MAIN TEXT ARTICLE

Generation of microbubbles in extracorporeal life support and assessment of new elimination strategies

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Abstract

Occurrence of microbubbles (MB) is a major problem during venoarterial extracorporeal life support (ECLS) with partially severe clinical complications. The aim of this study was to establish an in vitro ECLS setup for the generation and detection of MB. Furthermore, we assessed different MB elimination strategies. Patient and ECLS circuit were simulated using reservoirs, a centrifugal pump, a membrane oxygenator, and an occluder (modified roller pump). The system was primed with a glycerin solution of 44%. Three different revolution speeds (2500, 3000, and 3400 rpm) were applied. For MB generation, the inflow line of the pump was either statically or dynamically (15 rpm) occluded. A bubble counter was used for MB detection. The effectiveness of the oxygenator and dynamic bubble traps (DBTs) was evaluated in regard to MB elimination capacities. MB generation was highly dependent on negative pressure at the inflow line. Increasing revolution speeds and restriction of the inflow led to increased MB activity. The significant difference between inflow and outflow MB volume identified the centrifugal pump as a main source. We could show that the oxygenator's ability to withhold larger MB is limited. The application of one or multiple DBTs leads to a significant reduction in MB count and overall gas volume. The application of DBT can significantly reduce the overall gas volume, especially at high flow rates. Moreover, large MB can effectively be broken down for faster absorption. In general, the incidence of MBs is significantly dependent on pump speed and restriction of the inflow. The centrifugal pump was identified as a major source of MB generation.

KEYWORDS
bubble trap, extracorporeal life support, extracorporeal membrane oxygenation, microbubbles, oxygenator

Frank Born and Fabian König contributed equally to this study.

1 | INTRODUCTION

Venoarterial extracorporeal life support (ECLS) is a promising, yet a highly invasive therapeutic option in cardiovascular intensive care medicine.[1-3] It is associated with a high incidence of complications and recently attention has been directed to the emergence of microbubbles (MB) within the circulatory system.[4,5] MB can lead to neurological morbidity or mortality due to a cascade of pathophysiological reactions, including diffuse cerebral microischemia as well as inflammatory reactions and activation of the complement system.[6] There are two common ways of MB formation within the ECLS circuit: they can form via bubbling due to hypobaric conditions (according to the Henry's law)[7] or suction can lead to MB by centrifugal pumphead- or oxygenator-induced microsplitting.[8] As ECLS is based on a closed system, the induced MB cannot dissolve into the atmosphere as it is the case in an open HLM circuit. Due to potential negative sequelae, it is necessary to reduce the occurrence of MB to a minimum. The implementation of a venous compliance reservoir (eg, Better-Bladder, etc.) or a pressure controlled pump management has been proposed.[9,10] But to our knowledge, no strategy has been described so far to achieve a sufficient reduction of already existing MB.

Thus, the aim of this study was to mimic the MB formation in an experimental in vitro ECLS circuit and to evaluate different strategies for MB removal including dynamic bubble traps (DBTs) and the oxygenator (OXY) without the bubble trap.

2 | METHODS

2.1 | Basic experimental circuit

For comparability, one experimental in vitro circuit was used throughout the whole study (Figure 1). However, parts of the system were clamped off and bypassed for the sub-analyses. Standard 3/8″ silicone ECLS tubing (P.h.i.s.o.; Sorin Group, Mirandola, Italy) was used to connect the different parts. An arterial and venous tubing length of 180 cm each (typically used in clinical settings) was selected. The system was primed with 8 L of water-based media. To mimic the viscosity of human blood $(4.4 \pm 0.6\,cP;^{11})$, a glycerin solution (44% in distilled water; Roth GmbH, Karlsruhe, Germany) was used. The systemic blood volume of the patient was simulated by a cardiotomy reservoir (Inspire 6F M; Sorin Group S.p.A.). The venous inflow line was connected to the inferior outlet and the arterial tube at the top in order to minimize the entry of potential MB from the reservoir into the experimental circuit. The patient's systemic and the arterial cannula resistances were simulated by the compression of the outflow tube using a Hofmann clamp. Resistance was adjusted to generate an arterial cannula pressure of 220 mm Hg at a flow rate of 4 L/min to simulate typical clinical settings.

A commercially available ECLS system (LifeBox; Sorin GmbH) was used. The centrifugal pump was equipped with a specific magnetically driven head (Sorin Revolution 5; Sorin GmbH). The pump was operated at three different speeds during the study: 2500 and 3000 rpm as standard configurations for clinical use and 3400 rpm as the highest reachable speed in our setting. A polymethylpentene (PMP) diffusion capillary membrane oxygenator (ECMO adult; Eurosets S.r.l.; Medolla, Italy) was used for gas exchange. A clinically typical oxygen flow rate of 2 L/min was applied to the oxygenator in order to generate a counterpressure on the oxygenator membrane and to replace the outgassed volume.

2.2 | Generation of MB

It is known that the hypobaric MB formation can occur especially in hypovolemic patients.[10] Hypovolemia in ECLS
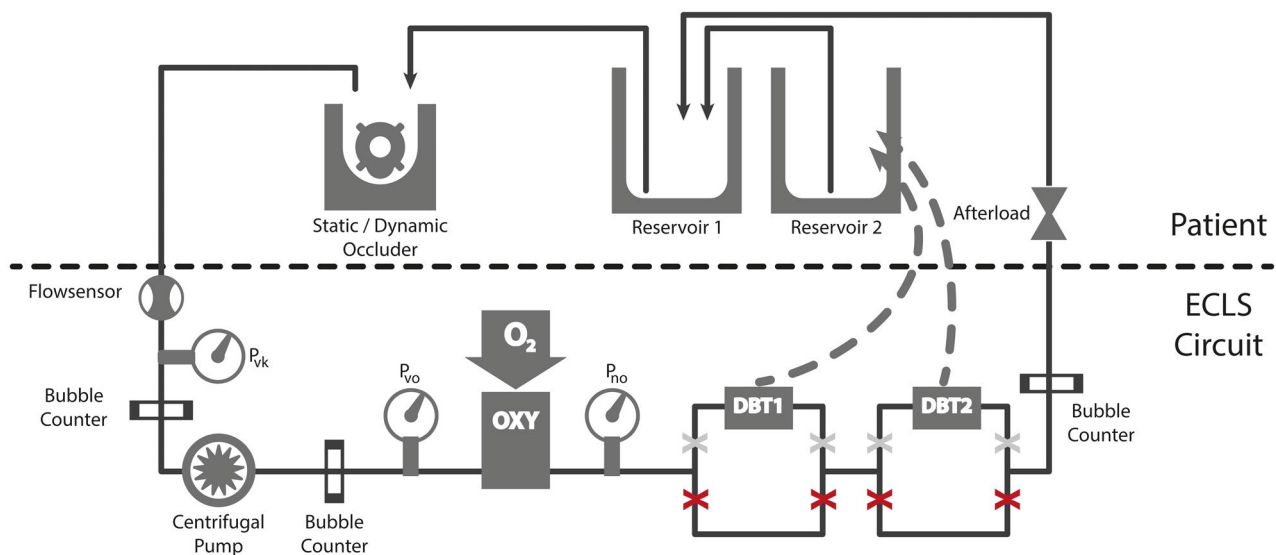

FIGURE 1 Experimental setup [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Flow and microbubble (MB) characteristics at different pump speeds and different types of flow restriction

	Pump speed (rpm)	Measuring point	Normal flow	Static flow restriction	Dynamic flow restriction
Flow [L/min]	2500	Inflow	4.03 ± 0.02	3.71 ± 0.01	3.78 ± 0.02
	3000	Inflow	4.97 ± 0.01	4.63 ± 0.01	4.69 ± 0.02
	3400	Inflow	5.78 ± 0.04	5.41 ± 0.04	5.52 ± 0.07
Pressure [mm Hg]	2500	Inflow	−66	−94	−76.72 ± 7.87
	3000	Inflow	−97	−143	−114.43 ± 12.38
	3400	Inflow	−127	−190	−148.15 ± 15.86
Count [1/L]	2500	Inflow	0.05 ± 0.01	0.13 ± 0.05	0.26 ± 0.16
		Outflow	1.41 ± 0.43	7.04 ± 6.36	8.55 ± 2.91
	3000	Inflow	0.94 ± 1.16	46.6 ± 36.75	5.8 ± 3.96
		Outflow	24.54 ± 29.51	289.21 ± 168.54	50.39 ± 47.75
	3400	Inflow	9.24 ± 12.65	900.43 ± 236.09	364.96 ± 271.77
		Outflow	128.63 ± 135.1	1262.51 ± 261.34	700.76 ± 460.15
MB volume total [nL/L]	2500	Inflow	1.88E-06 ± 1.49E-06	4.82E-06 ± 2.24E-06	1.08E-05 ± 8.32E-06
		Outflow	1.45E-04 ± 1.27E-04	2.79E-04 ± 2.23E-04	4.02E-04 ± 2.49E-04
	3000	Inflow	6.78E-05 ± 8.28E-05	3.62E-03 ± 3.03E-03	4.45E-03 ± 2.64E-03
		Outflow	1.64E-02 ± 2.15E-02	8.38E-01 ± 6.47E-01	1.98E-02 ± 3.54E-02
	3400	Inflow	5.42E-04 ± 7.49E-04	2.81E-01 ± 1.22E-01	7.69E-02 ± 3.76E-02
		Outflow	1.46E-01 ± 2.17E-01	5.25E+01 ± 1.69E+01	1.05E+01 ± 1.04E+01
Max. MB size [μm] n = >500 μm	2500	Inflow	56 ± 20.74	66 ± 13.42	76 ± 8.94
		Outflow	146 ± 77.01	124 ± 54.59	124 ± 8.94
	3000	Inflow	110 ± 30	148 ± 13.04	238 ± 30.33
		Outflow	270 ± 62.05	436 ± 66.56	238 ± 54.5
	3400	Inflow	114 ± 20.74	212 ± 13.04	234 ± 25.1
		Outflow	320 ± 60	>500[a] (573.4)	>500[a] (24.03)

Note: Measurements were performed at the inflow and outflow of the centrifugal pump. As the pressure was constant at "normal flow" and "static flow restriction", these values are presented without SD.

[a]Due to measurement device properties, sizes of MB >500 μm cannot be specified.

TABLE 2 MB reduction by different elimination strategies; MB were measured before and after the respective elimination device

MB size	Pump speed	Oxygenator		1 DBT		2 DBT	
		n [%]	V [%]	n [%]	V [%]	n [%]	V [%]
Total	2500	−54.57 ± 2.48**	−72.19 ± 4.39*	−46.77 ± 2.45**	−66.44 ± 4.75**	−71.29 ± 5.75***	−82.15 ± 2.97***
	3000	−14.13 ± 6.97**	−63.92 ± 8.35	−52.55 ± 17.31*	−95.81 ± 0.14	−73.15 ± 8.59*	−98.84 ± 0.12
	3400	2.2 ± 12.18	−40.87 ± 3.51*	−25.36 ± 9.02**	−96.81 ± 0.09	−49.1 ± 12.23*	−99.27 ± 0.02*
Very small <50 μm	2500	−49.8 ± 4.39**	−75.46 ± 8.22**	−43.14 ± 2.59**	−50.41 ± 16.54*	−68.75 ± 7.17**	948 ± 584.57
	3000	18.11 ± 24.1	184.79 ± 210.87	184.79 ± 210.87	156.11 ± 190.45	117.24 ± 154.05	−83.15 ± 10.23
	3400	18.19 ± 14.23	21.67 ± 12.64*	527.12 ± 164.04**	573.67 ± 191.62**	1016.77 ± 586.94*	948 ± 584.57*
Small 50-100 μm	2500	−79.19 ± 8.35*	−63.68 ± 22.36*	−71.49 ± 5.54***	−49.19 ± 23.8	−85.41 ± 3.58***	−85.41 ± 0***
	3000	43.65 ± 28.65*	42.77 ± 30.31	−13.6 ± 63.24	−36.98 ± 47.11	−98.6 ± 0.73*	−98.95 ± 0.54**
	3400	47.21 ± 3.88***	49.87 ± 4.93***	415.7 ± 288.83*	341.64 ± 276.17	120.47 ± 147.42	36.81 ± 95.54
Medium 100-150 μm	2500	−100 ± 0*	−63.68 ± 22.36*	−49.19 ± 23.8*	−100 ± 0*	−100 ± 0*	−100 ± 0*
	3000	16.76 ± 33.59	12.27 ± 33.41	−89.99 ± 6.68	−92 ± 5.16	−98.6 ± 0.73*	−98.95 ± 0.54*
	3400	66.42 ± 8.75***	66.73 ± 9.53***	12.14 ± 98.86	−9.07 ± 82.25	−92.35 ± 6.54***	−94.62 ± 4.66**
Large 150-300 μm	2500	–	–	–	–	–	–
	3000	−49.63 ± 19.76*	−60.94 ± 16.41*	−99.21 ± 0.14	−99.6 ± 0.22	−99.93 ± 0.05	−99.96 ± 0.03
	3400	34.45 ± 16.65*	25.55 ± 19.42*	−96.22 ± 3.68*	−98.43 ± 1.47*	−99.93 ± 0.06*	−99.97 ± 0.02*
Very large >300 μm	2500	–	–	–	–	–	–
	3000	−89 ± 8.36	−50.14 ± 8.17*	−100 ± 0	−100 ± 0	−100 ± 0	−100 ± 0
	3400	−38.39 ± 11.49**	−50.14 ± 8.17*	−100 ± 0	−100 ± 0	−100 ± 0	−100 ± 0

Note: Changes in amount and volume were expressed as percentage reduction. The Student's *t*-test was performed to calculate the significance of the reduction by comparison of the value sets measured before and after the elimination device.

– = no MB of the respective size were detected.

Abbreviations: DBT, dynamic bubble trap; n, number; V, volume.

*$P \leq .05$; **$P \leq .01$; ***$P \leq .001$.

therapy is normally accompanied by rhythmic occlusion of the inflow cannula due to recurrent drainage of the total blood volume in the blood vessel (eg, vena cava/caval vein) and subsequent suction to the vessel's wall. Another reason for hypobaric MB formation is the (static) complete or partial occlusion of the inflow cannula due to iatrogenic manipulation or nursing errors.[7] In order to simulate these conditions, we added a standard roller pump (type 10-00-00, Sorin Group S.p.A.) to the system and removed one roll. We adjusted the occlusion to reduce the tubing volume to approximately 20% (resulting from an occlusion of 114 mm leading to a gap size of 8 mm and standardized 1/8″ tubing). The inflow (venous) line of the ECLS unit was then either statically (stationary pump head) or dynamically (rotating pump head; 15 rpm) compressed by the roller pump.

2.3 | Different elimination strategies

In this study, different MB elimination strategies were tested. The first experimental setting (ES$_1$) measured the MB removal capacity of the oxygenator. In a second experimental setting (ES$_2$), a DBT (Kardialgut GmbH, Petersdorf, Germany) was included into the extracorporeal circuit. Finally, the third experimental setting (ES$_3$) evaluated the in-line connection of two DBTs. The DBT includes a helix and thereby induces an axial rotation of the blood flow. This results in a centrifugal concentration of the MB in the center of the bloodstream. There a bypass line separates the bubble-rich fraction of the blood flow. The DBT is made from polycarbonate, has a priming volume of 50 mL, and is usually used in the arterial line (3/8″) of a heart-lung-machine during the extracorporeal bypass. A second cardiotomy reservoir was used for the drainage of the bypass lines of the DBTs. These bypass lines were linked via Luer connections and a standard infusion line. The relatively low flow (275 mL/min at 4 L/min blood flow rate; manufacturer's specification) enabled a long resting time of the media in the second reservoir. This way, potential (micro-) bubbles were removed by gravitation. A connection tube ensured the passive hydrostatic backflow of the media to the main reservoir.

2.4 | Data acquisition

Flow (SCP, Sorin Group S.p.A.) and pressure (SCPC, Sorin Group S.p.A.) sensors were used for the assessment of flow and pressure characteristics (Figure 1). Both, flow and pressure transducers were attached to the inlet of the centrifugal pump. Two additional sensors measured the pressure before and after the oxygenator, respectively. The flow rates and pressures were measured every minute for the static flow restriction runs and every 30 seconds for the dynamic flow restriction runs.

Additionally, a specific micro bubble counter (BCC 200; GAMPT mbh, Merseburg, Germany) with two Doppler

effect-based ultrasound probes was used for MB detection, as described elsewhere.[12] The device is able to count as well as to measure the dimensions of the MB flowing by. Depending on the specific test run, the probes were either placed before and after the centrifugal pump (generation of MB) or before and after the bubble elimination section (evaluation of the elimination strategies). The total number, the volume distribution, and the overall volume of the MB were documented at the end of each run. Due to device properties, MB with a size >500 μm could not be differentiated and only their total number was counted.

For a more detailed assessment of the elimination strategies, the removed MB were classified into five different groups depending on their size (Table 2): <50 μm := very small; 50-100 μm := small; 100-150 μm := medium; 150-300 μm := large; >300 μm := very large.

2.5 | Experimental design

First, the occurrence of MB was quantified for each pump speed (2500, 3000, and 3400 rpm) either with static or dynamic compression of the inflow line. Thereafter, different MB elimination strategies were assessed during the static compression of the inflow line. This resulted in the continuous induction of MB and the elimination capacities of the different approaches could thus be determined precisely. All setups were tested for 20 minutes and repeated for five times ($n = 5$). To eliminate preexisting MB and influences of previous test runs, two techniques were applied: before data collection, the system was primed at a pump speed of 1200 rpm until no MB were detected for at least 5 minutes. Additionally, the system was shut down between two experimental runs and rested for at least one hour.

The data gathered from these experiments were then preprocessed for easier handling in the statistical analysis: the raw data collected with the two MB sensors were processed to calculate the removal efficiency of the respective strategy. The removal efficiency was defined as:

$$\text{removal efficiency} = 1 - \frac{\text{MB}_{\text{before}}}{\text{MB}_{\text{after}}}.$$

2.6 | Statistical analysis

The Anderson-Darling test is applied to evaluate if the measured data is adequately described by the normal distribution. Where applicable, data are reported as mean ± standard deviation. Differences between the groups were detected by performing the unpaired Student's *t*-test.

To compare the group means in regard to the statistical significance in detail, a multiple regression model with interactions was developed. A two-way ANOVA test was performed to evaluate the significance of the chosen predictors

FIGURE 2 Pressure and MB
generation depending on occlusion type
and pump speed. Measurements were
performed at the inflow and outflow of the
centrifugal pump. Increasing pump speed
leads to an increase in the negative pressure.
It is noteworthy that the type of occlusion
has a significant effect on the resulting
MB volume [Color figure can be viewed at
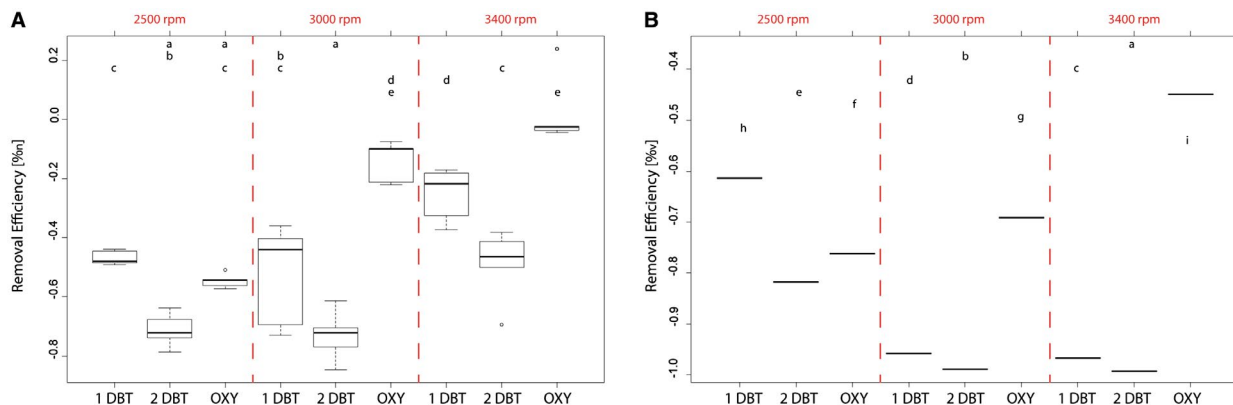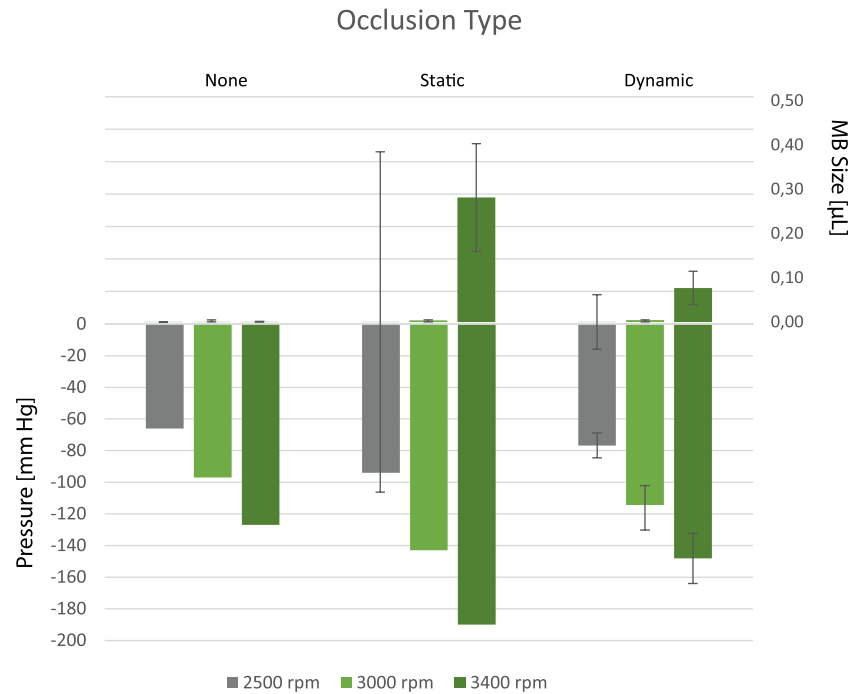wileyonlinelibrary.com]

FIGURE 3 Compact letter display for all pairwise comparisons in regard to MB removal efficiency in regard to MB count (A) and volume (B). While the use of an OXY might be a solution at low flow rates (<2500 rpm), its effectiveness decreases significantly at higher flow rates. Both DBT strategies show a significant increase in MB volume removal with increasing flow rates [Color figure can be viewed at wileyonlinelibrary.com]

(elimination strategy/pump speed). For post hoc analysis, a Tukey test was applied to perform a pairwise comparison of the elimination strategies. The α-level was set at 0.05. Data are displayed as box plots with compact letter display.

3 | RESULTS

3.1 | Flow characteristics and MB activity

Flow characteristics as well as MB formation, the total MB volume, and the maximum MB size were assessed at different pump speeds (see Table 1). At normal flow conditions, three different scenarios with flow rates 2500 rpm (4.03 ± 0.02 L/min), 3000 rpm (4.97 ± 0.01 L/min),

and 3400 rpm (5.78 ± 0.04 L/min) were evaluated. Simultaneously, the negative pressure at the inflow of the pump increased from −66 to −127 mm Hg. After the static compression of the inflow cannula, the flow rate decreased by $7 \pm 1\%$ at all pump speeds and consequently the negative pressure intensified by $46 \pm 4\%$. Dynamic flow restriction showed similar, yet weaker effects. The pulsatility of the compression is recognizable by varying flow rates (eg, 5.52 ± 0.11 L/min) and pressures (eg, -148.15 ± 15.90 mm Hg) where the pressure is affected (SD = 10.7% of mean) greater than the flow rate (SD = 2% of mean).

MB were detected in all experimental setups. However, we observed major and—to a vast extent—substantial differences in MB occurrence for the different pump speeds and

types of flow restriction (static vs. dynamic, Figure 2). From 2500 to 3400 rpm, we detected a mean increase in the total gas volume of $3.29 \cdot 10^6$%. Especially large and very large MB only occurred at flow rates ≥3000 rpm.

Furthermore, these differences correlated with the negative pressure in the experimental setup. Thus, in all inflow restricted settings an increase in the pump speed, resulting in increased negative pressure, led to an increase in MB generation and a higher overall gas volume. This behavior was also observable when static or dynamic flow restriction led to higher negative pressure with an increase of the number as well as the volume of the MB and of the overall gas volume. Consequently, static flow restriction led to the highest MB activity (number and volume). Compared to normal flow, static restriction at 3400 rpm increased the number of MB by the factor 97 and the total volume by the factor 518. However, when normalized to the negative pressure, MB activity was higher in dynamic flow restriction (stat.: −143 mm Hg → 289.21 MB; VS dyn.: 148.15 ± 15.90 mm Hg → 700.76 MB).

It is notable that the negative pressure alone is not an indicator for MB activity. Results show similar pressure for static occlusion at 3000 rpm and dynamic occlusion at 3400 rpm. The generated MB volume at dynamic occlusion is three orders of magnitude higher than at static occlusion (Figure 2).

It was further observed that the pump itself induced significant MB formation. For all experimental settings, a higher number of MB as well as higher overall gas volumes were detected after the pump was compared to prepump measurements. The largest difference was detected at normal flow and 3400 rpm. Here, the pump generated a 269-fold increase in the overall gas volume (inflow: 0.542 nL → outflow: 146 nL).

3.2 | Different strategies for MB removal

All tested strategies for MB removal showed an influence on the number of MB as well as on the total gas volume (see Table 2). The sole usage of an OXY (ES_1), however, was only effective at low flow rates. At 2500 rpm, more than half of the MB (54.57 ± 2.48%; $P < .01$) and 72.19 ± 4.39% ($P < .05$) of the total gas volume were removed from the fluid. With increasing flow speeds, the efficiency of the OXY decreased. The total number of MB was not affected, and the gas volume was only reduced by 40.87 ± 3.51% ($P < .05$) at 3400 rpm. The two MB removal approaches using DBTs showed different results. When using one DBT (ES_2), 46.77 ± 2.45% ($P < .01$) of the MB and 66.44 ± 4.75% ($P < .01$) of the gas volume could be removed at 2500 rpm. Increasing flow rates lead to an increased efficiency in gas volume reduction reaching 96.81 ± 0.09% at 3400 rpm. Simultaneously, the efficiency in MB count reduction decreased to 25.36 ± 9.02% ($P < .01$). The same tendencies, albeit less pronounced, were observed for two DBTs (ES_3) in-line. Here, the

maximum MB removal capacity was 73.15 ± 8.59% ($P < .05$) at 3000 rpm and at 3400 rpm the highest gas volume reduction (99.27 ± 0.02%; $P < .05$) was achieved.

The developed regression model provided an R^2 of 0.884 and an adjusted R^2 of 0.859, rendering it sufficiently accurate and generalizable. The two-way ANOVA test proofed statistical significance for both predictors (elimination strategy / pump speed) as well as for the interaction of those.

The post hoc Tukey test was performed in regard to elimination strategies for both the number of MB and their volume. Regarding the MB number, we showed a significantly better performance of two DBTs compared to an OXY ($P < .05$) as well as compared to only one DBT ($P < .001$). Relating to the MB volume, two DBTs proved most effective, followed by one DBT, rendering the OXY the least effective strategy (all $P < .001$). If the pump speed is considered (Figure 3) it can be clearly observed that with increasing flow rates, the efficiency of the OXY decreases while the efficiency of the DBT strategies increases. In regard to the MB volume, the OXY proved more efficient than one DBT at 2500 rpm. However, as soon as the flow rates exceed 3000 rpm, the picture changes drastically.

3.3 | Influence of experimental settings on DBT performance

With the above-described setup, we evaluated the generation of MB with focus on their size and total volume distribution. This allowed us to evaluate the effect of different elimination strategies on MB for a specific size range. In the case of ES_2, an overall reduction of 96.69% of MB volume could be achieved. However, if analyzed in regard to the MB size, a clear difference can be seen. Small and very small MB can be immediately removed (Figure 4A). Large and very large MB will be removed effectively as well, but cause an immense increase in small MB (Figure 4B).

4 | DISCUSSION

MB, also called gaseous microemboli, are a major problem in extracorporeal circulation.[5] Both, the evaluation of the formation as well as the effective removal of MB from ECLS circuits are a focus of current research. In the present study, we provide a structured assessment on the development of MB in a standard ECLS circuit. Additionally, we investigated widespread removal strategies.

We showed a proportional relation of MB formation to the pressure, respectively to suction at the venous (inflow) line. In our study, the applied negative pressure depends on the rotational speed of the centrifugal pump as well as on the (dynamic) restriction of the inflow. These findings are in accordance with previously published results.[7,10] The highest MB activity (number + gas volume) was observed after

FIGURE 4 DBT MB removal efficiency depending on the MB size. (A) Perfusion with mainly small and very small MB. (B) Perfusion with mainly large and very large MB. Measurements of the inflow sensor are displayed in blue and of the outflow sensor in red [Color figure can be viewed at wileyonlinelibrary.com]
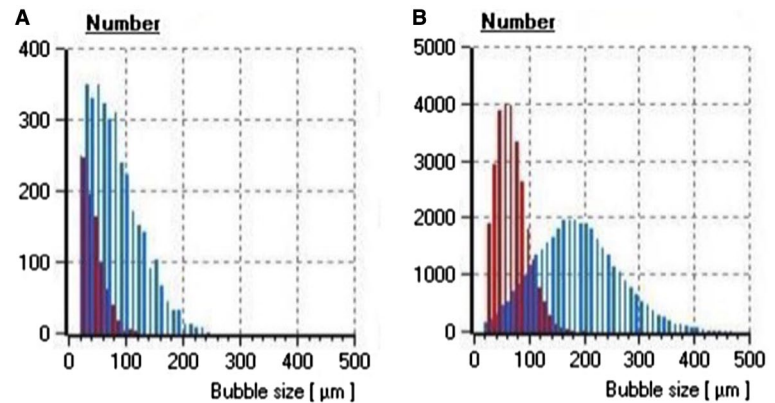
the static restriction of the inflow line. This is due to the higher mean levels of negative pressure. However, the peak negative pressure of the dynamic restriction can lead to the massive focal occurrence of MB with a low mean negative pressure level. As we demonstrated, especially a high MB activity is difficult to clear. Beside restriction of the inflow line, the pump itself induces a relevant amount of MB. The total gas volume as well as the number of MB was significantly increased after passing through the pump. Simons et al observed the same effect.[10] As MB and the total gas volume increased, we hypothesize that the occurrence of peak pressures inside the pump leads to the formation of additional bubbles.

As it is not possible to completely prevent MB formation in ECLS therapy, we focused on an effective removal strategy. Therefore, we investigated the effectiveness of a standard OXY compared to additional application of one or two DBT to withhold MB. In previous studies, oxygenators showed a high capacity to remove MB even at high counts and gas volumes. Simons et al could show a gaseous volume reduction of at least 92% and Qiu et al achieved a nearly complete removal of the gas volume.[10,13] In contrast to these findings, we were not able to achieve such high removal rates in our study. It was only possible to reduce the total gas volume by $72.19 \pm 4.39\%$ and the total count by $54.57 \pm 2.48\%$ at 2500 rpm. Higher flow rates resulted in an even worse result. However, it needs to be taken into consideration that microporous membrane oxygenators (Capiox Baby FX05, Terumo Corporation, Tokyo, Japan; Quadrox-series oxygenators, Maquet Cardiopulmonary AG, Hirrlingen, Germany) were used in the previously published studies. The Capiox oxygenator, for example, is only certified for up to six hours. The underlying reason is that microporous oxygenators are not completely tight to plasma and could thus lead to leakage after longer operation times. Therefore, we used a plasmatight PMP diffusion membrane oxygenator with an approved operation time of up to 14 days in our study. Even if less effective than the microporous oxygenators, the PMP oxygenator still showed beneficial effects. A significant reduction of the

MB at lower flow rates was achieved. At higher flow rates, the total reduction capacity was decreasing profoundly and bigger MB were probably split into smaller MB. However, smaller MB are considered less dangerous due to their shorter lifetime and their reduced risk of microvascular occlusion.[14]

As another completely new option for MB reduction in ECLS therapy, we investigated the removal capacity of a DBT. Usually, DBT are used in heart-lung machines during cardiac surgery on cardiopulmonary bypass.[15,16] Usage of a DBT successfully reduced the total amount of MB as well as the total gas volume drastically, even in a setting of high MB activity. The best capacity was observed for large and very large MB: $96.22 \pm 3.68\%$ reduction of MB >150 μm and 100% reduction of MB >300 μm. This supports previous publications.[17] Besides the reduction of large MB, the induction of new small MB was detected at higher flow rates. We hypothesize that turbulence inside the DBT leads to a partial fragmentation of MB. At first look, these results are contradictory to previous publications.[15,18] However, it needs to be highlighted that only MB with a diameter of up to 120 μm were measured in the published studies. When just focusing on MB <120 μm we also observed an overall reduction of the MB.

In conclusion, with a mean volume reduction of up to $96.81 \pm 0.09\%$, the application of a DBT leads to an effective reduction of MB activity. Though the reduction of the total number of MB decreased with higher flow rates, the gas removal capacity increased. As small MB are less dangerous and since it is more important to reduce the total gas load, the DBTs possess significant advantages compared to the sole use of an oxygenator.[13] Moreover, from this study it shows that the flow rate has a significant influence on MB removal strategies. While the use of an OXY is effective for low flow rates (2500 rpm), at increasing flow rates its effectiveness drastically declines. The effectiveness of DBTs, however, shows a significant increase with increasing flow rates (Figure 3) in regard to MB volume.

The application of a second DBT in a series connection setting achieved a further increased efficiency of up to $99.27 \pm 0.02\%$. However, in our opinion, the higher costs and
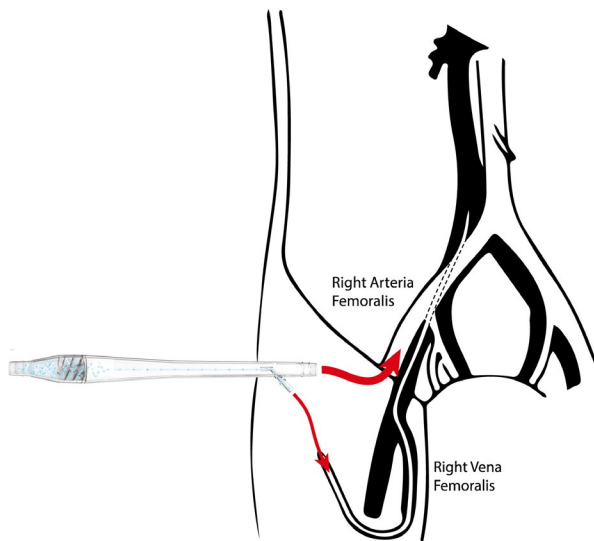
FIGURE 5 Possible drainage of the DBT bypass line in femoral ECLS cannulation via the Shaldon catheter [Color figure can be viewed at wileyonlinelibrary.com]

the additional bypassed flow outweigh the benefit of an efficiency increase of 2.46% in most cases.

As the ECLS circuit is a closed system, the bypass line of a DBT needs to be drained adequately. In femoral ECLS cannulation, drainage into the Shaldon catheter (Figure 5) might be an option. The distal limb tissue has a much higher ischemic tolerance compared to the brain or the heart and is able to resorb the gaseous volume.[19] To evaluate the feasibility and risk of this option will be the focus of further studies.

It needs to be highlighted that the efficiency of the DBT strongly depends on the fluid's viscosity. This was suggested earlier and we were able to experimentally prove it in this study.[17] In a clinical setting, this is of critical relevance, since the blood's viscosity can change due to temperature, hematocrit (volume status), blood flow, and other factors.[20] Using a water-glycerin solution we directly assessed the fundamental functionality of our approaches with less disturbing factors. The results should be transferrable to blood, as the physical principles are the same, but further evaluation will be needed.

5 | CONCLUSION

We were able to provoke the induction of microbubbles in an experimental ECLS circuit at different flow rates. This clearly shows the immense increase in MB generation at flow rates ≥3000 rpm. Evaluation of MB elimination strategies demonstrates that PMP diffusion membrane oxygenators are highly limited in retaining MBs, especially at higher flow rates. Dynamic bubble traps showed a significantly higher

MB removal efficiency and an increase in effectiveness with increasing flow rates. We recommend further investigation of this strategy in the setting of ECLS to provide a profound basis for the clinical establishment of this approach.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

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