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# *Surfactant Reduction in Embolism Bubble Adhesion and Endothelial Damage*

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***Background:*** **Surfactants may reduce the adhesion force holding bubbles to the vessel wall in gas embolism. The authors measured bubble adhesion force using excised microvessels. They assessed endothelial damage by measuring vessel reactivity and with microscopy.**

***Methods:*** **Microbubbles injected into arterioles resided for 5, 10, or 30 min, with intact or damaged endothelium. Perfusion was with rat serum alone (control) or with 1% Perftoran (OJSC SPC Perftoran, Moscow, Russia) or 1% Pluronic F-127 (Molecular Probes, Eugene, OR) added. Pressure across the bubble, bubble length, and bubble diameter were measured, and adhesion force per unit surface area, $K = \Delta PD/4l$, was calculated. Vessel reactivity was assessed using topical application of phenylephrine and acetylcholine.**

***Results:*** **With the endothelium intact, K was higher in controls than with Perftoran at 10 and 30 min or Pluronic F-127 at 10 min ($P < 0.05$). With surfactant added after air perfusion to damage the endothelium, K was lower ($P < 0.05$) at all times for both Perftoran and Pluronic F-127. With surfactant in the perfusate before air perfusion, K was lower at 10 and 30 min for Perftoran and at 10 min for Pluronic F-127 than for controls ($P < 0.05$). Phenylephrine-induced vasoconstriction was identical among groups. Acetylcholine-induced vasodilatation was the same among groups with an intact endothelium but was found to be lower in controls after air perfusion that followed surfactant exposure than in either surfactant group ($P < 0.05$).**

***Conclusions:*** **Surfactants reduced bubble adhesion force and preserved basic endothelial structure and vasodilatory function despite attempts to damage the endothelium. Surfactants seem to protect the endothelium from mechanically induced injury in addition to decreasing bubble adhesion forces.**

THE interfacial mechanics of a gas bubble adherent to a blood vessel wall has important implications in the development of therapeutic interventions for gas embolism. Gas microbubble embolization remains a significant threat in risk situations including space flight and diving and surgery using extracorporeal circulation. Gas embolism is associated with postoperative neurologic injury in cardiac surgery using cardiopulmonary bypass. Roughly one half of patients have a significant decline in cognitive function consistent with multifocal cerebrovascular gas embolization that limits blood flow to small regions of brain tissue.[1–3] Intravascular bubbles cause injury by obstructing blood flow, initiating clotting, and activating inflammatory pathways.[4] Our previous work has demonstrated that interactions between blood-borne macromolecules adsorbed to the bubble surface and the endothelial surface lead to the development of an adhesion force causing embolism bubbles to lodge in the vasculature.[5] One major unresolved question is what can be done to prevent or treat it. There are currently no clinically approved drugs available to treat gas embolism. The only treatment invoked is hyperbaric therapy, which is not preventative treatment. In addition, it is infrequently used except in the treatment of rare cases of massive gas embolism.

It has been suggested that the addition of surfactants to blood (or a blood-based perfusate in the case of an *in vitro* experiment) makes it feasible to manipulate interfacial stresses and prevent or reduce formation of the adhesion responsible for trapping intravascular gas bubbles, as occurs in intravascular gas embolism.[6–8] Surfactants preferentially populate gas-liquid interfaces. At a sufficiently high concentration, surfactant adsorption to a bubble interface could limit the interfacial area available for occupancy by those moieties that otherwise adsorb to the bubble surface and subsequently form adhesive attachments that tether the bubble to the vessel wall.

We hypothesize that a soluble surfactant compound added to a blood-based perfusate can alter interfacial mechanics sufficient to reduce the strength of adhesion that develops between the bubble and endothelial surfaces. One implication of this being true lies in the potential development of clinical pharmacologic therapy where none currently exists: Addition of a biocompatible surface-active drug to the intravascular perfusate might preserve or restore blood flow through bubble-embolized vessels to regions of brain or other tissue otherwise denied blood flow by lodged bubbles. To test this hypothesis, we have measured the force of adhesion that develops over time using an excised, perfused microvessel model of microvascular gas embolism. Experimental conditions included an intact endothelium and attempts to damage the endothelium by air perfusion, or macrobubble passage, before and after surfactant exposure. This study simulates vascular injury, characterizes the contribution of the endothelium to the force of adhesion, and identifies a potential protective role of surfactants to maintain endothelial structure and function in the face of attempts to induce endothelial damage. Experiments were conducted with serum to which

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was added either a physiologic salt solution (PSS) or one of two surfactant solutions: Perftoran (OJSC SPC Perftoran, Moscow, Russia), a perfluorocarbon-based emulsion; or Pluronic F-127 (PF-127; Molecular Probes, Eugene, OR), a nonionic block copolymer.

## Materials and Methods

The procedures for microvessel preparation, micropuncture gas embolization, and adhesion force measurement used in this study were all performed as previously described.[5] All animals used for this study were handled according to National Institutes of Health guidelines, and the University of Pennsylvania Animal Care and Use Committee (Philadelphia, Pennsylvania) approved the protocol.

### *Isolated, Perfused Microvessel Preparation*

Male Wistar rats (200–250 g) were anesthetized with halothane, and a portion of the mesentery including the proximal jejunum was removed. The tissue was pinned to a silicone pad in a dissection dish containing bicarbonate-buffered PSS (119 mM NaCl, 4.7 mM KCl, 1.8 mM $CaCl_2$, 24 mM $NaHCO_3$, 1.18 mM $KH_2PO_4$, 1.17 mM $MgSO_4$, 5.4 mM dextrose) at room temperature. The chemicals and salts used in the PSS were purchased from Sigma-Aldrich (St. Louis, MO). A 5- to 6-mm-long segment of first- or second-generation arteriole having an OD of 200–300 $\mu$m was isolated.[5] Tissue adherent to the vessel segment was carefully removed under a dissecting microscope (Stereozoom 7; Bausch & Lomb, Rochester, NY). A schematic of the apparatus for the mounted vessel studies has previously been published.[5] After being cleaned, the microvessel was transferred to a perfusion chamber (Living Systems, Burlington, VT) filled with PSS. One end of the microvessel was carefully threaded over the inflow cannula and secured with a suture ligature. To clear the vessel of any old or clotted blood, the perfusion pressure was increased to 10 mmHg using a pressure servo micropump system (Living Systems).[5,9] The free end of the microvessel was then mounted to the outflow cannula and secured with a suture ligature. A two-way stopcock connected to the outflow cannula was then closed, and the perfusion pressure was slowly increased to 60 mmHg to check for leaks with the pressure servo system set to manual mode. If a stable pressure were obtained, the system was free from leaks. If the pressure decreased, additional suture ligatures were added until the leak was stopped.

The vessel chamber was transferred onto the stage of an inverted microscope (CK40, 4× and 10× objectives; Olympus, Tokyo, Japan) and connected for superfusion. Mounted microvessels were superfused continuously using PSS delivered through the chamber at 25 ml/min. The superfusate was gassed with 5% $CO_2$, 21% $O_2$, and 74% $N_2$ and heated to 37°C (BAT-4; Baily Instruments, Saddle Brook, NJ; and IT-18; Physitemp Instruments, Clifton, NJ) using an in-line heat exchanger. The pH of the superfusing solution (7.35–7.45) was assessed (PH/T; Living Systems) before initiating the experimental protocol. A high-resolution black-and-white video camera (JE12HMV; Javelin Systems, Torrance, CA) connected to the microscope was used to record the magnified image of the microvessel onto S-VHS videotape (HR-S5900U; Victor Company of Japan, Tokyo, Japan) throughout the experiment. Postexperiment measurements of vessel diameter and microbubble size were made by reviewing the videotape with a high-resolution video monitor (PVM-14M4U; Sony, Tokyo, Japan) calibrated using a stage micrometer.

### *Intraluminal Perfusion Solution Preparation*

The intraluminal perfusion solution was rat serum with or without one of two study surfactants added. Serum was prepared from whole blood (5–7 ml) obtained from the donor rat by intracardiac puncture performed before dissecting out the mesenteric tissue. Blood was left stagnant for not less than 10 min at room temperature to permit clotting to occur. The sample was then centrifuged for 20 min at 3,000 rpm, and an aliquot of serum (2–3 ml) was carefully aspirated. Two prototypical surface-active agents were added to serum: PF-127 and Perftoran. Both surfactants have been shown to attenuate thrombin production in an *in vitro* model of gas embolism.[10] Small volumes of the concentrated surfactants were added to bring their final concentrations in the serum to 1% (volume/volume). These surfactant concentrations were chosen because they provided equally small volume dilutions of plasma.

### *Assessment of Endothelium Function*

Physiologic integrity of excised vessels was assessed by topical application of phenylephrine to produce smooth muscle–mediated vasoconstriction and by topical application of acetylcholine to produce endothelium-dependent vasodilatation. Relaxation from the phenylephrine-induced vasoconstriction caused by the addition acetylcholine is an indicator of an intact, functioning endothelium being present.[11] After a 15-min equilibration period, vessel OD ($D_{control}$) was measured. Superfusion was halted, and 50 nl phenylephrine ($10^{-2}$ M) was placed directly onto the vessel surface with a micropipette mounted on an automatic injector (Nanoject; Drummond Scientific, Broomall, PA). Fifteen seconds after the injection, the vessel OD ($D_{PE}$) was measured at the location of phenylephrine application. A 50-nl aliquot of acetylcholine ($10^{-2}$ M) was injected using the same method, and vessel OD ($D_{ACH}$) was remeasured

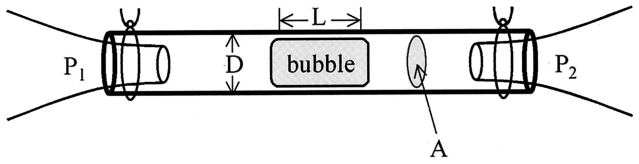


**Fig. 1. Schematic demonstrating the excised mesenteric artery mounted on micropipettes. An air microbubble has been injected using a small diameter micropipette to puncture the vessel wall. A = bubble cross-sectional area in the plane perpendicular to the axis of the vessel; D = vessel (and microbubble) internal diameter; L = microbubble length; $P_1$ = inflow pressure; $P_2$ = outflow pressure. Mounted vessel length was 3.5–4 mm.**

after 15 s. The percent decrease in vessel diameter in response to phenylephrine, $\Delta D_{PE}\%$, was calculated as[12]

$$\Delta D_{PE}\% = \frac{D_{control} - D_{PE}}{D_{control}} \times 100. \quad (1)$$

The percent recovery in vessel diameter in response to acetylcholine after vessel constriction precipitated by phenylephrine injection, $\Delta D_{ACH}\%$, was calculated as[12]

$$\Delta D_{ACH}\% = \frac{D_{ACH} - D_{PE}}{D_{control} - D_{PE}} \times 100. \quad (2)$$

*Air Microbubble Injection*

Air microbubbles were injected through a sidewall puncture into the mounted microvessel close to the outflow cannula tip using a micropipette mounted on a Nanoject injector. The injector was held in the stage of a micromanipulator, and the micropipette tip was viewed under the dissection microscope. The OD of the tip was in the range of 7–10 $\mu$m, and the tip of the micropipette was ground to a 20° bevel angle. The micropipette was backfilled with distilled water followed by aspiration of a small amount of air before vessel puncture. The microvessel was pressurized to 60 mmHg before micropuncture occurred and then depressurized to an intraluminal pressure of 0 mmHg just before bubble injection. An air microbubble having a volume of 2–4 nl, a diameter of 90–135 $\mu$m, and a length of approximately 200–400 $\mu$m was injected into the microvessel without permitting distilled water entry. The microbubble contacted the vessel walls but did not contact either mounting pipette. After bubble injection, the micropipette was withdrawn, and the outlet pressure was briefly increased to advance the bubble toward the inflow cannula and away from the puncture site. Figure 1 provides a schematic of the image of the intravascular bubble. Our previous work indicated that the micropuncture sidewall hole sealed itself, preventing leakage of gas, perfusate, or superfusate through the vessel wall.[5] Bubbles were left in place at a fixed axial position for 5, 10, or 30 min.

*Adhesion Force Measurement*

For the bubble to move, the axial force applied must exceed the adhesion between the bubble and the vessel wall. The force balance just before bubble movement is given by

$$\Delta P \cdot A = K \cdot S, \quad (3)$$

in which $\Delta P = P_1 - P_2$ is the pressure gradient across the bubble's length, A is the cross-sectional area of the bubble in the plane perpendicular to the vessel axis as shown in figure 1, S is the bubble surface area contacting the vessel wall, and K is the adhesion force per unit surface area. Substituting the vessel (or bubble) diameter, D, and bubble length, L, along with the two relations $A = \pi D^2/4$ and $S = \pi DL$ into equation 3 yields $K = \Delta P \cdot D/4$ l. K is determined from experiment as was done by Suzuki and Eckmann[5] by slowly increasing the inflow pressure ($<<$ 1 mmHg/s) and recording $\Delta P$ when microbubble movement begins. Calibrated measurements of D and L are made from videotape recordings of each experiment.

*Induction and Evaluation of Endothelial Damage*

Endothelial damage was induced using an established protocol with mounted vessels.[5,13] After reducing intraluminal pressure to 0 mmHg, the outflow stopcock was opened, and the microvessel was perfused with 4 ml air over 1–2 min. Perfusion subsequently commenced with the experiment-specific perfusate at an inflow pressure of 40 mmHg for 10 min to flush away any endothelial cells and debris that had separated from the wall. Vessel reactivity was reassessed to establish that endothelial damage had resulted, indicated by $\Delta D_{ACH}\% <$ 50%. Sodium nitroprusside ($10^{-6}$ M) was then added to the superfusate to demonstrate that vessel smooth muscle dilatory function was preserved.[5]

A subset of vessels were mounted and perfused with serum or serum plus one of the study surfactants. Vessel reactivity was assessed before and after the air perfusion and debris-flushing maneuvers. These vessels were then split longitudinally using microdissection scissors, placed flat on a glass microscope side, stained with hematoxylin to demonstrate endothelial nuclei, and photographed at 20× magnification.

*Experimental Protocol*

Vessels were divided into three serum-based perfusion groups: Serum alone (control), Perftoran, and PF-127. Vessel reactivity in response to phenylephrine and acetylcholine and adhesion force between the bubble surface and the vessel wall at 5, 10, and 30 min (each time duration was performed at a unique axial location) of the bubble residence time in the vessel were assessed following the methods previously published.[5] In some sets of experiments, the air perfusion maneuver was invoked to damage the endothelium before introducing a surfac-

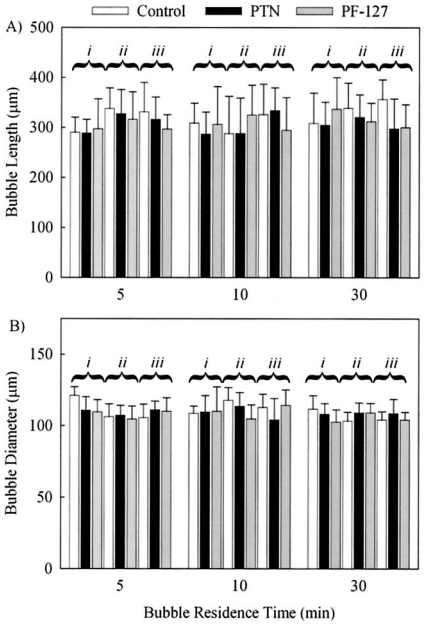


**Fig. 2. Bubble length (*A*) and diameter (*B*) values for each of the experimental conditions studied. Experimental conditions are with the endothelium intact (*i*), after air perfusion that followed surfactant exposure (*ii*), and after air perfusion that preceded surfactant exposure (*iii*). n = 4/group. PF-127 = Pluronic F-127; PTN = Perftoran.**

tant into the perfusate. In other sets of experiments, the air perfusion maneuver was invoked after the vessel had already been perfused with a serum–surfactant mixture. In either case, vessel reactivity and bubble adhesion force were measured again after air perfusion. A total of 58 vessels taken from 38 animals were studied, with 4 vessels tested for adhesion force at each experimental condition. Because some vessel reactivity measurement conditions overlapped between different experiments, 12 vessels were included in that analysis.

*Statistical Analysis*

Data are presented as mean ± SD of the values measured for each group. A one-way analysis of variance was performed for statistical comparison of results between surfactant groups and controls. Differences were considered significant at $P < 0.05$ using the Bonferroni method.

## Results

Dimensions of the bubbles studied in each group appear in figure 2. There were no differences in bubble length (fig. 2A; $P > 0.05$) or diameter (fig. 2B; $P > 0.05$) identified between groups. The results shown in figure 3 demonstrate the effect of surfactants on K, the adhesion force per unit surface area, for three different bubble residence times with an intact endothelium (fig. 3A), with air perfusion after surfactant exposure (fig. 3B), and

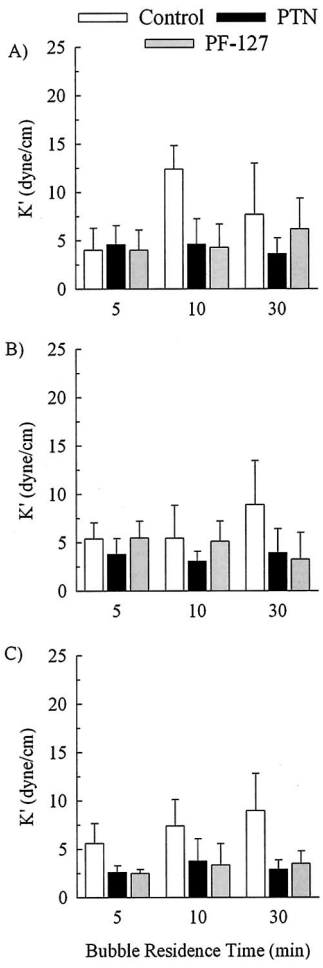


**Fig. 3. The adhesion force per unit surface area developed between embolism bubbles and the vessel wall at different contact times using serum (control) or serum plus either Perftoran (PTN) or Pluronic F-127 (PF-127). Experimental conditions are with the endothelium intact (*A*), after air perfusion that followed surfactant exposure (*B*), and after air perfusion that preceded surfactant exposure (*C*). n = 4/group. * $P < 0.05$ compared with control at the same time point.**

with air perfusion before surfactant exposure (fig. 3C). With the endothelium intact, values of K for the control group after residence times of 10 and 30 min were no different ($P > 0.05$) than the values previously reported for an intact endothelium and serum perfusion.[5] By comparison, K was significantly lower at 10 and 30 min residence time with Perftoran ($P < 0.05$) and at 10 min with PF-127 ($P < 0.05$) added to serum (fig. 3A). The values of K at 5 min contact time were not significantly different between the three different perfusate preparations ($P > 0.05$ for all comparisons).

For surfactant-containing perfusion solutions used before massive air perfusion, values of K measured after residence time of 10 min were significantly lower ($P < 0.05$) with Perftoran and PF-127 than values measured for controls (fig. 3B). At 30 min contact time, adhesion force in the Perftoran group was lower ($P < 0.05$) than in the control group, which was not different from the

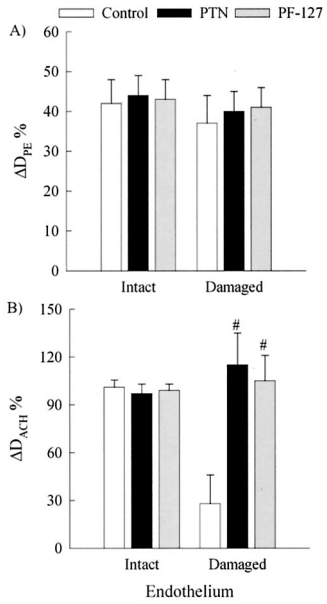


**Fig. 4. (*A*) The percent decrease in vessel diameter in response to phenylephrine before and after damaging the endothelium. (*B*) The percent recovery in vessel diameter in response to acetylcholine after vessel constriction precipitated by phenylephrine before and after damaging the endothelium. Vessels were perfused with serum (control) or serum plus Perftoran (PTN) or Pluronic F-127 (PF-127), with n = 12/group. # $P < 0.05$ compared with both Perftoran and PF-127 exposure that preceded air perfusion. Values of $\Delta D_{PE}$% and $\Delta D_{ACH}$% are calculated from equations 1 and 2, respectively.**

PF-127 group. At 5 min contact time, K was not different among the three different perfusates, with $P > 0.05$ for all comparisons. For surfactant-containing perfusion solutions introduced only after massive air perfusion, values of K measured at each time point were lower ($P < 0.05$ for all comparisons) for both the Perftoran and PF-127 groups than for controls (fig. 3C).

The results of the percent decrease in vessel diameter in response to phenylephrine appear in figure 4A. No differences were identified in $\Delta D_{PE}$% between the three groups either with the endothelium intact ($P > 0.05$ for all comparisons) or after air perfusion after surfactant exposure ($P > 0.05$ for all comparisons). Data for vessel dilation in response to acetylcholine appear in figure 4B. With the endothelium intact, the response in the control group was the same as in the Perftoran and PF-127 groups ($P > 0.05$ for both cases). After air perfusion to induce endothelial damage, $\Delta D_{ACH}$% was significantly lower in controls ($P < 0.05$) than in either the Perftoran or PF-127 groups when the surfactant–serum combination was used for vessel perfusion before air perfusion. The vessel dilating response to sodium nitroprusside was positive in all of these vessels in which the response to the acetylcholine was decreased, confirming that vascular smooth muscle dilatory function itself was still preserved.

Photomicrographs of representative vessels before and after air perfusion are shown in figure 5. Before air perfusion, numerous endothelial nuclei were present in the serum-perfused vessel (fig. 5A). After air perfusion, the endothelial nuclei were absent if the perfusate was serum alone (fig. 5B), indicating destruction of endothelial cells. Endothelial nuclei remained present after air perfusion if the vessel had first been perfused with serum containing either Perftoran (fig. 5C) or PF-127 (fig. 5D).

## Discussion

One approach to developing new therapy for vascular gas embolism is by modifying the bubble-endothelial surface molecular interactions responsible for the mechanical forces underlying bubble adhesion. The adhesion forces result in blood flow obstruction, which will ultimately cause ischemic injury unless flow is restored. The addition of surfactants to blood may provide a pharmacologic intervention targeted at the molecular cause of adhesion. This intervention is aimed at preserving or

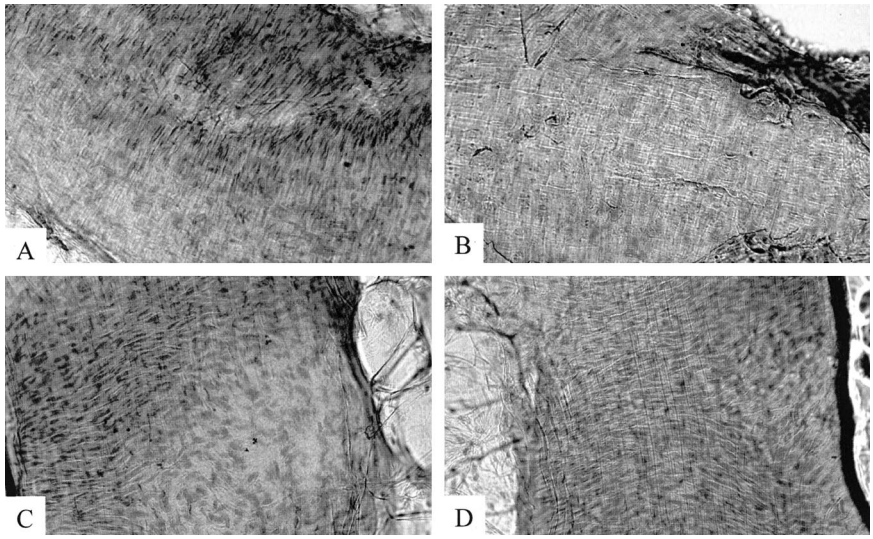


**Fig. 5. Hematoxylin stained vessel sections at 20× magnification to demonstrate endothelial nuclei. (*A*) Vessel perfused with serum, no air perfusion performed. (*B*) Vessel perfused with serum, air perfusion performed. (*C*) Vessel first perfused with serum plus Perftoran, then air perfusion performed. (*D*) Vessel first perfused with serum plus Pluronic F-127 (PF-127), then air perfusion performed.**

restoring blood flow in gas embolized tissues. We have hypothesized that surfactants can reduce the adhesion force leading to bubble arrest within the vasculature. Previous *in vitro* studies have shown that bubbles adherent to a tube wall can be induced to detach by the addition of a surfactant to the liquid perfusate,[14,15] and *in vivo* studies have shown that the addition of surfactants favorably alter the patterns of deposition and accelerate the rates of clearance of embolic bubbles.[7,8] Mechanistically, intravascular gas bubbles provide the interfacial surface area needed for adsorption of circulating molecules. Blood-borne macromolecules, particularly proteins, can adsorb onto gas–liquid interfaces,[16,17] where they have mechanical implications for adhesion interactions between the bubble surface and the vasculature[5,6,18] as well as biochemical implications such as activation of clot formation.[10] In relation to gas embolization and interruption of blood flow, surface adsorption is an important contributor to the adhesion force developed between embolism bubbles and the vessel wall.[5] Our previous work indicates that adhesion results from interactions between blood-borne macromolecules residing on the bubble surface and elements of the endothelial surface.[5] Results of the current study indicate that addition of surfactants to the perfusate can reduce the force of bubble adhesion, as has been shown before for liquid drops,[19] as well as help to preserve endothelial function in the face of gas embolization.

One way to assess adhesion between the bubble-blood interfacial surface and the luminal endothelial cell surface is by measurement of K, a global measure of the strength of surface–surface interactions.[5] Surface–surface interactions between bubbles and endothelial cells are likely the cause of gas embolism lodging, interruption of blood flow, and development of ischemia. Although the specific details of the binding interactions between various adhesion elements of the glycocalyx and bubble surface elements (adsorbed blood-borne macromolecules) remain unknown, it is evident that the resultant forces can be manipulated pharmacologically. Our results indicate that both PF-127 and Perftoran, if present, contributed greatly to the reduction of adhesion force. With the endothelium intact, the value of K at 10 min was reduced by 63.6% with 1% Perftoran present in serum and by 69.4% with 1% PF-127 present. At this time point under the same experimental conditions but without any surfactant present, we have previously shown that this is associated with the largest adhesion force generated.[5] After steps intended to damage the endothelium, K at 10 min was reduced with Perftoran (50.7%) or PF-127 (38.8%) added to the perfusate before air perfusion. Concomitantly, endothelial structure (figs. 5C and D) and endothelial-mediated vasodilatory function (fig. 4B) were also preserved under these conditions. Although the adhesion force was also reduced at all time points in those cases in which PF-127 and Perftoran were added to serum only after induction of endothelial damage (fig. 3C), the additional endothelium protecting effects of previous addition of surfactant were absent.

The effects of the surfactants to attenuate the adhesion force associated with bubble embolism could have several causes, including direct competition with proteins for interfacial adsorption, interference with the formation of adhesion interactions between moieties present on the bubble surface, and those present in the endothelial surface layer, or direct interaction with structural elements of the glycocalyx. Many different types of compounds, including solvents, colloids, and polymers, are surface active. The two chemically dissimilar compounds we have included in this study are a perfluorocarbon (Perftoran) and a nonionic polyol (PF-127). Specifically, Perftoran is a perfluorocarbon emulsion of perfluorodecalin ($C_{10}F_{18}$, molecular weight = 462 D) and perfluoromethylcyclohexylpiperiden ($C_{12}F_{23}N$, molecular weight = 595 D). PF-127 is a polyethylene oxide-polypropylene oxide-polyethylene oxide block copolymer with two hydrophilic polyethylene oxide chains and a single hydrophobic polypropylene chain. PF-127 forms gels, making it a useful delivery vehicle for controlled drug release. We have previously shown that Perftoran enhances the rate of arteriolar embolism bubble clearance *in vivo*[8] and that both Perftoran and PF-127 reduce thrombin formation in an *in vitro* model of blood clot formation initiated by gas embolization.[10]

One major experimental finding was that lower adhesion forces were measured in experiments in which a surfactant was present. One mechanism explaining this is preferential adsorption of surfactant molecules to the bubble interface. Surfactant occupancy of the interface can reduce or prevent population of the interface by other, larger plasma-borne molecules participating in the formation of adhesion interactions with the vessel wall. Studies of adsorption of mixed systems of surfactants and proteins illustrate that surfactant–protein interactions change protein structure, alter surface coverage, and modify mechanical properties of the interface.[20,21] Surfactants can displace proteins already occupying the interface.[22] Our results are consistent with the concept that bubble surface coverage with the surfactant outcompetes protein adsorption and reduces formation of adhesion interactions with the vessel wall. The expected finding is lower adhesion force by effectively shielding blood from exposure to a surface that elicits macromolecule adsorption or by secondary modification of an adsorbed layer.

One expected consequence of this should be a shorter residence time for arteriolar embolism bubbles with a surfactant present. When adhesion forces are smaller than the forces tending to advance the bubble, it will move downstream. By decreasing the adhesion force, surfactants should speed the course of bubbles through the vasculature. This behavior has previously been ob-

served with an *in vivo* model of bubble clearance from embolized vessels.[7,8] The current study complements those findings by showing that two chemically different surfactants reduce the adhesion force that develops between bubbles and the endothelial surface. This is particularly important to the treatment of gas embolism because, depending on the conditions, small bubbles ($<$ 100 nl) can persist for hours.[6,23–25]

Passage of very large bubbles can also damage or even denude the endothelium, which is the reason that air perfusion has been invoked as a common laboratory technique for stripping the endothelium in excised vessel preparations. In our experiments, however, initial perfusion with surfactant-laden serum followed by air perfusion intended to strip the endothelium results in a vessel preparation in which the endothelium-mediated dilatory responses and endothelial structural elements are preserved. The extent to which other aspects of endothelial function (*e.g.*, water transport barrier function, endothelium-derived nitric oxide release) are maintained is unknown. This finding, nonetheless, is encouraging that some protection from the damaging effects of air bubbles to vascular endothelium might be conferred by addition of even small quantities of surfactants to blood. The mechanism of protection remains unclear.

Although no direct molecular interactions between endothelial surface structures and either PF-127 or Perftoran are known, it is possible that integration of the surfactants into the endothelial surface layer could, in part, explain the weaker adhesion forces measured after attempts to damage the endothelium. It could also be that the endothelial surface layer structure itself was altered by the air perfusion maneuver. In either case, the endothelium itself was not removed if surfactant exposure preceded air perfusion. The protective effect of the surfactants may be a result of their maintaining hydration of the luminal endothelial surface during air perfusion, thus reducing any drying effects. We believe it is more likely that the mechanism of action is alteration of the direct mechanical effect of shear stress at the cell surface. During air perfusion, it is possible that a thin liquid layer remains in place between the endothelial surface and the gas phase. As a result, very large shear stresses potentially injurious to the endothelium could develop. The surfactants could act as drag-reducing agents,[26,27] altering the shear forces so that mechanical stresses sufficiently large to damage the endothelial surface, disrupt cellular function, or kill and denude the cells do not develop.

The presence of endothelial nuclei and the preservation of endothelium-mediated vasodilatation after massive air perfusion along with the reduction in bubble adhesion force found in these experiments have clinical implications in the treatment and protection of patients at risk for gas embolism. We have demonstrated that both Perftoran and PF-127 prevented loss of endothelial structure and function normally associated with air perfusion. That bubble adhesion and cellular injury can be attenuated by surfactants opens the possibility that targeted therapy based on competition with plasma proteins for bubble interfacial occupancy is a rational approach for the future study of the pathophysiology and treatment of gas embolism–related injury.

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