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Microbubble dispersions of natural lung surfactant

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ABSTRACT

Microbubbles played an important role in R. E. Pattle's discovery of lung surfactant and E. M. Scarpelli's finding of the foam structure that forms the alveolar surface network. Today, colloidal dispersions of microbubbles coated with the same lipids found in natural lung surfactant are being used for a variety of biomedical applications, including ultrasound imaging, targeted drug delivery and injectable oxygenation. The purpose of this review is to introduce the reader to these two lines of research, in hope that an understanding of the biophysics of natural lung surfactant may inform the materials science of synthetic biomedical microbubbles, and vice versa. Clearly, one can gain a better understanding of synthetic lipid-coated microbubbles by studying Pattle's classical descriptions of lung bubbles, as many of the same properties have been observed in these two systems. For example, lung surfactant films on both natural lung bubbles and synthetic microbubbles fracture as they expand and reseal as they compress back to their original area. Additionally, the wrinkle-to-fold collapse transition can be observed on both systems, as it has been on the Langmuir trough and other surface film techniques. Use of the experimental microbubble platform may allow future measurements of lung surfactant permeability to gases and other solutes, as well as surface dilatational mechanics. Conversely, the study of natural lung surfactant monolayers may provide insights into new colloidal dispersions of synthetic microbubbles for medical imaging or drug delivery.

1. Introduction

Lung surfactant is a mixture of lipid and protein that coats the gas/liquid interface of the lung bronchioles and alveoli and functions to prevent alveolar collapse, reduce the work of breathing and provide uniform lung inflation [1•,2,3•,4•,5•]. Transport across the lung surfactant monolayer is also a critical step in pulmonary drug delivery [6]. Deficiency or dysfunction of lung surfactant leads to respiratory distress syndrome (RDS). In premature infants, RDS is treated with the instillation of exogenous lung surfactant. The advent of lung surfactant therapy has drastically reduced RDS-related mortality rates in neonates. Most surfactant therapy products are derived from processed animal lung extracts, and there is great interest in developing purely synthetic formulations. However, it appears that more work on the biophysics of natural lung surfactant and on the materials science of synthetic biomimetic compounds must be done in order to formulate effective synthetic alternatives. Such work may also lead to innovative microbubble colloidal dispersions for ultrasound imaging, targeted drug delivery and injectable oxygen therapies, as described below.

Lung surfactant was first discovered by R. E. Pattle in 1953 by the observation of extremely stable 40–50 μm diameter bubbles obtained from the lung [7••]. Epstein and Plesset demonstrated just a few years earlier that such small bubbles are unstable, even in an air-saturated medium, owing to surface tension-driven dissolution [8••]. Thus, Pattle reasoned that these lung microbubbles must be coated with a surfactant that achieves nearly zero tension [9••]. The discovery of a natural surfactant also conveniently addressed von Neergaard's much earlier observation that a properly functioning lung must have a very low surface tension at the air/water interface of the bronchioles and alveoli. Thus, it was established that lung surfactant found on microbubbles liberated from the lung is a critical part of healthy lung function. In the six decades since Pattle's discovery, however, much of the research into the biophysics and materials science of lung surfactant has centered on studies involving the Langmuir trough, pendant drops and captive (or pulsating) bubbles.

The Langmuir trough is a relatively simple technique involving compression of a flat monolayer while reading the surface tension with a wetted force transducer. Unfortunately, this technique deviates from the physiological reality in a number of ways, including (i) the flat, macroscopic surface (cm^2) bounded by hydrophobic barriers, (ii) anisotropic unidirectional compression and (iii) the inability to accurately measure surface tension as the monolayer transitions from a fluid to a solid that can hold compressive stress [10••]. The pendant drop and captive bubble methods allow calculation of surface tension from an axisymmetric drop shape analysis during buoyancy driven deformation. The ratio of buoyancy to surface tension forces can be captured by the Bond number (Bo), defined as:

$$Bo = \frac{\rho g R^2}{\gamma} \tag{1}$$

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where ρ is the liquid density, g is the gravity, R is the bubble radius and γ is the surface tension. The technique requires a Bo near unity to achieve a relative balance between these forces, which allows a smaller area (mm^2) than the Langmuir trough, as well as a curved, continuous surface and isotropic compression. However, the surface area is still much larger than the real alveoli (μm^2), and the technique loses accuracy as γ approaches zero and the bubble significantly flattens. Perhaps it would be worthwhile to revisit Pattle's microbubbles as a platform for studying the biophysics and materials science of lung surfactant.

The opinion in favor of the microbubble platform is bolstered by physiological studies implicating the importance of bubbles in the lung. In the early 1970s, E. M. Scarpelli used a new method to preserve the intact lung for microscopic observation, from which he discovered that the architecture of the bronchioles and alveoli comprised micron-scale "unit bubbles" separated from one-another by thin fluid films and Plateau borders [11••]. This foam architecture, which he called the "alveolar surface network", was in stark contrast to open-surface models proposed by J. Clements and others [12]. Over the next three decades, Scarpelli went on to publish many more experimental studies substantiating his initial observation and providing a biophysical interpretation of the foam structure.

More recently, colloidal dispersions of synthetic microbubbles coated with the same lipids found in natural lung surfactant have found widespread use in ultrasound imaging and therapy, as well as injectable oxygen delivery. The reader may be interested to know that there are several common properties between these synthetic biomedical microbubbles and the original natural lung microbubbles described by R. E. Pattle and E. M. Scarpelli, as discussed below. It seems that we still have much to learn from Nature! It is therefore advisable for researchers who are engineering microbubble formulations or studying their acoustic and biomedical properties to consult some of the prior literature on natural lung microbubbles. Therefore, one major goal of this review is to provide a brief introduction to the vast body of knowledge developed in Pattle's and Scarpelli's work on lung surfactant bubbles and foams. Inspired by their work, as well as more recent Langmuir trough and captive bubble studies on lung surfactant monolayers, my research group has begun to investigate the properties and biomedical performance of microbubbles generated from natural lung surfactant. We simply call these "lung surfactant microbubbles". The results of these studies are summarized in the second part of this review, followed by a brief outlook on possible future directions for this fascinating material.

2. Natural lung microbubbles

2.1. Pattle's discovery of the alveolar lining layer

In 1955, R. E. Pattle published his discovery of the alveolar lining layer (lung surfactant) based on the observation of stable microbubbles derived from instilling saline into the lung [7••]. Natural lung microbubbles were found to stabilize at 40–50 μm diameter. Unlike microbubbles formed from serum or other foams, microbubbles taken from the lung persisted for hours (Fig. 1). Pattle used the mathematical model of Epstein and Plesset for surface tension-driven dissolution [9••] to estimate a very low surface tension (<0.1 mN/m) for lung surfactant. It is now well established that the ability of lung surfactant to diminish surface tension at the air/water interface in the lung provides the necessary conditions to prevent alveolar collapse, reduce the work of breathing and provide uniform lung inflation.

2.2. Early lung bubble experiments

In 1958, R. E. Pattle reported his work on lung microbubbles to the Royal Society of London, where he opens with the statement [13••]: "The properties of foam and bubbles arising in the lung have been studied, and evidence has been obtained as to the nature of the alveolar lining." Pattle first noted that rodents experiencing acute lung edema produced foam in the trachea, and this foam was stable, even to detergents or silicone anti-foaming agents. Similar stability was found in the foam expelled from the lungs after instilling blood serum into the trachea of an anesthetized animal, while the foam formed by shaking blood serum with air was unstable to anti-foaming agents. Interestingly, the foam was found even when respiratory motion was blocked, indicating that the bubbles and surfactant arise directly from the bronchioles and alveoli. However, lung microbubbles are not only liberated from the pathological lung. Pattle explained that lung microbubbles could easily be isolated in the laboratory by simply squeezing a specimen of excised lung into a droplet of water.

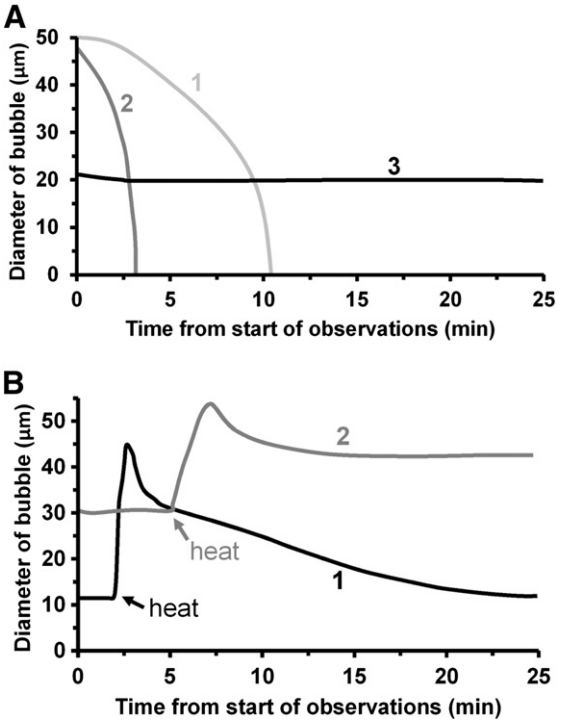

Fig. 1. A) Pattle's bubble dissolution curves in air-saturated liquid. Curve 1: Oxalated whole guinea pig blood. Curve 2: Distilled water. Curve 3: Lung microbubble liberated from the trachea of a rabbit suffering lung edema and transferred to water for observation. Curves 1 and 2 follow the expected trend for a bubble dissolving owing to surface tension, as predicted by Epstein and Plesset [8••]. The long stability of the lung microbubble in Curve 3 can only be explained by a surface tension that is effectively equal to zero [9••]. B) Pattle's bubble dissolution in air-saturated liquid following heat treatment. Curve 1: Stable lung microbubble expanded by heat during the third minute, and later resuming its original diameter. Curve 2: Stable lung microbubble expanded by heat after 5 min, and later becoming stable at a greater diameter. These curves demonstrate that the lung surfactant that coats the microbubbles can rupture and reseal, in the latter case incorporating additional surfactant onto the surface. Adapted from R. E. Pattle [13••].

Pattle reported that the tracheal foams are remarkably stable [13••]. They can be washed with several cycles of water without losing their stability, and they would not start to break under vacuum until subjected to only ~24% atmospheric pressure. Individual lung bubbles were stable indefinitely in air-saturated media, even when mixed with anti-foaming agents. However, they dissolved when placed in degassed water, often leaving behind a "ghost" of translucent material with an irregular shape. Based on these observations, Pattle concluded that the alveolar lining is insoluble, solid and permeable to gas.

To assess the composition of the alveolar lining, Pattle performed a series of experiments in which he incubated the tracheal foam to protein digesting enzymes, such as trypsin and pancreatin [13••]. He found that exposure to enzymes made the tracheal foam unstable to antifoaming agents, indicating an important role for protein in maintaining the integrity of the alveolar lining. A gravimetric analysis led Pattle to conclude that the protein film was 4–5 nm thick. It was later