Embolism Bubble Adhesion Force in Excised Perfused Microvessels

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Background: The mechanics of gas embolism bubble adhesion to the vessel wall is poorly understood. New strategies to treat gas embolism may result from an understanding of adhesion forces, including the molecular determinants of bubble adhesion. The authors conducted experiments to measure the adhesion force of bubbles contacting the vessel wall.

Methods: Microbubbles were injected into excised arterioles. Bubbles resided for 5, 10, 20, or 30 min with the endothelium intact or damaged and with a physiologic salt solution, physiologic salt solution with 5% bovine serum albumin, or rat serum as the perfusate. Inflow pressure was raised until the bubble dislodged. The differential pressure across the microbubble, ΔP, was recorded at the moment of bubble movement. Bubble diameter, D, and length, L, were determined by videomicroscopy. The adhesion force per unit surface area of a bubble contacting the vessel wall, K = ΔPDL, was calculated for each experiment.

Results: K at 10 min contact time (physiologic salt solution, 141 ± 29; serum, 153 ± 57 dyne/cm²) was higher than at 5 min (physiologic salt solution, 56 ± 22; serum, 71 ± 29 dyne/cm²), 20 min (physiologic salt solution, 46 ± 29) and 30 min (physiologic salt solution, 14 ± 5) (P < 0.05). Endothelium removal reduced K at 10 min (physiologic salt solution, 68 ± 46; serum, 60 ± 14 dyne/cm²) (P < 0.05). K was higher with 5% bovine serum albumin present at 10 min (349 ± 149, P < 0.05), correlating with in vivo estimates.

Conclusions: The adhesion force developed between a microbubble and the vessel wall depends on multiple factors, including bubble residence time, presence of the endothelium, and perfusion solution.

GAS bubbles are introduced into the body both accidentally and deliberately. The accidental, or unwanted, occurrence of intravascular gas in vivo occurs often and poses a health threat regardless of the source. The avoidance of intravascular gas bubbles is of vital importance to the health of end organs, primarily the brain and the heart. For instance, gas microembolism continues to be an unavoidable major cause of increased neurocognitive dysfunction in cardiopulmonary bypass surgery. Many basic physiologic studies provide insight into the phenomena provoked by intravascular emboli. Intravascular air bubbles cause damage by vessel occlusion, diminished tissue perfusion, and initiation of thrombotic and inflammatory pathways. Thus, the effects of intravascular gas bubbles can result in local, regional, and global systemic responses. In the case of cerebrovascular gas embolization, the resultant pathophysiology can devastate the patient. Cerebrovascular occlusions of short duration (a few minutes) may cause transient or permanent brain injury. Finding a therapy to minimize the magnitude and duration of blood flow obstruction caused by bubbles may prevent the development of end-organ injury.

Therapy for both the accidental and deliberate forms of gas lesion disease currently is limited to hyperbaric oxygen therapy. This is often only undertaken days after the event because of the lack of specific diagnostic criteria for gas embolism and the scarcity of hyperbaric chambers. Hyperbaric therapy is not preventative, nor does it address the mechanism of blood flow obstruction: the adhesion of bubbles to the vessel wall as the cause of the resultant ischemic injury. In fact, the molecular basis of the mechanical mechanism by which bubbles adhere to the vessel wall is unknown. New strategies to treat gas embolism will depend on knowledge of the molecular mechanisms by which surface–surface interactions between the bubble interface and vascular endothelium lead to bubble adhesion and obstruction of blood flow. Clinically applicable methods of prevention and treatment for gas embolism based on an understanding of the molecular mechanisms involved could perhaps be developed to reduce morbidity, mortality, and the cost of care.

We hypothesized 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 within the vasculature. Our main objective was to quantify the relative contribution of those constituents that determine the resultant force of adhesion. We therefore measured the force of adhesion that develops over time by means of an excised, perfused microvessel model of microvascular gas embolism. Experimental conditions included both intact and damaged endothelium to simulate a severe circumstance of vascular injury and to characterize the contribution of the endothelium to the force of adhesion. Experiments were also conducted in the presence and absence of 5% bovine serum albumin (BSA) in physiologic salt solution (PSS) and with serum to quantify the specific contribution of serum proteins to the adhesion force.
A, a,

The perfusion pressure was increased to 10 mmHg with a pressure servo micropump system (fig. 1, A, b and c, Living Systems, Burlington, VT). The free end of the microvessel was then mounted to the outflow cannula after the microvessel was cleared of any old or clotted blood. After vessel mounting, the two-way stopcock connected to the outflow cannula was closed and perfusion pressure was slowly increased to 60 mmHg. The pressure servo system was placed in manual mode, in which a stable pressure value indicated that the system was free from leaks. If a leak were detected, the system was fixed by applying additional suture ligatures. For these experiments, the intraluminal perfusion solution was the PSS previously described, PSS with 5% BSA, or rat serum. To prepare the serum, blood (5–7 mL) was obtained from the donor rat using an intracardiac puncture before dissecting the mesenteric tissue. The specimen was left for at least 10 min at room temperature for clot formation and then centrifuged for 20 min at 3,000 rotations per min. A 2- to 3-ml aliquot of serum was aspirated carefully.

The vessel chamber was transferred to an inverted microscope (fig. 1, A, d, CK40; ×4 and ×10 objectives, Olympus, Tokyo, Japan). The magnified image of the microvessel was recorded throughout the experiment using a high-resolution black and white video camera (fig. 1, A, e, JE12HMV; Javelin Systems, Torrance, CA) and a S-VHS video cassette recorder (HR-S5900U; Victor Company of Japan, Tokyo, Japan) connected to the microscope. Measurements of the vessel diameter and the size of the microbubble were made after the experiment to review the videotape with a high-resolution video monitor (fig. 1, A, f, SVM-14 M4U; Sony, Tokyo, Japan) calibrated using a stage micrometer.

Microvessels were continuously superfused (fig. 1, A, g) with PSS flowing through the chamber at a rate of 25 ml/min. The superfusing solution was bubbled with 5% CO2, 21% O2 and balanced nitrogen and warmed to 37°C (IT-18; Physitemp Instruments, Clifton NJ, and BAT-4; Bailey Instruments, Saddle Brook, NJ) using an in-line heat exchanger. The pH of the superfusing solution (7.35–7.45) was measured (PH/T; Living Systems, Burlington, VT) before starting the experimental protocol. Total volume of the superfusion system was 100 ml. Chemicals were added to the reservoir to test the vessel physiologic integrity, and final concentrations are reported.

Air microbubbles were injected into the mounted microvessel through a puncture in the sidewall made near the outflow cannula tip with a micropipette under dissection microscope. The micropipette was mounted on automatic injector (Nanoject, Drummond Scientific, Broomall, PA) held in the stage of a micromanipulator. The micropipette tip was beveled at an angle of 20 degrees and the outside diameter of the tip was in the range of 7–10 μm. Distilled water was first backfilled

Materials and Methods

Isolated, Perfused Microvessel Preparation

Animals for this study were handled according to the University of Pennsylvania Animal Care and Use Committee. Mesenteric tissue (the proximal jejunum) was removed from halothane-anesthetized male Wistar rats (200–250 g). The tissue was pinned in a silicone-lined dissection dish containing bicarbonate buffered physiologic salt solution (PSS, NaCl 119 mM, KCl 4.7 mM, CaCl2 1.8 mM, NaHCO3 24 mM, KH2PO4 1.18 mM, MgSO4 1.17 mM, dextrose 5.4 mM) at room temperature. All salts and chemicals used in the PSS were obtained from Sigma-Aldrich, St. Louis, MO. The proximal artery was followed distally and a 5–6 mm long segment of first- or second-generation arteriole (200–300 μm OD) was isolated. The segment was carefully dissected free and cleaned from the adhering tissue under a dissecting microscope (Sterezoom 7, Bausch & Lomb, Rochester, NY).

The microvessel was transferred to a vessel perfusion chamber (fig. 1, A, a, Living Systems, Burlington, VT) containing PSS. One end of the microvessel was mounted to the inflow cannula and secured with a suture ligature. The perfusion pressure was increased to

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into the pipette, and a small amount of air was then aspirated into the pipette tip. The microvessel was pressurized to 60 mmHg for micropuncture and then depressurized to an intraluminal pressure of 0 mmHg for bubble injection. An air microbubble in the range of 2–3 nl (length, ~ 500 μm), but not the distilled water, was injected to the microvessel. The microbubble conformed to the vessel shape, contacting the vessel walls without being in contact with either the inflow or outflow mounting pipettes. After bubble injection, the micropipette was removed and the outlet pressure was momentarily increased slightly to move the bubble away from the puncture site. A more detailed schematic of the video image of the bubble in the vessel appearing in figure 1 (A, f) is shown enlarged in figure 1, B. A videomicrograph of a bubble in a vessel taken from a videotaped experiment appears in figure 1, C. Preliminary dye studies indicated that the hole in the vessel wall made by this procedure was self-sealing and there was no leakage of gas, perfusate, or superfusate across the vessel wall. Additional leakage testing was performed by measuring the intraluminal pressure after puncture but without bubble injection. Five min postpuncture, intraluminal pressure was raised to 20 mmHg, the pressure servo system was turned off, and the rate of pressure decrease was measured. This study was performed with all three intraluminal perfusates and repeated with intact and damaged endothelium. The rate of pressure loss was less than 2 mmHg/min under all experimental conditions.

The physiologic integrity of the vessel was tested by periodic application of a maximal dose of phenylephrine in a concentration of 10^{-6} M, which produced a smooth muscle mediated constriction of the vessel. Vasodilation induced by acetylcholine is known to be endothelium dependent,11 and relaxation from the phenylephrine induced vessel constriction by acetylcholine is taken as an indication of intact endothelium. A maximal dose of acetylcholine (10^{-6} M) was added to the phenylephrine-containing bath after the phenylephrine concentration had stabilized (~ 3–5 min) to test the integrity of the endothelium.12 The OD of the microvessel was measured before injection of phenylephrine (D_{control}), 2–5 min after phenylephrine injection (D_{pe}), and 5 min after injection of acetylcholine (D_{ACH}). The percent decrease in vessel diameter in response to phenylephrine, \( \Delta D_{PE}\% \), was calculated as:

\[
\Delta D_{PE}\% = \frac{D_{control} - D_{PE}}{D_{control}} \times 100
\]  

Using a previously reported metric,13 \( \Delta D_{ACH}\% \), the percentage recovery in vessel diameter in response to acetylcholine after vessel constriction precipitated by phenylephrine injection, was calculated as:

\[
\Delta D_{ACH}\% = \frac{D_{ACH} - D_{PE}}{D_{control} - D_{PE}} \times 100
\]

For those experiments in which endothelial removal was required, an established protocol was used to damage the endothelium from the mounted vessels.14 Intraluminal pressure was first reduced to 0 mmHg and the outflow stopcock was opened. The microvessel was then perfused with 2 ml air, followed by perfusion with the experiment-specific perfusate at 40 mmHg for 10 min using the pressure servo micropump system to flush out any of the endothelial layer that separated. The outflow stopcock was closed and the pressure was increased to permit microbubble introduction as described above. Measurement of \( \Delta D_{PE}\% \) and \( \Delta D_{ACH}\% \) was performed after endothelial damage to confirm that the phenylephrine-elicited response was retained whereas the acetylcholine-elicited response was eliminated.

**Adhesion Force Measurement**

At the moment the bubble dislodges, the axial force applied to the bubble exceeds the force of adhesion of the bubble to the vessel wall. Using figure 1, B as a reference, the axial force applied is the product of the intraluminal pressure gradient and the cross-sectional area of the bubble, whereas the adhesion force can be represented as the product of the surface area of the bubble in contact with the vessel lumen and an adhesion force per unit surface area. This can be expressed as:

\[
\Delta P \cdot A = K \cdot S
\]  

in which \( \Delta P = P_1 - P_2 \) is the static pressure gradient across the axis of the bubble, \( A \) is the cross-sectional area of the bubble in the plane perpendicular to the axis of the vessel, \( S \) is the surface area of the bubble in contact with the vessel wall, and \( K \) is the adhesion force per unit surface area. If the vessel (or bubble) diameter, \( D \), and bubble length, \( L \), are known, then substituting the two relationships \( A = \pi \cdot (D/2)^2 \) and \( S = \pi \cdot D \cdot L \) into equation 3 gives the following expression for \( K \):  

\[
K = \Delta P \cdot D/4 \cdot L
\]

\( K \) can be determined with equation 4 from experiment by slowly raising the inflow pressure (< 1 mmHg/sec) and recording \( \Delta P \) at the moment of microbubble movement. The values of \( D \) and \( L \) are determined by calibrated videomicrometer measurements made from videotape recordings of each experiment.

**Bubble Effects on Endothelial Function**

An experiment was performed to assess the effects of air bubbles to alter endothelial function. Following a 15-min equilibration period after vessel mounting, the vessel OD was measured. Superfusion was stopped and 50 nl of phenylephrine (10^{-2} M) was injected directly onto the vessel surface with a micropipette mounted on a Nanoject injector. The vessel OD was measured at the point of phenylephrine injection 15 sec after the injection. A 50 nl aliquot of acetylcholine (10^{-2} M) was...
then injected the same way, and vessel OD was remeasured after 15 sec. The percent decrease in vessel diameter in response to phenylephrine, $\Delta D_{P_{E1}}\%$, and $\Delta D_{A_{C1}}\%$, the percent recovery in vessel diameter in response to acetylcholine after vessel constriction precipitated by phenylephrine injection, were calculated using equations 1 and 2. An air microbubble was subsequently injected as described in Materials and Methods, and the outflow stopcock was opened. The bubble resided for 30 min at the location of vessel reactivity assessment and was then flushed. The outflow stopcock was closed and vessel reactivity was again assessed at the same location. This experiment was performed using rat serum (n = 3) intraluminally.

**Evaluation of Adhesion Force**

Six separate protocols were undertaken to measure adhesion force. The first was performed to determine the effect of bubble residence time within the vessel on the magnitude of the adhesion force that developed in the absence of any protein in the perfusate. Following a 15-min equilibration period after vessel mounting and perfusion with PSS alone, vascular reactivity was assessed by application of phenylephrine and acetylcholine as detailed in Materials and Methods. Those microvessels that did not respond to the phenylephrine ($\Delta D_{P_{E1}}\% < 15\%$) or to the acetylcholine ($\Delta D_{A_{C1}}\% < 50\%$) or did not develop spontaneous tone were considered damaged and were not studied further. After establishment of a mounted, intact, and reactive vessel (n = 3 per group), an air microbubble was injected as described in Materials and Methods. The outflow stopcock was then opened. One bubble per vessel was allowed to reside at a fixed axial location for four different durations (5, 10, 20, or 30 min) randomly assigned. After each preselected time, the adhesion force was measured. Microbubble adhesion was assessed at four different nonoverlapping axial positions, yielding four measurements for each residence time using 8 vessels.

A second set of experiments was performed with rat serum as the intraluminal perfusion solution. After confirming normal vascular reactivity, the endothelium was damaged as detailed in Materials and Methods. Those microvessels that did not respond to the phenylephrine and acetylcholine application after completion of the force measurement, followed by induction of endothelial damage by massive air perfusion as previously detailed. The loss of endothelial function was confirmed pharmacologically before a second microbubble was injected. The measurement of adhesion force was then repeated.

A fifth experiment was devised to quantify the contribution of a single protein, BSA, to the magnitude of the adhesion force. In this experiment, PSS plus 5% BSA was used for intraluminal perfusion. Eight microvessels were obtained from animals and divided into two groups, endothelium intact (n = 5) and endothelium damaged (n = 3). The expected decrement in endothelial function was confirmed after induction of endothelial damage with the massive air perfusion method. Assessment of the adhesion force generated was then made using a 10-min bubble residence within the vessel lumen.

**Statistical Analysis**

Data are presented as mean ± SD. Repeated measure analysis of variance with the Bonferroni post hoc testing was used to analyze the effect of bubble residence time on adhesion force with PSS for the intraluminal perfusion solution. The Student unpaired t test or one-way analysis of variance were used for the other experiments. Differences were considered significant for $P < 0.05$.

**Results**

Table 1 lists the results of the vessel reactivity tests after a 30-min bubble residence period. Responses to phenylephrine ($P = 0.93$) and acetylcholine ($P = 1.0$) were unchanged.

The results shown in figure 2 demonstrate the effect of bubble residence time on the adhesion force per unit surface area (K) under three different perfusion conditions: using a PSS with an intact endothelium, using...
Table 1. Effects of Air Microbubble Residence in the Vessel for 30 min on Vessel Reactivity, Using Serum as an Intraluminal Perfusate Solution

<table>
<thead>
<tr>
<th>Agent</th>
<th>Control</th>
<th>After 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylephrine</td>
<td>42 ± 6</td>
<td>34 ± 8</td>
</tr>
<tr>
<td>(ΔDPE%, n = 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>(ΔDACH%, n = 3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values of ΔDPE% and ΔDACH% are calculated from equations 1 and 2, respectively.

serum with an intact endothelium, and using serum with a damaged endothelium. For PSS, K was maximal at 10 min of bubble contact, reaching a value of 141 ± 29 dyne/cm². This was significantly higher (P < 0.05) than each of the values of K obtained with PSS after residence times of both shorter (5 min, 56 ± 22 dyne/cm²) and longer (20 min, 46 ± 29 dyne/cm²; 30 min, 14 ± 5 dyne/cm²) duration. With the endothelium intact and using serum, the adhesion force after 10 min of bubble residence (153 ± 57 dyne/cm²) was significantly higher than after a shorter residence time (5 min, 71 ± 29 dyne/cm²; P < 0.05). The adhesion forces at longer contact times (20 min, 100 ± 55 dyne/cm²; 30 min, 80 ± 48 dyne/cm²) were not significantly different (P > 0.05 for both comparisons). For the endothelium-damaged condition with serum, the adhesion forces (5 min, 62 ± 9 dyne/cm²; 10 min, 60 ± 14 dyne/cm²; 20 min, 56 ± 8 dyne/cm²; 30 min, 60 ± 13 dyne/cm²) were not different (P > 0.89 for all comparisons). At 10 min residence time with serum as the perfusate, the adhesion force with intact endothelium was significantly higher than with damaged endothelium (P < 0.05). The vessel contractility data for serum perfusion presented in table 2 shows that responses to phenylephrine and acetylcho-

Table 2. Vessel Reactivity before and after Endothelial Damage

<table>
<thead>
<tr>
<th>Perfusate/Agent</th>
<th>Before Damage</th>
<th>After Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylephrine (ΔDPE%)</td>
<td>30 ± 6</td>
<td>22 ± 6*</td>
</tr>
<tr>
<td>Acetylcholine (ΔDACH%)</td>
<td>100 ± 0</td>
<td>28 ± 12*</td>
</tr>
<tr>
<td>Physiologic salt solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylephrine (ΔDPE%)</td>
<td>22 ± 10</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Acetylcholine (ΔDACH%)</td>
<td>92 ± 17</td>
<td>35 ± 26*</td>
</tr>
<tr>
<td>Physiologic salt solution + bovine serum albumin (n = 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylephrine (ΔDPE%)</td>
<td>43 ± 3</td>
<td>30 ± 15</td>
</tr>
<tr>
<td>Acetylcholine (ΔDACH%)</td>
<td>100 ± 0</td>
<td>31 ± 5*</td>
</tr>
</tbody>
</table>

Values of ΔDPE% and ΔDACH% are calculated from equations 1 and 2, respectively. *P < 0.05 compared with before damage.

line were decreased (P < 0.05) after induction of endothelial damage. The vessel dilating response to sodium nitroprusside (10⁻⁶ M) was positive in all of these vessels.

Endothelial damage resulted in the reduction of K at 10 min of contact time with a BSA-free perfusate, to 68 ± 46 dyne/cm² from its baseline value of 156 ± 50 dyne/cm² obtained with the endothelium intact (P < 0.05, fig. 3). This baseline value of 156 ± 50 dyne/cm² was not different from the value of 141 ± 29 dyne/cm² determined in the residence time experiment and re-

Fig. 2. The adhesion force per unit surface area developed between embolism bubbles and the vessel wall at different contact times with physiologic salt solution and intact endothelium (n = 3), with serum and intact endothelium (n = 6), and with serum and damaged endothelium (n = 4). *P < 0.05 compared with 10 min with physiologic salt solution and intact endothelium; #P < 0.05 compared with 10 min with serum and intact endothelium.

Fig. 3. K, the adhesion force per unit surface area developed between embolism bubbles and the vessel wall after 10 min contact time. The two groups (n = 4) have the endothelium either intact or damaged with physiologic salt solution. The other groups have the endothelium either intact or damaged with physiologic salt solution plus 5% bovine serum albumin. *P < 0.05 compared with intact endothelium with physiologic salt solution group; #P < 0.05 compared with damaged endothelium with physiologic salt solution group.
ported above ($P = 0.66$). Table 2 lists data for PSS perfusion showing that vessel contractility in response to phenylephrine was the same before and after induction of endothelial damage ($P = 0.21$). As expected, endothelial damage did impair the acetylcholine-induced vasodilation in comparison with the magnitude of vasodilation measured with the endothelium intact ($P < 0.05$).

The addition of 5% BSA to PSS significantly increased the adhesion force parameter $K$. At 10 min of contact time with an intact endothelium, $K$ rose from $156 \pm 50$ dyne/cm$^2$, obtained with the BSA-free perfusate described above, to $349 \pm 149$ dyne/cm$^2$ ($P < 0.05$, fig. 3). After induction of endothelial damage and using PSS plus BSA as the perfusion solution, $K$ was determined to be $322 \pm 101$ dyne/cm$^2$ at 10 min bubble residence time, also graphed in figure 3. This value was significantly higher than the value of $K$ ($68 \pm 46$ dyne/cm$^2$) found after endothelial damaging but without BSA in the perfusate ($P < 0.05$). There was no difference between the result of this experiment (endothelium damaged, albumin present) and the result of the experiment having conditions of intact endothelium and BSA in the perfusate ($P = 0.80$). Vessel contractility, reported in table 2 (PSS + BSA), was not different in response to phenylephrine before and after endothelial damage ($P = 0.24$), but endothelial damage obliterated the acetylcholine-induced vasodilation response ($P < 0.05$).

**Discussion**

One approach to developing new therapy for vascular gas embolism is first to identify the underlying mechanical behavior of bubble adhesion to the vessel wall, thereby obstructing blood flow and causing ischemic injury. The molecular cause of adhesion may provide potential “targets” for therapeutic interventions that impair bubble adhesion. Such interventions, then, are aimed at preserving or restoring the desired physiologic effect: maintenance of blood flow.

We have hypothesized that the adhesion force leading to bubble arrest within the vasculature results from interactions between blood-borne macromolecules adsorbed to the bubble surface and the endothelial surface. Proteins are known to adsorb to gas-liquid interfaces.\(^{15,16}\) Gas bubbles in the bloodstream provide interfacial surface area for adsorption of circulating molecules. Macromolecules such as albumin, present in blood, have regions that can bind to gas-liquid interfaces through ordinary hydrophobic interactions. The specificity of this process is poorly understood, largely because of the lack of systematic study. Albumin, for example, adsorbs to the interface because it has a broad hydrophobic surface formed as a result of its tertiary structure,\(^{17,18}\) and because the energetics favor unfolding to expose hydrophobic surfaces that can then bind the interface.\(^{19}\)

Interfacial adsorption of albumin becomes essentially an irreversible process,\(^{20}\) leading to formation of a surface-bound protein layer. With an albumin concentration above 0.7 mg/ml, well within the normal human physiologic range, the adsorption process occurs very rapidly. Saturation of the surface occurs within a second.\(^{21}\) In addition, adsorbed protein surface layers have mechanical implications for direct surface-surface adhesion interactions within the vasculature\(^{22,23}\) and may also retard gas efflux from embolism bubbles.\(^{24}\)

Numerous studies modeling the reabsorption of intravascular gas bubbles\(^{25-32}\) have been published. This body of work illustrates that very small bubbles (\(< 100$ nl) can persist for many minutes or hours, essentially leaving distal microcirculatory units without blood flow. In this study we have examined the adhesion characteristics of small bubbles (2–3 nl) over a broad range of times (5–30 min). The results begin to provide a basis for interpreting the mechanical basis of adhesion so that novel or newly proposed therapeutic strategies can be interpreted. There have been many studies of pharmacologic interventions, such as the intravenous administration of the anticoagulant heparin, the local anesthetic lidocaine, or the antibiotic doxycycline, directed at mitigating the neurologic damage caused in animal models of cerebral gas embolism.\(^{33-35}\) There have also been many investigations of the mechanical behavior of drops and bubbles in vitro.\(^{36,37}\) Only recently has literature been published on the detachment of adherent drops and bubbles,\(^{38-39}\) including in vivo work showing that exogenous surfactants can accelerate the rate of bubble clearance from embolized vessels.\(^{40}\) None of these studies is a de novo investigation of any of the underlying mechanics of bubble adhesion and blood flow obstruction ultimately responsible for tissue injury.

The physics of bubble arrest (and subsequent detachment) within the vasculature will depend on the formation (and breakage) of adhesion between the bubble and the vessel wall. A rigorous formulation of the force balance, as derived previously,\(^{57}\) may be expressed as:

$$\Delta P \cdot A + \int_{\text{surface}} \tau \, dA = \int_{\text{surface}} \gamma \cos(\phi) \, dl$$

$$+ \int_{\text{surface}} \kappa \, dA + \Delta p gV \sin \omega \quad (5)$$

In equation 5, the two terms on the left side are the pressure and viscous drag terms, respectively. The three terms on the right side are the contact line adhesion, surface adhesion, and buoyancy force, respectively. $\Delta P$ is the axial pressure drop across the bubble, $A$ is the
projection of the cross-sectional area of the bubble in the radial plane, \( \tau \) is shear stress, \( \gamma \) is the surface tension, \( \phi \) is the contact angle in the liquid phase, \( k \) is the spatially dependent local adhesion force, \( \Delta \rho \) is the gas–liquid density difference, \( g \) is acceleration due to gravity, and \( \omega \) is the angle of inclination relative to horizontal. Not all terms must be preserved in the force balance. In the case of blood flow obstruction (zero velocity), if the bubble interface lacks a distinct contact line with the vessel wall and if the buoyancy is negligible (e.g., a horizontal vessel), only the first and fourth terms are retained, reducing this force balance to equation 4. The experimental model presented, then, sufficiently parallels the adhesion biomechanics of in vivo gas embolization to permit quantification of the adhesion parameter, \( K \).

The magnitude of \( K \) indicates the strength of surface-surface interactions. One surface involved is the bubble-blood interface, including any adsorbed material. The interface between blood and the luminal endothelial cell surface constitutes the other surface. In the experiments with an intact endothelium, this should be the endothelial cell surface layer, or glycocalyx. It is a fluffy-appearing, polysaccharide-rich, thin (< 1 \( \mu \)m) extracellular matrix of membrane-bound proteoglycan and glycoprotein macromolecules.\(^{44}\) Its structure is known to vary spatially in thickness and in composition in the vasculature.\(^{12,45}\) The endothelial glycocalyx provides a microenvironment with considerable functional importance: it influences resistance to blood flow,\(^ {44-46}\) and likely mediates many aspects of mechano-transduction important in endothelial cell function. It has been shown that the glycocalyx can be disrupted by hypoxia,\(^ {47}\) which could potentially explain our finding of the reduction in \( K \) seen at longer times (20 and 30 min), despite the evidence that the pharmacologically-evoked endothelial-mediated responses are maintained (table 1).

Structural elements of the glycocalyx play a major role in cellular adhesion, although relatively little is known about specific molecular components of the endothelial surface that allow it to bind effectively with circulating cells. There are ligand-receptor binding interactions mediated by known endothelial surface molecules such as P-selectin, ICAM-1, and ICAM-2. These form focal adhesion sites for immune cell recruitment into inflammatory sites.\(^ {41}\) Nonspecific adhesion interactions occur between cells, and these may be strongly influenced by glycocalyx glycoprotein and proteoglycan head groups. For example, expression of leukosialin (CD43), a large molecule found on many leukocytes, is inversely proportional to adheriveness.\(^ {48,49}\) Surface-surface interactions between circulating cells and the endothelial glycocalyx determine whether cell–cell adhesion and rolling arrest occur. Similarly, surface-surface interactions between endothelial cells and intravascular bubbles are likely determinants of gas embolism arrest (bubble lodging), blood flow obstruction, and onset of ischemia. In the case of albumin-encapsulated microbubbles, extensive degradation of the glycocalyx induced by ischemic injury has been shown to increase bubble residence time within the coronary circulation, where the glycocalyx is damaged by cardioplegia during cardiopulmonary bypass.\(^ {22}\) Presumably, bubble sticking is due to an increased adhesive interaction between the microbubbles and the surface-altered endothelium. The nature and temporal aspects of the binding relationship between specific and nonspecific adhesion elements of the glycocalyx and bubble surface elements (adsorbed blood-borne macromolecules) remain unknown.

The results of the experiments presented indicate that albumin, if present, contributed greatly to the adhesion force regardless of the state of the endothelium. With the endothelium intact, the value of \( K \) was 2.1–2.5 higher with BSA present in the perfusate. For purposes of comparison, it has been shown in previous in vivo experiments of embolism bubble entrapment in the rat cremaster microcirculation that the mean diameter of lodged emboli was approximately 65 \( \mu \)m and their mean length was 2,523 \( \mu \)m.\(^ {40}\) Assuming the arteriolar blood pressure distal to the bubble obstruction equilibrates with capillary pressure, the pressure gradient across the bubble is expected to be 30–40 mmHg. Using these values, the value of \( K \) is estimated to be in the range of 300–400 dyne/cm\(^ 2 \), which compares favorably with the value of 3.49 ± 1.49 dyne/cm\(^ 2 \) determined in the experiments conducted with an intact endothelium and 5% BSA. These values exceed those obtained with serum, suggesting that other blood-borne proteins may prevent albumin from reaching the bubble surface or may form complexes with surface-adsorbed albumin resulting in structures that interfere with its formation of adhesion with the luminal surface.

We chose to use serum for these experiments because this did not require the addition of exogenous substances (e.g., calcium chelators or heparin) to plasma or blood for anticoagulation. Such compounds may themselves contribute to, or interfere with, establishment of adhesion between the surfaces and confound the results. In their absence, significant plugging of the apparatus occurs because of clot formation, making it impossible to obtain reliable data. Although it preserves many features of the blood, serum lacks clotting proteins and platelets that, once activated, may contribute significantly to the adhesion interactions between the bubble and luminal surfaces.

The magnitude of \( K \) after endothelial damage was nearly five times greater with BSA in the perfusate versus without BSA. Coupled with the intact endothelium results, this suggests that BSA alone increases the adhesion force between the bubble and the vessel by the formation of a significant binding interaction between adsorbed BSA and surface elements present on the endothelium or the exposed basement membrane. This was...
not the case with serum, yet K was still the same order of magnitude as estimated from the in vivo data. Further, with or without an intact endothelium, K was not zero in the absence of protein, which indicates that adhesion still occurred. This may have resulted from the adsorption of hydrophobic surface elements tethered to the vessel wall onto the otherwise clean bubble surface. Such bridging of surface elements might be expected to be greater with the endothelium intact because the endothelial surface layer has a multitude of lengthy surface-tethered molecules available for bridging, which the collagen- and laminin-laden basement membrane does not. Our experimental results showed that K was smaller in the endothelium-damaged groups (figs. 2 and 3), a finding consistent with this explanation. The similarity in adhesion force developed by having protein in the perfusate, with or without the endothelium damaged, suggests a similar strength of binding interaction between proteins on the bubble surface and the exposed molecular structure of the vessel lumen. The specific nature of this binding interaction between bubble surface-bound macromolecules and vessel-wall elements (e.g., electrostatic interactions, steric effects) bears further investigation.

The time required for maximal adhesion to develop between an intact endothelium and a bubble might also indicate the time necessary for vessel wall surface elements to mobilize and adsorb to the bubble surface. The subsequent reduction of adhesion force with increasing time may stem from some aspect of endothelial injury not reflected by the response to acetylcholine, or it may indicate local changes in the surface layer structure. Experimental and numerical models indicate that the biophysics of glycocalyx transport barrier and cellular adhesion functions is highly dependent on both the electrochemical and molecular microenvironment. The balance of short-range and long-range cell-cell attraction and repulsion forces depends on adhesion molecule intermolecular distance and position within the thickness of the glycocalyx. How this balance is altered by the presence of a bubble is unknown, but it may ultimately contribute to endothelial cell injury or death.

It remains to be shown whether manifestations of endothelial injury such as changes in vascular permeability or alterations in vessel reactivity resulting from gas embolism may be the result of traumatic mechanical disruption of endothelial surface elements forming adhesion. These experiments demonstrate that such adhesion between bubble and vessel wall does form and that its quantification is possible. Yet this demonstration that the adhesion force is constituent dependent opens the possibility that targeted therapy based on competition with albumin for bubble interfacial occupancy is a rational approach for the future study of adhesion mechanics.

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