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COMMENT

Reply to the 'Comment on "The promise of microfluidic artificial lungs"' by G. Wagner, A. Kaesler, U. Steinseifer, T. Schmitz-Rode and J. Arens, *Lab Chip*, 2016, 16, DOI: 10.1039/C5LC01508A

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This response explores and discusses the critiques of Wagner et al. in their "Comment on 'The promise of microfluidic artificial lungs' by Joseph A. Potkay, *Lab Chip*, 2014, **14**, 4122-4138".

The goal of the "The promise of microfluidic artificial lungs" was to investigate the promising potential of microfluidic artificial lungs and provide the tools necessary to design and analyse this new class of devices. In their Comment [1], Wagner et al. provide a contrast to the Tutorial Review by focusing on the current challenges of microfluidic artificial lung technology. Wagner et al. raised a number of points that deserve further comment and exploration. These points have been summarized and addressed below and, where appropriate, specific comments of Wagner et al. are discussed. Table 1 below updates the data in "The promise of microfluidic artificial lungs" [2] with more consistent calculations for the performance parameters for all devices and the natural lung.

Calculation of the surface area and blood volume of microfluidic artificial lungs: In the Tutorial Review [2], the goal was to compare the regions of *gas exchange* in microfluidic artificial lungs, a commercially-available artificial lung, and the human lung. For microfluidic devices, the gas exchange surface area was thus calculated as the *actual* surface contributing *effectively* to gas exchange. Blood volume was calculated as the blood volume in the gas exchange area. In contrast, Wagner et al. [1] provide estimates for the total gas exchange surface area (effective and non-effective) and total blood volume of each device and used these values to calculate the various performance metrics.

Both of these approaches have merits. The original approach highlights that microfluidic artificial lungs with small diameter artificial capillaries can potentially achieve excellent gas exchange efficiency and small priming volume. The approach by Wagner et al. [1] highlights the current challenges of microfluidic devices and where commercial devices currently have an advantage. An updated table is provided below that combines the data in the Tutorial Review [2] and the comments by Wagner et al. [1] to compare both the gas exchange region and the total (gas exchange and distribution regions) device performance of microfluidic artificial lungs, a commercial artificial lung, and the human lung. Columns were added to investigate the percent of blood contacting surface area and blood volume that are used for gas exchange.

Performance of the natural lung: Wagner et al. [1] propose new numbers for the surface-area-to-blood-volume ratio of the human lung. The properties of the natural lung listed in the Tutorial Review [2] were copied from [3]. Wagner et al. are likely correct that the values listed in the Tutorial Review [2] for the natural lung are incorrect. Wagner et al., however, compare the lung at rest (and using only a fraction of its surface area and gas exchange ability) to artificial devices operating using their full capacity. In the updated table below, performance of the lung is provided for exercise conditions to provide a more fair comparison to current technology. Wagner et al. also omit information on sweep gas in their table. As the sweep gas is critical for gas exchange, it is included in Table 1.

An estimate of the surface-area-to-blood-volume ratio (SAV) of the capillaries in the lung can be calculated by assuming that each pulmonary capillary is approximately cylindrical with a diameter (D) of 10 μm and that three quarters [4] the surface area of each capillary contributes to gas exchange. In this case, SAV = 3/D = 3000 for the capillaries in the lung, or approximately the number arrived at by Wagner et al. [1]. For more precise calculations in the Table, below, we use information from the literature [3, 5, 6, 7, 8]. The blood volume of the pulmonary capillaries is approximately 100 ml during rest and 250 mL during exercise (due to recruitment of more capillaries and increase in capillary diameter). Total pulmonary blood volume is approximately 400 mL (rest) and 550 mL (exercise). The *alveolar* surface area is between 70 and 100 m^2. A value of 85 m^2 is assumed. The alveoli are surrounded by a dense network of capillaries. The structural matrix and tissue components of the alveolar-capillary

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network decrease gas exchange area slightly (~10%). The lung can exchange between 2 and 5 L/min of O2 and CO2 in exercise conditions. A value of 3.5 L/min is assumed. The numbers in the Table below are calculated using these assumptions.

Pressure drop and blood flow of microfluidic artificial lungs: Wagner et al. [1] cite early work in PDMS sheet artificial lungs to suggest that microfluidic artificial lungs cannot be designed with physiologic flows or pressure drops compatible with pumpless clinical operation. Several recent papers have described blood flow networks that exhibit physiologic shear stress, pressures, and flow fields [9, 10, 11, 12]. Regarding pressure drop, Hoganson et al. [10] described a branched vascular network with 100 μm tall artificial capillaries that achieved measured pressure drop of 35 mmHg at a blood flow of 14 mL/min. This results in a fluidic resistance of 2.6 $mmHg \cdot mL^{-1} \cdot min^{-1}$. Similarly, Kovach et al. [12] recently described a microfluidic lung with 10 μm tall capillaries and a branching blood distribution network that implemented Murray's law and was compatible with pumpless support. The entire blood flow network exhibited a measured pressure drop of 6 mmHg at the device's rated flow (0.4 mL/min) or a fluidic resistance of 15 $mmHg \cdot mL^{-1} \cdot min^{-1}$. Scaling to larger membrane areas will require that many of these devices be combined in parallel. When combined in parallel, rated blood flow will increase without significantly impacting pressure drop.

Scaling of membrane thickness: Wagner et al. [1] describe work from the 1960s and 1970s on PDMS sheet membranes where "reducing the thickness of PDMS membranes, even by half, showed no considerable effect" on gas exchange. As Wagner et al. note, this is because "gas transfer was found to be limited by diffusion within the blood" and was due to the large blood channel heights (the first PDMS sheet membranes in the had 80 μm tall artificial capillaries with heights a 127 μm-thick PDMS membrane [13]). This concept is illustrated in Fig. 5 of the Tutorial Review [2]. With the blood channel height set to 80 um, changing the membrane thickness has minimal effect on gas exchange. However, if the blood channel height is 10 um, reducing the membrane thickness has a large impact on gas exchange. Thus, as the blood channel height is decreased, the membrane thickness has to be decreased to maximize gas exchange performance.

Thrombosis in microfluidic artificial lungs: Wagner et al. [1] describe early work in PDMS sheet membranes in which reducing the blood channel height in order to increase gas exchange (without otherwise modifying the blood flow network) led to large blood side pressures and thrombolytic events. The large pressures and throbolytic events were seen in early microfluidic artificial lung work as well [14]. More recent works have produced microfluidic blood flow networks with physiologic blood flow [9, 10, 11, 12], pressure drops compatible with pumpless operation [10, 12], and coatings to significantly decrease thrombolytic events [15, 16]. Further research underway is expected to increase the blood compatibility of these microfluidic devices further.

Scaling of membrane area: Recent work from several research groups has developed technologies aimed at creating larger area microfluidic artificial lungs [11, 17, 18]. Specifically, Riepert et al. [18] developed a stacked microfluidic artificial lung consisting of up to 10 blood layers and 11 air layers. Due to the stacked nature of the device, gas transfer occurred from both sides of the device, increasing the fraction of blood contacting surface area that contributes to gas exchange. The rated blood flow of the device was approximately 83 mL/min for the 10 layer device, the largest rated flow for a microfluidic artificial lung to date. Pressure drop was 80 mmHg at a flow of 5 mL/min per layer (50 mL/min for the 10 layer device). An adult device was estimated to require 833 layers and a priming volume of 580 mL. Both numbers could be decreased through smaller blood channel heights or thinner membranes, but care would need to be taken so that pressure drop remained small. The overall assembly process was designed to be easily automated in the future.

Limitations of 2D Microfabrication Techniques: Microfluidic artificial lungs have demonstrated superior gas exchange efficiency. Gas exchange efficiency generally increases as the diameter of the artificially capillaries decrease (due to decreased diffusion distance). However, as artificial capillary height decreases in microfluidic artificial lungs, the percent of blood contacting surface area contributing effectively to gas exchange generally decreases (see Table 1). This is specifically pronounced in the work of Potkay et al. [14] and Kovach et al. [12]. In these to works, a large array of parallel, small diameter and short length artificial capillaries was used to increase gas exchange efficiency and maintain a small pressure drop. These large arrays of artificial capillaries required a branching blood distribution network. This distribution network contained a relatively large blood contacting area, but did not effectively contribute to gas exchange (Fig. 1). The main culprit for this is current microfabrication techniques, which limit fluidic designs to a two dimensional (2D) plane, thereby resulting in a large use of area. Stacking techniques (described above) have combined many 2D layers, but have not implemented truly 3D blood flow networks (as in the natural lung) which could potentially overcome the limitations and area inefficiency of these 2D microfluidic devices.

"It seems therefore reasonable to assume the gas transfer takes place in both the capillary network and the distributing network" in microfluidic artificial lungs: Although this is strictly true, gas exchange efficiency is much smaller in large diameter artificial vessels (Fig. 5 in [2]). Thus, only the artificial capillary network *effectively* contributes to gas exchange. This is further shown in Fig. 1. Initial attempts at manufacturing the devices described by Kovach et al. in [12] resulted in an x-axis shift of the 60 μm-tall artificial arterioles. This shift resulted in blood flow bypassing all the artificial capillaries in these early devices. Before the error was realized and fixed, gas exchange

in the devices was tested. Figure 1 shows gas exchange of the device with the artificial capillaries bypassed (open diamonds) compared with a fully functional device (filled diamonds) from [12]. Gas exchange without the artificial capillaries was approximately a factor of 10 less than with them (at the flow rates tested). In the updated Table, gas exchange efficiencies are reported for only the gas exchange region as well as for the combined gas exchange and distribution regions, when applicable.

"Hoganson et al. described the membrane surface area in the capillary network as accounting for 30 % of the total blood contacting membrane": Hoganson et al. stated "The vascular channels accounted for 30% of the surface area of the device" [10]. Based on the terminology of Hoganson et al. in the rest of the manuscript, "vascular network" refers to the entire blood network, not just the artificial capillaries. In the statement above, Hoganson et al. appear to be describing the entire surface area of the device, not the gas exchange area. Thus, the assumptions that Wagner et al. use in their calculations are incorrect. Updated Table 1 below fixes this calculation error, where possible.

Performance of the natural lung vs artificial lungs: The human lung is an amazing organ that exhibits both very efficient gas exchange and efficient transport of blood to the pulmonary capillaries. However, there is one area where artificial lungs can outperform the natural lung. Specifically, the natural lung only participates efficiently in gas exchange during half of the in-and-out breathing cycle. Artificial lungs, on the other hand, perform effective gas exchange constantly due to the constant one directional flow of the sweep gas. The Tables in [2] and provided by Wagner et al. [1] show that some artificial lungs already outperform the natural lung in terms of gas exchange efficiency ($mL \cdot min^{-1} \cdot m^{-2}$).

Final thoughts: As described in the original Tutorial Review [2], hemocompatibility and scaling remain challenges for microfluidic artificial lungs that are currently being researched. As highlighted in this Response, another challenge for microfluidic artificial lungs with small diameter artificial capillaries is the relatively large blood contacting area that is not contributing effectively to gas exchange. New 3D manufacturing techniques under development [11, 17, 18] should enable 3D microfluidic topologies which help to remedy this issue. These 3D microfluidic designs are likely to more closely mimic the efficiency and 3D structure of the natural vasculature than previous devices, resulting in a reduction in blood contacting area and priming volume.

Table 1 A revised version of Table 1 from [2] incorporating the Comment of Wagner et al. Gas exchange data represent the maximum values reported. Blood flow is the flow rate at which the maximum oxygen exchange was achieved. H is blood channel height. δ_M is membrane thickness. SAV is surface-area-to-blood-volume ratio. SAGE is the percentage of the total blood contacting Surface Area that is contributing effectively to Gas Exchange. VGE is the percentage of the total blood Volume that is contributing effectively to Gas Exchange. Values were calculated for the gas exchange region. Values in parentheses were calculated for the combined gas exchange and distribution regions, if different from that of the gas exchange region. Items that have been updated since the original Tutorial Review are *italicized*.

Source	H (μm)	δ_M (μm)	SAV (cm^{-1})	*SAGE (%)*	*VGE (%)*	O_2 Exchange ($mL \cdot min^{-1} \cdot m^{-2}$)	CO_2 Exchange ($mL \cdot min^{-1} \cdot m^{-2}$)	Blood Flow ($L \cdot min^{-1} \cdot m^{-2}$)	Sweep Gas
Lee 2008 [19]	15	130	476	*34*	*100*	256	-	22.5	O_2
	15	130	476	*34*	*100*	85	-	9.1	Air
Hoganson 2010 [9]	200	12	*45*	*23*	*100*	136	111	4.9	O_2
	200	15	*45*	*23*	*100*	145	86	4.9	O_2
	200	63	*45*	*23*	*100*	151	40	4.9	O_2
Potkay 2011 [14]	20	15	400 (*184*)	*33 (9)*	*100 (6.5)*	137 *(37)*	346 *(94)*	6.4 (*1.7*)	Air
	10	15	800 (*202*)	*36 (6)*	*100 (2.1)*	225 *(39)*	492 *(85)*	9.0 (1.6)	Air
Hoganson 2011 [10]	100	9	100	*25*	-	(41)	(191)	(9.1)	O_2
Kniazeva 2012 [11]	50	30	200	*36*	*100*	*358*	-	11.7	O_2
	100	30	100	*30*	*100*	*346*	-	11.7	O_2
	50	117	200	*36*	*100*	*243*	-	11.7	O_2
Wu 2013 [20]	80	15	125	*44*	*100*	12	108	2.6	Air
	80	15	125	*44*	*100*	19	92	2.6	Air
	80	6	125	*44*	*100*	13	90	2.6	Air
Rochow 2014 [17]	80	20	125	*44*	*100*	104	101	2.6	O_2
	80	20	125	*44*	*100*	31	140	2.6	Air
Kovach 2015 [12]	*10*	*15*	*800 (109)*	*34 (4)*	*100 (0.8)*	*133 (15)*	*478 (54)*	*6.3*	*Air*
Riepert 2015 [18]	*100*	*90*	*100*	*92*	*100*	*33*	*33*	*0.7*	*O_2*
Novalung iLA [21]	200	30	*111 (74)*	*98(92)*	*100(67)*	177	154	3.5	O_2
Human lung [3]	10	2	*3060 (1390)*	-	*100(40)*	*46*	*46*	0.6	Air

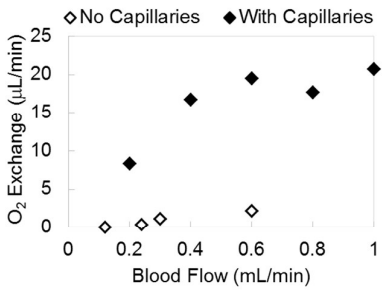

Fig. 1: Comparison of gas exchange with and without artificial capillaries in the work of Kovach et al. [11].

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