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Surfactants Reduce Platelet–Bubble and Platelet–Platelet Binding Induced by In Vitro *Air Embolism*

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Background: **The effect of gas bubbles on platelet behavior is poorly characterized. The authors assessed platelet–bubble and platelet–platelet binding in platelet-rich plasma in the presence and absence of bubbles and three surface-active compounds.**

Methods: **Platelet-rich plasma was prepared from blood drawn from 16 volunteers. Experimental groups were surfactant alone, sparging (microbubble embolization) alone, sparging with surfactant, and neither sparging nor surfactant. The surfactants were Pluronic F-127 (Molecular Probes, Eugene, OR), Perftoran (OJSC SPC Perftoran, Moscow, Russia), and Dow Corning Antifoam 1510US (Dow Corning, Midland, MI). Videomicroscopy images of specimens drawn through rectangular glass microcapillaries on an inverted microscope and Coulter counter measurements were used to assess platelet–bubble and platelet–platelet binding, respectively, in calcium-free and recalcified samples. Histamine-induced and adenosine diphosphate–induced platelet–platelet binding were measured in unsparged samples. Differences between groups were considered significant for $P < 0.05$ using analysis of variance and the Bonferroni correction.**

Results: **Sixty to 100 platelets adhered to bubbles in sparged, surfactant-free samples. With sparging and surfactant, few platelets adhered to bubbles. Numbers of platelet singlets and multimers not adherent to bubbles were different ($P < 0.05$) compared both with unsparged samples and sparged samples without surfactant. No significant platelet–platelet binding occurred in uncalcified, sparged samples, although 20–30 platelets adhered to bubbles. Without sparging, histamine and adenosine diphosphate provoked platelet–platelet binding with and without surfactants present.**

Conclusions: **Sparging causes platelets to bind to air bubbles and each other. Surfactants added before sparging attenuate platelet–bubble and platelet–platelet binding. Surfactants may have a clinical role in attenuating gas embolism–induced platelet–bubble and platelet–platelet binding.**

IT is well known that intravascular gas bubbles can severely affect a patient[1] and that even short duration occlusion of cerebral or cardiac blood flow as a result may cause transient or permanent injury.[2–4] Nonetheless, very little specific research has demonstrated the molecular mechanisms by which gas embolism can precipitate pathophysiologic responses. Intravascular gas can injure or denude the endothelium,[5] and bubbles adherent to the luminal endothelial surface can completely obstruct microvascular blood flow.[6–8] Bloodborne macromolecules affect the strength of adhesive bonds formed between the bubble and the endothelial surfaces,[5,9] and recently, platelets have been shown to contribute as much as 61% of the total adhesion force generated between bubbles and the vessel luminal surface.[10] Therefore, it seems warranted to investigate the effects of pharmacologic agents aimed at reducing blood flow obstruction resulting from the initiation of coagulation processes caused by blood contact with bubbles.

The current standard treatment for gas embolism is hyperbaric oxygen. Emergency therapy commonly begins several hours after gas embolism has occurred or after thorough clinical assessment. This happens both because there are no specific diagnostic criteria for gas embolism and because of the limited access to hyperbaric chambers. Because hyperbaric therapy does not prevent the onset of blood coagulation or other responses to embolism bubbles, we seek new treatment strategies to interrupt or block tissue responses after bubble exposure has occurred. Clinical application of preventive methods applied in advance to individuals at high risk for gas embolism could potentially improve outcomes and decrease the costs of patient care.

We hypothesize that interactions between blood and its components and the surface of an embolism bubble provide biochemical signaling that results in platelet adhesion to bubble surfaces and leads to platelet–platelet binding. We further hypothesize that surfactants, by preferentially occupying the gas–liquid interface and decreasing the interfacial area available for platelet–bubble signaling, reduce platelet–bubble and platelet–platelet binding stimulated by exposure to bubbles. Our main objective is to assess the effect of exposure to gas embolism bubbles on platelet–bubble and platelet–platelet binding. We also seek to assess the extent to which three novel surface-active compounds, added to platelet-rich plasma (PRP) samples before gas embolization, attenuate platelet binding to bubbles or to other platelets. We have therefore measured platelet–bubble and platelet–platelet binding using *in vitro* gas-embolized, recalcified human PRP, with and without the addition of three chemically distinct surfactants. Additional experiments were conducted to assess each surfactant's effect on adenosine diphosphate (ADP) and histamine-induced platelet–platelet binding and without recalcifying specimens to help eliminate the effects of any thrombin and fibrin generated *in vitro*.

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Materials and Methods

The Institutional Review Board of the University of Pennsylvania, Philadelphia, Pennsylvania, approved this study. Written, informed consent was obtained from all study volunteers.

Platelet-rich Plasma Preparation

Whole blood was drawn from 10 healthy, aspirin-free volunteers on two occasions each and from 6 healthy, aspirin-free volunteers on three occasions each to provide 8 samples per experimental group. Samples were immediately citrated (nine parts blood to one part citrate) and spun in a Sorvall Super T21 centrifuge (Thermo Electron Corporation, Asheville, NC) at 750 rpm (130*g*) for 15 min. The supernatant PRP was drawn off into Eppendorf tubes and diluted to 3×10^5 cells/μl using platelet-poor plasma. Platelet-poor plasma was obtained by centrifuging the residual blood for 15 min at 2,600 rpm (1,500*g*). Cell counts were obtained using a Coulter Multisizer II (Beckman Coulter, Inc., Fullerton, CA). The aperture orifice was set at 100 μm accurately to count particles in the size range of 2–60 μm.

In studies requiring recalcification of PRP, calcium chloride was added to a final calcium concentration of 10 mM to neutralize the excess citrate for each sample studied. A study surfactant, if called for, was added. The three experimental surface-active agents we used were Pluronic F-127 (Molecular Probes, Eugene, OR), Dow Corning Antifoam 1510US (Dow Corning, Midland, MI), and Perftoran (OJSC SPC Perftoran, Moscow, Russia). The surfactants, if used, were delivered along with sufficient HEPES buffer to provide a total 10% volume dilution for all specimens. The final surfactant concentrations used were 0.1% (vol/vol) for Pluronic F-127, 1.5% (vol/vol) for Dow Corning Antifoam 1510US, and 10% (vol/vol) for Perftoran. The surfactant concentrations chosen match those used in a previous *in vitro* study of surfactant effects on thrombin production in the face of gas embolization.[11] Also, in previous work, it was shown that these concentrations reduce blood's surface tension halfway between its native value of approximately 52 mN/m and that of the neat (or pure) surfactant.[7,8]

Samples were sparged for 10 min or sat for 10 min. Sparging was performed by air delivery at 200 μl/min through a 34-gauge nonmetallic syringe needle (MicroFil, World Precision Instruments, Sarasota, FL) using a Harvard 22 syringe pump (Harvard Apparatus, Holliston, MA). Samples were continuously stirred with Fisherbrand Teflon microstirring bars (Fisher Scientific International Inc., Hampton, NH).

Microcapillary Flow Chamber Apparatus

Microcapillary flow chambers were used to assess platelet–bubble binding. Flow chambers were constructed using rectangular glass microcapillaries (Vitrocom, Mountain Lakes, NJ) having a cross-section of 0.2 × 2.0 mm, a length of 7 cm, and a wall thickness of 0.15 mm. Microcapillaries were incubated overnight in 20% nitric acid, extensively washed with deionized water, rinsed with 100% dry ethanol, and dried under argon. During perfusion, PRP was drawn through the flow chamber at 100 μl/min by a Harvard 22 syringe pump. The shear stress imposed on the wall and the shear rate to which the flowing fluid is subjected are easily calculated if the volume flow rate, perfusate viscosity, and flow chamber dimensions are known. For these experiments, the imposed perfusate flow rate of 100 μl/min corresponded to a shear stress of 1.25 dyn/cm^2 and a wall shear rate of 125 s^{-1}. These are both consistent with venous shear levels not explicitly associated with platelet activation.[12]

For imaging during flow experiments, flow chambers were mounted on an Axiovert 135 inverted microscope (Carl Zeiss AG, Oberkochen, Germany) equipped with a 63× (numerical aperture 1.40) oil immersion objective lens (Plan Apochromat; Carl Zeiss AG) and an oil immersion condenser (numerical aperture 1.4) for differential interference contrast microscopy. Videomicroscopy images of flow in the microcapillary chambers were intensified, and video images showing platelets bound to bubbles were frame-grabbed. Numbers of visible bubble surface-bound platelets were manually counted from these two-dimensional images because no computer algorithm or measurement instrument was available to perform accurate counts of bound platelets in the actual three-dimensional structure.

To assess numbers of platelet singlets and multimers resulting from platelet–platelet binding away from bubble surfaces, aliquots of PRP were collected into a solution of 0.5% glutaraldehyde diluted in Isoton (Beckman Coulter, Inc., Fullerton, CA). These were analyzed with the Coulter Multisizer II, setting the aperture orifice at 100 μm as previously described. Using a sizing cutoff of 11.5 fl (clinical standard for upper limit of normal size platelets used by Department of Pathology and Laboratory Medicine at the Hospital of the University of Pennsylvania), the percentages of total number of platelets appearing as singlets and multimers were determined using the volume distribution histograms obtained. Measurement of singlet platelet count before and after various study steps (*e.g.*, sparging) allowed discrimination of the magnitude of the decrease in the number of singlet platelets as well as the production of platelet multimers present after each step. An example of a histogram appears in figure 1. Particles such as bubbles and any associated aggregates that were too large to traverse the aperture (100 μm) remained undetected.

Histamine- and ADP-induced Platelet–Platelet Binding

In separate experiments, histamine- and ADP-induced platelet–platelet binding were assessed in unsparged

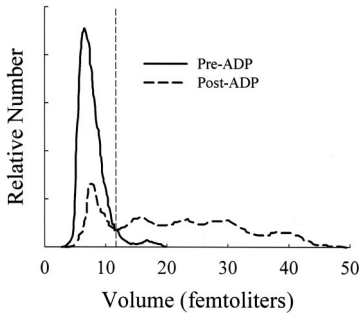

Fig. 1. Coulter multisizer histograms for a single surfactant-free, recalcified platelet-rich plasma sample before and after addition of 10 μM adenosine diphosphate (ADP). *Vertical dashed line* at 11.5 fl is used to delineate platelet singlets from multimers.

samples with and without surfactant present. ADP is a well known direct platelet agonist, whereas histamine does not have a direct agonist effect, but rather it potentiates effects of other agonists (*e.g.*, thrombin) on formation of platelet multimers. After PRP sample preparation, addition of surfactant, if specified, and recalcification, either histamine (H 7125; Sigma-Aldrich Chemicals, St. Louis, MO) was added to a final concentration of 10 μM or ADP (A 5285; Sigma-Aldrich Chemicals) was added to a final concentration of 10 μM. Samples were analyzed using the Coulter multisizer. This arm of the study was performed to determine whether any of the surfactants had a direct inhibitory effect on platelet–platelet binding.

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 for $P < 0.05$ (two tailed) using the Bonferroni correction applied to the Student *t* test.

Results

The microscopy image presented in figure 2A shows a large number of platelets adherent to a microbubble surface and bound to each other. The number of obvious platelets manually counted on such images was typically in the range of 60–100 platelets. This represents a minimum number under these conditions because the full three-dimensional structure cannot be fully appreciated. Several platelet pairs and single platelets are also visible. No surfactant was added to the sparged sample in this experiment. Figure 2B shows the effect of adding Pluronic F-127 before sparging. Each of the two microbubbles visible has only a sparse population of platelets adherent to its surface. Numerous singlets, but no multimers, also appear in the frame. Figure 2C is an image from a sparging experiment without surfactant in which PRP was not recalcified. Some 20–30 platelets (lower limit, not appreciating full three-dimensional aspect of the bubble) are bound to the bubble surface. The layer of platelets adherent to a bubble's surface seemed to be only one platelet thick. The presence of this thinner layer and fewer multimers not directly adherent to bubbles indicated that less platelet–platelet binding had occurred.

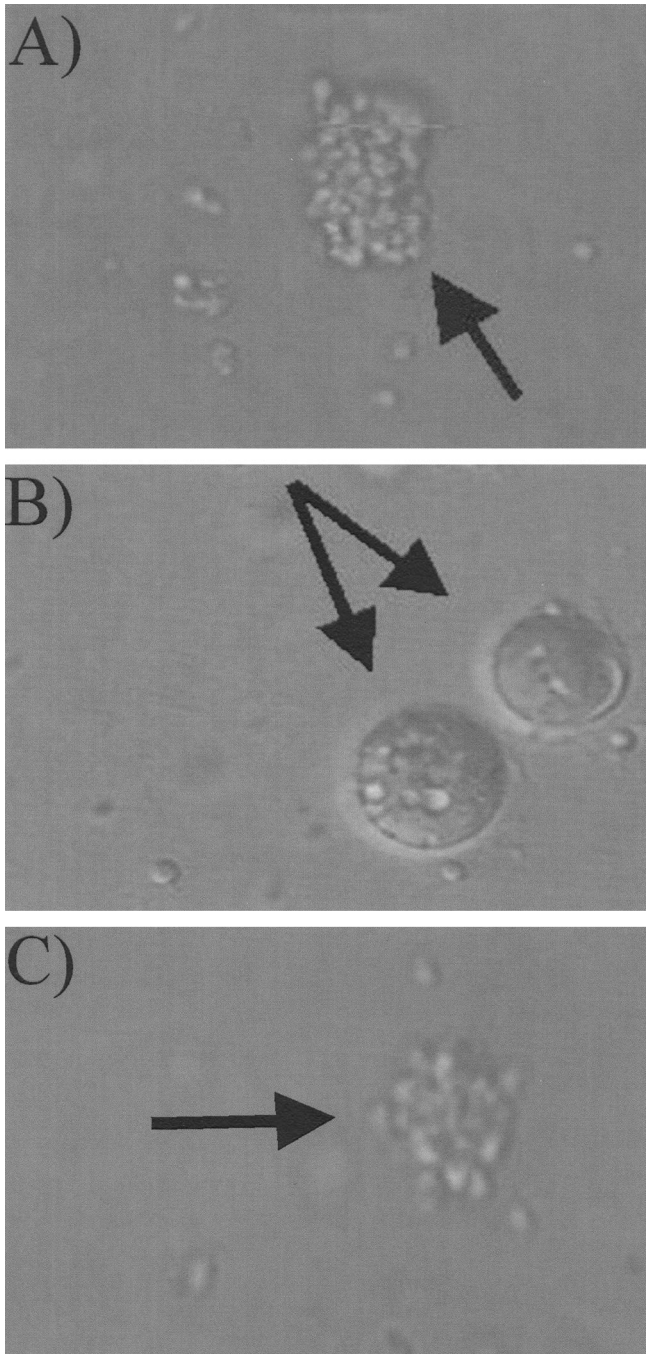

Fig. 2. Videomicroscopy images of platelet-rich plasma samples during flow in parallel flow plate microchambers. (*A*) Recalcified, sparged sample without surfactant added. (*B*) Recalcified, sparged sample with surfactant Pluronic F-127 added. (*C*) Citrated (not recalcified), sparged sample without surfactant added. *Arrows* indicate presence of microbubbles.

Quantitative results of the Coulter multisizer experiments to assess platelet–platelet binding are shown in

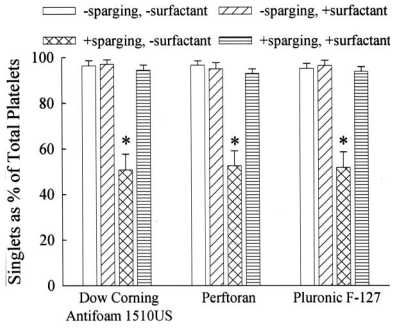

Fig. 3. Coulter multisizer percentages of total platelets appearing as singlets under the four experimental conditions for each of the three surfactants studied using recalcified platelet-rich plasma. Surfactants were Dow Corning Antifoam 1510US, Perftoran, and Pluronic F-127. * $P < 0.05$ compared with other conditions within the same surfactant group (n = 8/group).

figures 3–6. In figure 3, the percentages of total platelets appearing as singlets is plotted for each of the different surfactants under each of the experimental conditions using recalcified PRP. Without sparging, and with or without any of the surfactants added, platelet singlets were found to comprise 95–97% of the total, with platelet multimers constituting the remaining 3–5%. Sparging in the absence of surfactant decreased the number of platelet singlets to 51–53% of the total ($P < 0.05$ for all cases), with a concomitant increase in platelet–platelet binding resulting in increased platelets forming multimers.

Figure 3 also demonstrates that the addition of each of the surfactants attenuated the number of platelet singlets consumed to form multimers otherwise initiated by PRP exposure to microbubbles. Compared with the +sparging/−surfactant case, there were a significantly greater number of platelet singlets present, with the increase ranging from 77 to 86% ($P < 0.05$ for all comparisons), and a corresponding decrease in multimer formation. The percentages of total number of singlets present in the +sparging/+surfactant cases were not significantly different from the percentages measured in unsparged samples with or without surfactant present.

In figure 4, the percentages of total platelets appearing as singlets are shown for the four experimental conditions using each surfactant and without recalcifying PRP. Platelet singlets comprised at least 98% of the total for each experiment, and there were no significant differences between the various groups.

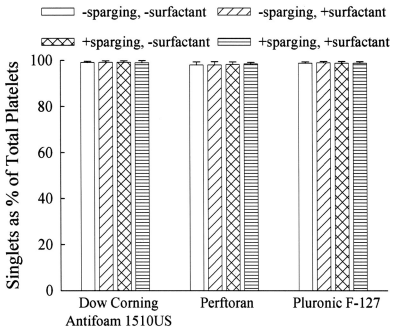

Fig. 4. Coulter multisizer percentages of total platelets appearing as singlets under the four experimental conditions for each of the three surfactants studied using citrated (not recalcified) platelet-rich plasma. Surfactants were Dow Corning Antifoam 1510US, Perftoran, and Pluronic F-127 (n = 8/group).

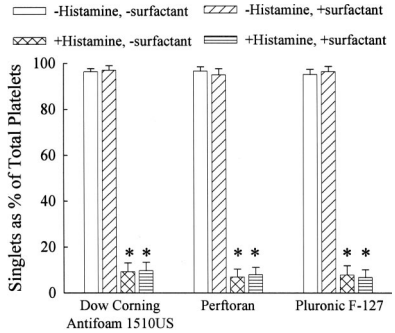

Fig. 5. Coulter multisizer percentages of total platelets appearing as singlets with and without histamine and with and without surfactant added to recalcified platelet-rich plasma. Surfactants were Dow Corning Antifoam 1510US, Perftoran, and Pluronic F-127. * $P < 0.05$ compared with the group having the same surfactant condition without histamine added (n = 8/group).

In figure 5, the effects of histamine added to surfactant-free and surfactant-laden, unsparged but recalcified PRP samples are demonstrated. Histamine caused consumption of significant numbers of singlets for formation of multimers. Specifically, the decreases in percentage of total of platelets appearing as singlets ranged from 90 to 93% without surfactant present and from 90 to 93% with a surfactant added ($P < 0.05$ for all groups). The degree of platelet–platelet binding to form multimers stimulated by histamine was preserved, with the percentage of total of platelets appearing as multimers having increased 18- to 27-fold without surfactant present and from 17- to 28-fold with a surfactant added ($P < 0.05$ for all groups). With no differences between groups, platelet–platelet binding elicited by histamine was not significantly enhanced or diminished by the presence of the any of the surfactants.

The effect of ADP added to surfactant-free and surfac-

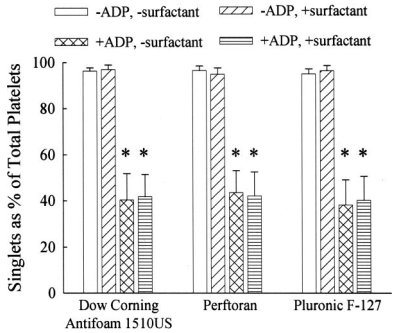

Fig. 6. Coulter multisizer percentages of total platelets appearing as singlets with and without adenosine diphosphate (ADP) and with and without surfactant added to recalcified platelet-rich plasma. Surfactants were Dow Corning Antifoam 1510US, Perftoran, and Pluronic F-127. * $P < 0.05$ compared with the group having the same surfactant condition without ADP added (n = 8/group).

tant-laden, unsparged but recalcified PRP samples is demonstrated in figure 6. Addition of ADP significantly reduced the percentage of total platelets appearing as singlets, ranging from 55 to 60% without surfactant present and from 56 to 68% with a surfactant added ($P < 0.05$ for all groups). ADP-stimulated platelet–platelet binding to form multimers was preserved. The percentage of total of platelets appearing as multimers increased in the range of 12- to 16-fold without surfactant present and from 10- to 18-fold with a surfactant added ($P < 0.05$ for all groups). No significant differences were identified between groups, indicating that platelet–platelet binding stimulated by ADP was not further enhanced or diminished by the presence of the any surfactant.

Discussion

We have hypothesized that blood contact with the bubble surface provides an important biochemical signal that promotes clotting by providing an interface for adsorption of blood-borne molecules. These may include molecular components of the surface of cells. Large molecules, particularly those proteins that are either free in the plasma or bound to the external aspect of the cell membrane, can adsorb to gas–liquid interfaces through ordinary hydrophobic interactions.[13,14] Alterations in molecular conformation results from surface adsorption, and this can expose portions of the molecule that stimulate a cascade of biologic activity, including activation of immune responses or blood clotting.

Intravascular bubbles provide surface area for contact with such circulating cells and molecules. Previous studies have examined thrombin production stimulated by blood contact with microbubbles introduced by the same sparging technique used here.[11] In those experiments, each of the three surfactants also used here attenuated thrombin production. Surfactants have also been shown to accelerate clearance of intravascular gas bubbles *in vivo* [7,8] and to reduce the adhesion force that develops between bubbles and the endothelium in excised perfused vessels.[5,9]

We have used a microscopy imaging technique and a Coulter counter to assess platelet–bubble and platelet–platelet binding in response to exogenous chemicals (*e.g.*, histamine, ADP) and microbubble gas embolism exposure in the presence and absence of surfactants. Although we cannot conclude that microbubbles were completely excluded from entering the microsizer, the relative paucity of microbubbles observed in the flow chamber experiments leads us to believe that the presence of any microbubbles in the microsizer data do not make an appreciable contribution to the results reported. Experiments performed using parallel plate flow chambers were conducted under conditions of low shear rate to minimize shear-dependent aggregation.[15] Although other experimental methods, such as whole blood (or PRP) aggregometry[16] or flow cytometry,[17,18] have the potential to facilitate identify specific aspects of platelet adhesion,[19,20] activation,[21,22] and aggregation[23,24] resulting from the interactions of PRP with gas bubbles, other configurations, including parallel plate flow chambers, have been used successfully to investigate various aspects of platelet hemostatic function under flow conditions.[25] Also, the use of imaging provides compelling evidence that bubble–platelet binding occurs with and without recalcification and is attenuated by the surfactants. *In vitro* microbubble embolization stimulated platelet binding events on the bubble surface (fig. 2). Bubbles also induced platelet–platelet binding away from the bubble surface (fig. 3), perhaps indirectly through thrombin generation or other biochemical signaling. Although specific elements of the platelet response (*e.g.*, P-selectin expression, glycoprotein IIb–IIIa concentrations) to bubble contact have not been quantified, our data do show that addition of surfactants attenuates the overall effects of bubble exposure on the occurrence of platelet–bubble and platelet–platelet binding.

We have previously demonstrated that *in vitro* microbubble gas embolization increases thrombin production in a shear rate–dependent fashion and that addition of surfactants attenuates this response.[11] Thrombin provides a potent signal for platelet activation.[22,26] We have attempted to isolate the contribution of thrombin-mediated responses in this study by incorporating those experiments using PRP without recalcification. Eliminating the recalcification step prevents significant thrombin formation from occurring within those samples and also leads to conditions of low ionized calcium concentrations under which, in the absence of exogenous agonists, formation of unstable platelet aggregates occurring at low shear stress and disaggregation occurring at higher shear stress have previously been described.[27] Under such conditions, our results show that platelet–bubble binding still occurs (fig. 2C), but platelet–platelet binding does not (fig. 4). This is different from the decrease in singlets and enhancement of multimer formation found in sparged, recalcified samples without surfactants present (fig. 3). It suggests that a calcium-dependent process, most likely thrombin formation stimulated by microbubble exposure,[11] led to the platelet multimer formation away from the surface. This would allow for the formation of platelet multimers not in direct contact with a bubble surface. Nonetheless, platelet binding to the bubble surface itself occurs regardless of whether the PRP is recalcified.

One possible mechanism for stimulation of platelet–bubble binding in our experiments is direct platelet stimulation induced by platelet contact with bubbles. The signaling for how this might cause increased surface expression of the GIIb–IIIa glycoprotein complex is subject to further research. In addition, other surface mol-

ecules could adsorb onto the bubble surface, binding the platelet to the bubble, as seen in figures 2A and C. Direct platelet activation could also have occurred by platelet contact with bubbles, leading to release of potent platelet activators such as ADP from dense granules or thrombin from α granules.[12,28] We did not perform release assays in these experiments. Another mechanism for platelet–bubble binding may involve fibrinogen adsorption to the interface. An adsorbed fibrinogen layer would provide substrate normally suited for cross-bridging GI-Ib–IIIa receptors but, in this case, holding a platelet aggregate onto the bubble. Other promoters of platelet activation such as collagen had no obvious source in these experiments and are unlikely to have caused the platelet response.

Even without detail of the specific molecular events, the effects of the surfactants to attenuate platelet aggregate formation in the presence of bubbles could have several causes, including direct inhibition of platelet–platelet binding, or inhibition of bubble–induced signaling activating clot formation. As shown in figure 3, without addition of microbubbles, the platelets were present primarily as singlets with a very small fraction forming multimers. These percentages are consistent with studies of platelet aggregation and disaggregation kinetics.[29] The relative fractions of singlets and multimers were unaffected by surfactant addition, indicating that the surfactants themselves did not promote multimer formation. The results of the histamine and ADP experiments (figs. 5 and 6) show that the surfactants also did not inhibit multimer formation stimulated by various mechanisms occurring in the bulk specimen and not at the bubble surface. The primary effect of the surfactants was to adsorb to the bubble surface and effectively render it inert as an aggregation stimulus. This would account for the profound reduction in numbers of platelets bound to bubbles as demonstrated in figure 2B, and it would reduce the magnitude of any conducted responses leading to platelet–platelet binding (not bubble associated) in the plasma, as shown in figure 3.

Unique physicochemical surfactant characteristics confer this protection. Several different classes of compounds are surfactants, including solvents, colloids, and polymers. The three classes of compounds included in this study are perfluorocarbons (Perftoran), polydimethylsiloxanes (Dow Corning Antifoam 1510US), and nonionic polyols (Pluronic F-127). Although they represent distinct chemical families (halogenated solvents, antifoaming agents, and long-chain polymers), they have common features. Their physical characteristics (*e.g.*, aqueous solubility, vapor pressure, boiling point, viscosity) have been well studied. They are chemically inert, essentially nontoxic, and stable. Their physical properties vary little with temperature, which is important to consider for application to cardiopulmonary bypass. More specifically, Dow Corning Antifoam 1510US is a nonionic, silicone emulsion–type surfactant. Perftoran is a proprietary 10 vol% perfluorocarbon emulsion, which contains perfluorodecalin ($C_{10}F_{18}$, molecular weight = 462 Da) and perfluoromethylcyclohexylpiperiden ($C_{12}F_{23}N$, molecular weight = 595 Da). Dow Corning Antifoam 1510US[7] and Perftoran[8] accelerate arteriolar embolism bubble clearance *in vivo*. Pluronic F-127 is a polyethylene oxide–polypropylene oxide–polyethylene oxide block copolymer. It contains two hydrophilic polyethylene oxide chains and a single hydrophobic polypropylene chain. It easily forms gels and is often used as a pharmaceutical delivery vehicle to modulate drug release. More specific information about each of these compounds appears in several publications.[5,7,8,11,30]

Our findings indicate that the surfactants are inert as direct inhibitors or stimulants of platelet–platelet binding and multimer formation. Their presence resulted in the consumption of fewer platelet singlets to form multimers with sparging. One mechanism for this is surfactant adsorption onto the bubble surface. As the surfactant molecules occupy the interface, they reduce or prevent population of the interface by other plasma-borne molecules having the potential to initiate clotting. Studies of competitive adsorption of proteins and surfactants have shown that the protein structure changes, which modifies surface coverage and changes the mechanical properties of the interface.[31–33] An extreme example is that proteins already occupying the interface can be displaced by a surfactant.[34] The reduction in platelet multimer formation and platelet–bubble binding we have found in relation to surfactant administration is consistent with the concept that the surfactant coverage of a bubble's surface prevents initiation of the molecular events that signal platelets to bind to the bubble surface and to each other.

We are not the first to observe that blood exposure to air provokes platelet binding interactions as part of the clotting response,[35] but our attempt to render the interface less of a stimulant to platelet aggregation by adding surfactants to PRP is new. Work showing various endothelial protective and antiadhesive[5] and antithrombotic[11] effects of exogenous surfactants as well as their potential to accelerate bubble clearance from embolized vessels[7,8] is complemented by our findings that surfactants attenuated platelet multimer formation and platelet–bubble binding stimulated *in vitro* by microbubbles.

Platelet activation in the cerebral circulation of patients undergoing cardiopulmonary bypass has recently been linked to postoperative cognitive decline.[36] In addition, although not specifically to gas embolism, there is a recognition that platelet microembolization is an important contributor to microvascular obstruction and unfavorable long-term prognosis in cardiovascular disease in general.[37] In the setting of cardiopulmonary bypass, atheromatous debris also produces vascular injury, tissue factor expression, and leads to thrombin

generation on damaged endothelium and platelets. Although the specific mechanisms leading to platelet–bubble and platelet–platelet binding in the clinical scenario of gas embolization have not been determined, clearly any protective therapy that can be instituted should be considered for incorporation into clinical practice. This includes the possibility that a surfactant could be added to the pump prime or cardioplegia solution before initiation of extracorporeal circulation or that a surfactant could be used during rewarming when bubble formation in the circuit is prominent.

In conclusion, we have demonstrated that platelet binding is greatly enhanced by gas embolizing PRP samples and that large numbers of platelets accumulate on the surface of microbubbles. Surfactant addition before gas embolization reduced platelet multimer formation significantly, with the appearance of many fewer platelets adherent to microbubble surfaces.

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