	134,42
3	27,8	7,42	0,128	47,4	86,76	63,2	94,42	181,69
4	27,5	7,43	0,128	47,4	86,76	62,6	94,24	236,46
5	26,8	7,41	0,128	47,4	86,76	61,5	93,93	283,14

AD1/Run 3

Q [mL/min]	Temp [ºC]	pH	[Hb] mg/L	*Po2 inlet (mmHg)	*SO2 inlet(%)	*Po2 outlet (mm Hg)	*SO2 oulet(%)	**O2 transfer [mL/min*m2]
0,1	33,5	7,45	0,139	38,8	77,11	127,9	99,43	19,70
0,3	35,2	7,43	0,139	38,8	77,11	66,4	95,23	46,14
0,5	35,8	7,42	0,139	38,8	77,11	58,7	93,01	67,18
0,7	34,2	7,41	0,139	38,8	77,11	53,6	90,76	80,54
1	34,1	7,42	0,139	38,8	77,11	49,3	88,15	92,87
2	27,4	7,43	0,132	61,7	93,99	82,8	97,63	63,05

AD2-5/Run 1

Q [mL/min]	Temp [ºC]	pH	[Hb] g/mL	*Po2 inlet (mmHg)	*SO2 inlet(%)	*Po2 outlet (mm Hg)	*SO2 oulet(%)	**O2 transfer [mL/min*m2]
0,1	31,5	7,42	0,142	39,3	77,94	138,2	99,56	13,31
0,3	32,2	7,42	0,142	39,3	77,94	128,7	99,44	39,46
0,5	30,6	7,41	0,142	39,3	77,94	110,6	99,08	63,88
0,7	30,5	7,39	0,142	39,3	77,94	81,5	97,51	81,28
1	31,2	7,4	0,142	39,3	77,94	64,5	94,76	98,74
2	25,8	7,4	0,146	56,0	91,53	108,8	98,93	97,00
3	23,5	7,39	0,146	56,0	91,53	111,5	99,01	147,72
4	23,5	7,38	0,146	56,0	91,53	87,1	97,82	159,76
5	23,1	7,4	0,146	56,0	91,53	94,4	98,31	217,75

AD2-5/Run 2

Q [mL/min]	Temp [ºC]	pH	[Hb] g/mL	*Po2 inlet (mmHg)	*SO2 inlet(%)	*Po2 outlet (mm Hg)	*SO2 oulet(%)	**O2 transfer [mL/min*m2]
0,1	37,2	7,39	0,136	33,2	66,94	111,9	99,11	18,39
0,3	35,8	7,38	0,136	33,2	66,94	115,5	99,20	55,43
0,5	33,7	7,4	0,136	33,2	66,94	98,4	98,65	90,07
0,7	32,7	7,39	0,136	33,2	66,94	69,1	95,79	113,18
1	31,2	7,41	0,136	33,2	66,94	62,3	94,15	152,09
2	29,3	7,42	0,141	51,5	89,18	85,0	97,65	102,66
3	32	7,41	0,141	46,5	85,51	70,9	95,85	183,20
4	31,1	7,43	0,141	46,5	85,51	67,2	95,10	225,82
5	28,7	7,42	0,141	46,5	85,51	66,6	94,97	278,33

AD2-5/Run 3

Q [mL/min]	Temp [ºC]	pH	[Hb] mg/L	*Po2 inlet (mmHg)	*SO2 inlet(%)	*Po2 outlet (mm Hg)	*SO2 oulet(%)	**O2 transfer [mL/min*m2]
0,1	38,1	7,41	0,136	34,1	68,93	116,4	99,22	17,40
0,3	37,4	7,39	0,136	34,1	68,93	103,5	98,85	51,23
0,5	36,3	7,38	0,136	34,1	68,93	95,8	98,52	84,11
0,7	35,7	7,37	0,136	34,1	68,93	66,5	95,24	103,22
1	35,1	7,4	0,136	34,1	68,93	57,3	92,45	131,26
2	27,4	7,43	0,142	52,5	89,74	92,9	98,22	104,75
3	29,6	7,38	0,142	44,8	83,98	67,4	95,15	198,47
4	29,3	7,39	0,142	44,8	83,98	62,6	93,91	234,44
5	29,8	7,41	0,142	43,0	82,44	61,5	93,59	327,98

[*]Expressed at standard conditions, i.e. 37ºC, pH of 7.4 and PCO_2 of 40 mm Hg.

[**] The O_2 transfer rates, $O_{2transf}$ ($mL \cdot m^{-2} \cdot min^{-1}$), through the membrane was calculated according to the following Equation:

$$O_{2transf} = \frac{\alpha \cdot \Delta P_{O2} + Hbs \cdot \Delta S_{O2} \cdot [Hb]}{S_A} \cdot Q$$

Where: α is the solubility coefficient of O_2 in blood plasma at standard conditions (see Table S3), ΔP_{O2} [mbar] is the difference between the outlet and inlet values of partial pressure that corresponds to the dissolved oxygen measured and expressed at standard conditions. Thus, the term $\alpha \cdot \Delta P_{O2}$ corresponds to the concentration of dissolved oxygen in plasma at standard conditions. ΔS_{O2} [-] is the increment in oxygen saturation in blood expressed at standard conditions, Hbs is the O_2 binding capacity of haemoglobin (see Table S3), [Hb] is the haemoglobin concentration in blood (see Table S2.A). The term $Hbs \cdot \Delta S_{O2} \cdot [Hb]$ corresponds to the concentration of oxygen in blood that is bound to haemoglobin. Q is the blood flow rate and S_A is the effective gas exchange membrane surface area.

Table S2.B Estimation of the rated blood flow on AD2-5 type design from the average incremental oxygen saturation values

Blood Flow Rate	DSO2 (%)	
[mL/min]	average	error
0,1	**28,03**	**4,60**
0,3	**27,89**	**4,62**
0,5	**27,48**	**4,57**
0,7	24,91	3,91
1	22,52	4,30

Table S3 Summary of the main blood and oxygen related parameters at 37ºC

Parameter	Symbol	Value	Units	Ref
Solubility coefficient of O_2 in plasma	α	$1.25 \cdot 10^{-3}$	$mol \cdot m^{-3} \cdot mmHg^{-1}$	[8]
Diffusion coefficient of O_2 in plasma	Db	$1.8 \cdot 10^{-9}$	$m^2 \cdot s^{-1}$	[8]
Number of binding sites of Hb	n	3.23*	-	[9]
Partial pressure of O_2 at 50% blood saturation	P_{50}	26.82*	mm Hg	[10]
O_2 binding capacity of Hb	Hbs	$1.34 \cdot 10^{-6}$	$m^3 \cdot g^{-1}$	
O_2 density	d	1331	$g \cdot m^{-3}$	
Blood density	ρ	1050	$kg \cdot m^{-3}$	[8]
Blood viscosity	μ	$2.9 \cdot 10^{-3}$	Pa·s	[8]
O_2 permeability, PDMS	Perm	$1.49 \cdot 10^{-11}$	$mol \cdot m \cdot m^{-2} \cdot s^{-1} \cdot mmHg^{-1}$	[11]

*It is important to mention that the parameter "n" of the Hill equation is constant only for particular blood species; n=3.23 for sheep blood according to [9]. The parameter P50 [mbar] depends upon pH and temperature, being 35.76 mbar at 37ºC, pH of 7.4 and PCO_2 of 40 mm Hg [10].

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