EDITORIAL

Gaseous Micro Emboli: Concepts and Considerations

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Introduction

The production of gaseous micro emboli associated with bubble oxygenators during extracorporeal perfusion has prompted considerable attention regarding product design and arterial filtration needs. There are a variety of routes by which gas bubbles are introduced into the vasculature. As invasive diagnostics and extracorporeal perfusion are used more widely, likewise, the incidence of gas embolism increases and thus the need arises for improved product design and standards. Recent studies[1–3] have attempted to evaluate many of the commercially available devices to determine both the degree of gaseous emboli production and the extent of removal by arterial blood filters. A review of the various testing protocols and equipment used in these evaluative studies indicates an apparent lack of consistency in methodology. Before discussing these inconsistencies, however, it is desirable to review the sources and pathophysiological effects of intravascular bubbles as well as any physical characteristics inherent to a gas bubble in blood.

It has been demonstrated that micro aggregates and micro gas emboli form in blood as a result of extracorporeal bypass and both have been implicated in the pathogenesis of organ dysfunction.[4] The major concern of this paper is with gaseous micro emboli production by bubble oxygenators and their removal by arterial blood filters. It is intended that standarized procedures will be recommended for evaluation of bubble oxygenators or arterial filters with regards to emboli production or removal. Accidental administration of an embolus either by injection or mechanical malfunction will not be discussed.

Sources of gaseous micro emboli

The source of gaseous micro emboli in bypass perfusion circuits has been attributed to: a) suction of blood and air for cardiotomy reserve,[5] b) cavitation of bubbles at turbulent regions of the circuit,[6] c) mechanical blows to devices which release retained bubles,[6] d) cavitation of oxygen bubbles when the saturation of hemoglobin (Hb) and the limit of dissolved oxygen in blood is exceeded,[7] e) cavitation of bubbles when hypothermically saturated blood is warmed with subsequent gas phase separation,[8] f) with the direct, often inadvertent, injection of gas into blood, or entrainment of gas trapped in the circuit.[9]

Sources *a, c* and *f* are self evident as the gas phase is already present and is merely mixed with the blood. The other sources involving cavitation are the result of complex biophysical processes, a summary of which is presented.

Cavitation

A fundamental concept concerning gas phase separation from a liquid is Henry's law, whereby at equilibrium the dissolved gas concentration is directly proportional to the partial pressure of the gas in the liquid[10], as expressed in units of pressure (P), the same as hydrostatic pressure in liquid is measured. From this pressure one determines the gas tension (t) in the liquid. The difference of (t-p), being equal to ΔP, expresses any tendency for gas to come out of solution in the form of a

bubble. As gas molecules are introduced in solution to a fixed volume of liquid at a low hydrostatic pressure (P), supersaturation will develop with subsequent gas phase separation from the liquid when ΔP exceeds a critical value. Certain physical constraints may alter the degree of supersaturation as well as the *de novo* formation of bubbles.

Likewise, if a mass of gas is introduced or already is present in the liquid, then growth of bubbles is predicted as diffusion of gas molecules from the supersaturated solution occurs. A likely comparison to this phenomenon is that of crystal growth following seeding, whereby growth continues until the degree of supersaturation is diminished. Sources *d* and *e* above are partially explained by this type of cavitation phenomenon, while local pressure differences in turbulent regions where the velocity component of Reynolds' numbers is excessively high can account for source *b* above.

Pathophysiology

The basic pathology associated with gas embolism is that of tissue ischemia or infarction due to the occlusion of nutrient blood vessels. The location, size and quantity of gas bubbles are primary determinants of the degree of pathology involved. Obviously, arterial bubbles pose a greater threat than venous, and where cerebral or coronary artery involvement occurs there is a greater likelihood for residual impairment or organ dysfunction.

Lethal doses of air injected into coronary arteries of dogs have been reported[11] as low as 0.05 ml., while 0.025 ml caused myocardial ischemia. In contrast, slow venous injections totaling as much as one liter[12] may be tolerated in dogs. These data demonstrate the remarkable capacity of the lungs to filter or trap venous gas emboli, further protecting arterial vessels. Compromise of this pulmonary filtration mechanism has been reported experimentally following oxygen toxicity,[13] administration of pharmacologicals,[14] or most commonly in gas volume overload conditions, where venous gas infusion rates exceeded 0.35 ml/kg/min.[15] These arterial bubbles can prove to be fatal, especially if nitrous oxide anesthesia is used.[16] This is due to the transfer of nitrous oxide molecules from the blood into the bubbles, causing them to increase in size.

Vascular reactivity to circulating bubbles generally demonstrates reflex dilatation in systemic beds, while the opposite holds true (i.e., constriction) for pulmonary vessels. These reflex changes in vessel tone have been attributed to neurogenic mechanisms[17] as well as humoral responses.[18] Further consequences of circulating bubbles include various interactions with blood products, the effects of which are discussed below.

Physical concepts

Certain physical features inherent to gas bubbles in blood warrant discussion as they may influence both pathophysiological consequences as well as results from evaluative studies. When addressing problems associated with gaseous micro emboli, references are often made regarding the size and counts of emboli. Another important characteristic is that of total gas volume which, if the other data are properly obtained, is the product of the two. Whereas total gas volume can be obtained as the product of the count and size of the emboli, a more accurate determination can be made using compression techniques (see below) which provide absolute values. In this way, the final value is not dependent upon faulty or restricted data obtained from other devices such as Doppler ultrasonics or particle data counters.

Total count data can be useful, although severe limitations may be imposed on interpreting the actual data or in the ways by which they are collected. Counts expressed per unit time or volume can offer a good index of emboli production or removal, however, coincidental counting which occurs with both Doppler and particle counters can bias the results to a significant degree. When employing such devices for size measurement, comparable restrictions can be placed on the resulting data. Very careful use with precise calibration of particle data counters can be reliable if all limitations are considered. Other physical considerations concerning bubble data include bubble:bubble interactions; blood:bubble interaction; and bubble stability or growth/resolution characteristics.

Intravascular gas represents a two-phase system with the gas embolus representing one

phase and blood (the liquid) representing the second. The physical properties of the two phases as well as the relative volume of the gas helps to determine the extent of distribution of the gas phase as embolic entities. Dispersion of the gas phase as microbubbles (14) is likewise a primary factor regarding the intravascular distribution of the emboli.

Further influences affecting the extent of distribution of microbubbles include the angle of plane of the particular vessel. In horizontal tubes or vessels, larger bubbles are observed to remain in the upper portion as they manifest the properties of bouyancy—especially in static regions. Bubbles traveling in vertical tubes or vessels usually remain more evenly dispersed within the fluid phase, as verified by *in vivo* observations[19] for superficial cerebral vessels. The bubbles one visualizes with the naked eye are large, while those below 75–100 microns are often not seen. Bubbles predictably travel through a vessel until they reach a segment whose caliber matches their own and the frictional forces tend to retard further advancement. Eventually the bubbles will be retained in this segment while any additional bubbles carried to the occluded site may contribute to the formation of a gas plug. When uniform microbubbles are used, the bubbles will travel to the initial retention site where a chain of bubbles will be formed or coalesce if the appropriate forces are available.[19] Coalescence is a complex physical process involving hydrostatic pressure gradients and interfacial tensions.

Coalescence

Following an exhaustive *in vitro* and *in vivo* study of gas plug formation, using microbubble production techniques, Grulke[19] arrived at three important conclusions: 1) bubbles failed to coalesce in extracorporeal capillary tubing, 2) bubbles failed to coalesce within blood vessels while blood flow was sustained and the bubbles remained dispersed within the fluid phase and 3) when several intravascular bubbles came into intimate contact with one another in static regions, coalescence was observed in less than one minute's time. The requirements for coalescence to occur included: 1) sustained intimate contact between the bubbles, 2) actual confinement by the vessel wall, and 3) the existence of a hydrostatic pressure gradient transmitted across the collection of bubbles. Intravascular gas plug formation is of major concern because of the potential for tissue infarction. While it is difficult to accurately predict whether intravascular coalescence of microbubbles will occur, consideration is given to cover this possibility.

Continued accumulation of new bubbles at the site of initial retention could develop into a gas plug whose length increases with each additional bubble. The plug could conceivably develop to a point such that the leading surface lies in a different environment than that of the trailing surface. While frictional forces would tend to increase as the coalesced plug grows in length, forward forces may develop to such a degree that movement through the vessel is maintained.

For bubbles in intimate contact with one another, coalescence will occur if the fluid film surrounding each bubble is ruptured. Another means for coalescence is when two bubbles of different diameter are in contact with one another and the gas within the smaller one diffuses through the liquid boundary layer and into the larger one.[20] The greater internal pressure of the smaller bubble, as predicted by the LaPlace relation[24] would provide the required driving force for diffusion. Another factor of primary importance regarding coalescence is the number of collisions between the gas bubbles. While certain factors such as hydrostatic pressure gradients and bubble: bubble interaction would tend to favor coalescence, certain surface phenomena would likewise occur which restrict such interactions.

Gas Bubble Stability

The adsorption and alignment of surfactant molecules to an air: aqueous interface are an example. The influence of these molecules is characterized by a decrease in the net attraction of the aqueous molecules towards the hypophase causing a reduction in the surface energy. However, preceeding the adsorption of surfactant molecules, intravascular bubbles become "coated" with a layer of protein molecules and other blood products including platelets and leukocytes. The protein molecules are rapidly denatured as electrostatic forces acting at the surface disrupt their tertiary struc-

ture.[21] This protein "coat" possesses a relatively low level of surface activity[22] and can inhibit the adsorption of surfactant molecules which manifest higher levels of surface activity. However, mechanical perturbations of the bubble surface, such as blood pressure or pump pulsations may conceivably disrupt and dislodge these proteins, revealing binding sites for surfactant molecules. While the adsorption of surface active molecules would help in stabilizing gas bubbles and thus, restrict coalescence, they also would contribute to the force balance relationship tending to force bubbles through vessels or possibly filter pores.[23]

Bubbles accumulated as a foam undergo specific interactions when collected in a particular segment of a vessel or perfusion circuit. Essentially, two types of foam can be characterized, wet and dry, for which the fluid content (film) between the individual bubbles and their shape are major characteristics.[24] The thickness of this fluid film contributes to the stability of the foam. As the film thins due to the hydrostatic pressure component across the bubble which pushes the fluid out, the activation energy of the surface tends to decrease; hence, the threshold for coalescence, as expressed by the critical energy of the surface, drops. Once the critical energy of the surface exceeds the activation energy, coalescence may proceed. Coalescence between bubbles represents a transition to a lower energy state with regards to surface energy. Once the transition exceeds the activation stage of increased energy, coalescence is predicted at the critical energy stage or point of surface film rupture. Adsorption of ordered molecules to the surface of bubbles stabilizes these energies; hence coalescence is more difficult.

Spherical bubbles with thick fluid films represent a wet foam which possesses a high degree of stability. As surface active molecules become adsorbed to the bubble surfaces, the fluid film viscosity increases, the hydrostatic pressure gradient may decrease and thus, the rate of film thinning is reduced and stability enhanced. Likewise, it has been suggested[25] from experimental work with venous air emboli injected into dogs, that surfactant molecules may be recruited to the intravascular bubbles or stagnant vessels resulting in a reduced blood surface tension. With a lower surface tension one may postulate that bubbles may "squeeze" through vessels or filter pores if the driving pressure is sufficient to overcome the restrictive forces holding the bubble in place. This expression of capillarity may be represented[25] by:

$$\Delta P = 2\gamma \cos\theta/r$$

Where ΔP is the pressure difference in front of and behind the bubble, γ is surface tension, θ is the contact angle at the triple point between the air:liquid:solid interface and r is the pore or vessel radius. When ΔP exceeds the value of the right hand side of the equation (i.e. restrictive forces) then forward movement of the bubble is predicted.

If no contact angle exists between the bubble and the adjacent solid surface eliminating θ from the above equation, then the following equation would apply when

$$r_2 > r_1: \Delta P = 2\gamma \left(\frac{1}{r_2} - \frac{1}{r_1}\right)$$

where r_1 and r_2 are radii at the trailing and leading edges.

Qualitative and Quantitative Methodology

In discussing physical features of microbubbles one begins to conceptualize how they may be counted or sized.

Sizing

Several techniques have been employed for sizing microbubbles: photographic, with a scaled micrometer for dimension reference[26]; terminal rise velocity, relating to buoyancy[27]; ultrasonic Doppler interpretation of reflected signals;[28,1,2] and particle counters,[14,27] which utilize the electric gating or Coulter Principle. Photographic applications are limited due to special lighting and magnification requirements and time delays in development. In addition, only a small representative sample can be obtained in any one given photo.

The terminal rise technique relies upon the translatory motion of a gas bubble in liquid as manifested by bouyancy. A gas bubble will rise in a fluid column achieving a terminal velocity as expressed in a balance equation whereby size and bouyancy are directly related and resistive forces (e.g., viscosity and gravity) are inversely related. This relationship has been expressed as:

$$R^2 = \frac{V\ (n)\ (9)}{g(p - p^1)\ (2)}$$

Where R is radius, V is velocity, n is viscosity, g is gravity, p is fluid density and p^1 is gas density. This equation was originally described by Stokes[29] for a metal ball bearing falling through a fluid column and applied to a gas bubble by Rybizynski[30] which allowed for non-rigidity. Lieberman[26] determined that smaller bubbles (<200 microns) behaved as rigid spheres, exhibiting a velocity of rise correctly determined by Stokes. Even though accurate determinations of bubble size can be made using terminal rise velocity, the fact that only one or a few bubbles can be measured at any one given time severely limits its application.

Ultrasound

Attenuation by diffraction of ultrasonic signals reflected from intravascular bubbles provides a method for detection and localization. The vast differences in density and bulk modulus between tissue, blood and gas bubbles allow for precise discrimination concerning changes in sound propagation. Application of ultrasonic attenuation of intravascular bubbles was first introduced by Stubbs and Kidd[31] for monitoring decompression bubbles.

Continuous-wave ultrasonic detection of intravascular bubbles was reported by Cannon[32] while earlier work by Fry and Dunn[33] mentioned observable changes in the propagation of ultrasonic waves if bubbles persisted in the sound field.

The theory of Doppler ultrasonics is based on the "Doppler Effect" whereby sound frequency reflected from a moving surface is proportional to the velocity of the moving surface.[28] Movement of the reflecting surface (e.g., bubble in blood) towards the source of the sound wave (ultrasonic transceiver) causes an increase in received frequency due to the increased number of pulse waves reflected. Movements away from the transceiver reduce the frequency for the opposite reason. If the ultrasonic beam is aimed towards a stationary bubble then the reflected beam is received at the same frequency as the incident beam.

The Doppler transceiver consists of two piezo electric crystals one of which continually emits ultrasonic waves while the other acts as a receiver for reflected and scattered waves. The back scattered or reflected waves from moving particles (e.g. bubbles in blood) originally transmitted from the crystal at frequency (f) are received at a different frequency (Δf) due to the velocity component of the moving particle. This shifted frequency is proportional to velocity as expressed in the following relationship:

$$\Delta f = \frac{2fv}{c} \cos\theta$$

where Δf is the shifted frequency, f is the transmitted frequency, v is the velocity of the fluid and hence the particle detected, c is the sound velocity in the fluid while θ is the angle of incidence of the sound wave with the fluid flow.[28] By electronically mixing the received shifted frequency (Δf) with the transmitted frequency (f) the resultant sound can be detected in the audible range.

With regard to sound reflection from a gas bubble, the sound velocity component (c) is determined by the elastic properties of the surrounding medium (e.g., bulk modulus, E) and the inertial properties of the material through which it must travel (e.g., density of the material, P, as represented by:[28]

$$c^2 = E/P$$

Thus, the vast difference in density between a liquid:gas interface is a major criteria for the excellent resolution obtained from an intravascular bubble. The incident ultrasonic beam, as it passes through a bubble, sets up sinusoidal variations in the local hydrostatic pressure within the bubble, and the liquid: gas interface pulsates as the bubble expands and contracts. These variations within the bubble help scatter the incident beam. This scattering and absorption of the incident beam is maximal at the reasonance frequency of the bubble.[28] However, due to the large acoustic impedance mismatch between the gas and the liquid, bubbles can effectively scatter the incident beam at many different frequencies. It is the physical difference in the two media (liquid and gas) that allows for effective sound reflection. The acoustic impedance for any material is thus a function of the velocity of sound through the material

which it passes and the density of the material as expressed in the relationship:

$$Z = c \cdot P$$

where Z is the acoustic impedance, c is the velocity and P is the density.[34]

All particles in blood will reflect sound waves to some degree. However, as particulate matter (e.g., thrombi, aggregates) have densities not too dissimilar to that of the surrounding medium, the reflection characteristics are less than those of bubbles.[35] There are inherent problems associated with the use of Doppler for accurate size measurement due to coincident signals and mismatch of the incident beam with the entrained microbubble in a vessel or tube. Often proper calibration techniques are not utilized experimentally, such as using particles other than calibrated microbubbles, flow conditions different than occur in clinical practice or the use of non-blood perfusates. Thus, without proper and rigorous validation, quantitative data obtained from Doppler devices may be questionable.

Electronic

An electronic method of sizing microbubbles utilizes the electric gating or Coulter principle.[36] The Coulter-counter, first reported for microbubble calibrations by Grulke,[27,14] was found to produce immediate and highly accurate bubble size measurements. The speed at which it can be operated as well as the advantage of a direct oscillographic display of bubble volume make this instrument ideally suited for microbubble calibration. The counting mechanism allows for size distributions to be made from given volumes of bubbles collected as a foam or individually.

The basic theory behind the use of the Coulter-counter or other particle data counters for particle size determination draws upon the Coulter principle. In an applied manner, microbubbles act as resistors when suspended in an electrolyte (e.g., normal saline). When the microbubbles are suctioned through an aperture tube, which is immersed in an electrolyte reservoir, through which a high density current is applied, then each bubble as it passes through the tube displaces a given volume of electrolyte. This displacement of electrolyte causes a sudden current drop which is directly proportional to the volume of the bubble.[36] The current change is fed into an oscilloscope and the subsequent spike produced for each bubble has a pulse height which relates directly to the volume of the bubble. The voltage has a variable threshold which can be set to discriminate microbubbles within a given size range. The aperture tube selection is dependent upon the size range of the particles measured. Appropriate data interpretation with computer software, now available with many commercial particle data instruments, can give fairly reliable results of bubble sizes. It should be mentioned, however, that inherent difficulties with the use of whole blood in these instruments can restrict the ability to properly interpret the results, although it is quite possible to obtain relative size distributions.

Counting

The above mentioned techniques and devices may also be utilized for counting microbubbles. The limitations previously discussed can also affect the results involving total counts. Thus, it may be helpful to suggest a different technique which can give absolute values to quantitate the extent of bubble formation or removal. This technique involves the concept of total gas volume.

Total Gas Volume

The volume of gas dispersed as microbubbles can be determined using a compression technique.[37] This principle involves the application of pressure to a compressible medium (gas bubbles) dispersed within a non-compressible medium (liquid). Taking a bubble oxygenator as an example, if one collects or isolates a representative aliquot of blood from the perfusion circuit containing microbubbles then the total volume of gas, dispersed as bubbles, can be determined by pressurizing that liquid, which essentially crushes the bubbles. As the void left by the gas space is filled by blood, the total fluid volume effectively decreases and this change can be measured. The calculation of the gas volume may be accomplished using Boyle's law (assuming an ideal gas and isothermic compression).

$$P_1V_1 = (P_1 + \Delta P) \cdot (V_1 - \Delta V)$$

where P_1 is the initial pressure, V_1 the initial

volume, ΔP is the pressure change and ΔV is the volume change. Water vapor pressure may be subtracted from P_1 above if the compression is slow enough for equilibrium. By collecting the fluid aliquot in a fixed volume container with a calibrated fluid-filled capillary tube connected, then the change in fluid level can be measured as the meniscus in the capillary tube moves.

An advantage of total gas volume measurement is that it gives an absolute reading, that is not dependent upon the limitations previously discussed for the other methods used. If one obtains total gas volume per unit time or volume of blood and determines bubble size distributions with a particle data instrument, then reasonable conclusions can be drawn as to the degree of bubble formation by an oxygenator or the extent of removal by a filter.

Conclusion

The problems associated with micro emboli formation, be they particulate or gaseous, are well recognized and appreciated. A considerable amount of effort has been expended by manufacturers, perfusionists, surgeons and researchers in attempting to fully identify these problems in hopes of providing adequate clinical solutions. Upon close examination of these problems several questions arise. While it is not the intention of this editorial to provide specific answers, it is intended that a broader understanding of the problem of gaseous micro embolism is achieved and more specific methodologies are proposed.

Previously, qualitative answers to questions concerning emboli production or removal were adequate for an evaluation of various products and procedures. However, current state of the art technology and knowledge require quantitative data which will specifically isolate problem areas.

One question often raised concerns the total number of emboli produced by various oxygenators or removed by filters. Ultrasonic devices and particle data counters are routinely used to answer this question but inherent weaknesses to these methods, as previously discussed, limit the confidence one can place on the results. Using these devices one must adequately distinquish between particulate and gaseous microemboli. Further limitations may be imposed due to coincidental counting and inadequate calibration procedures.

A recommended solution to this problem using a compression technique is proposed so that a greater degree of confidence is achieved in interpreting emboli counts. By determining count data before and after compression one can separate non-gaseous particles from microbubbles. This same analogy applies to the sizing of particles. It is imperative that blood particulate matter be differentiated from gaseous emboli. Compression techniques have been employed previously for gas volume measurements[6,37] and more specifically[1,6] to problems associated with extracorporeal bypass therapy. It should be appreciated, however, that when the analysis of a representative sample from a larger volume is made, bubbles of all sizes must have an equal opportunity to be captured in the test sample and that adequate volumes are used to avoid artifact from micro air contaminant. Sophisticated bubble separators[1] must be validated in so far as their capacity to isolate the microbubbles (<100 microns) that tend to be entrained within the fluid and are near impossible to visualize with the naked eye. Any recording device used to ensure complete isolation of bubbles in a test circuit must retain sensitivity to the 30–100 micron range.

An alternative procedure might include an inline compression apparatus which would periodically sample fixed volume aliquots of the perfusion circuit blood for gas volume measurement. Likewise, the use of a particle data counter to give size distributions from comparable samples would provide both qualitative and quantitative data regarding the amount of gaseous micro emboli.

Another area of concern in the evaluation of extracorporeal bypass problems is the lack of consistency in the various methods used by investigators. Ideally, certain procedures or devices could be standardized, such that comparisons would be more reliable and clinically useful. Such standardizations may include for example, the use of like fluids[2,38] for *in vitro* studies. While some analogies can be made to true clinical situations from data collected with a non-blood perfusate, these analogies are limited and potentially misleading. Studies evaluating blood filters must consider the possibility that flow characteristics may differ when non-blood perfusates are used. Fur-

thermore, non-blood perfusates do not contain surface active molecules or other material present in whole blood that may affect filtration. Furthermore, when counting or sizing devices are used for the collection of quantitative data, considerable attention must be paid to the calibration procedures. Particles of comparable material[39] and size should be used and the degree of confidence in such devices should be considered. Biffusion of gases into or out of a bubble[40] must also be considered, as this effect relates directly to volume and, therefore, size.

In closing, considerable improvements have been made in extracorporeal technology over the last decade. As the needs of patient care increases, so does the need for improved procedures and devices; hence, the importance of continued research and development cannot be overstressed.

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