Title: Alveolar Surface Mechanics

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SUMMARY

Alveolar Surface Mechanics: The major component of the recoil forces that tend to deflate the lungs is the surface tension of a thin liquid layer that lines the alveoli. This surface tension is well below the value for a clean air/water interface, indicating the presence of a surfactant. The surface tensions in situ specify that the surfactant films must have certain characteristics. Following large expansions of the air/water interface during deep inhalations, the surfactant must adsorb rapidly to form the interfacial film. When compressed by the shrinking surface area during exhalation, the films must be sufficiently rigid to resist the tendency to collapse from the interface. Materials washed from the lungs show that pulmonary surfactant is a mixture containing mostly lipids with some proteins that is synthesized and secreted by the type II pneumocyte. The disorder in which an abnormality of pulmonary surfactant most clearly plays a role is the Respiratory Distress Syndrome of premature babies, although altered surfactant function may also contribute to the Acute Respiratory Distress Syndrome that occurs in patients of all ages.

DESCRIPTION

The importance of alveolar surface mechanics is readily evident from the pressure-volume (P-V) characteristics of the lungs. Pressures required to maintain any given volume are substantially higher for lungs inflated with air than with saline. The fundamental difference between the two procedures is that saline eliminates an air/water interface, the surface tension of which contributes to the inward recoil forces for lungs inflated with air. The importance of surface tension implies that the curved surfaces of the pulmonary air spaces are lined by a layer of liquid. Electron microscopy has demonstrated that such a layer coats the alveoli, and that it is thin, with an average thickness of 0.2 μm, and continuous. The surface tension resulting from the air/water interface of this alveolar lining represents the major component of contractile forces in the lungs.

Surface tension results from an imbalance of forces on molecules close to an interface between two separated phases. For molecules deep within a substance, attractive forces towards neighboring constituents are equal in all directions (Figure 1). Within a few molecular diameters of the interface, however, components experience a reduced attraction towards constituents in the adjoining phase, resulting in a net inward pull and a force per unit length, or surface tension, that tends to contract interfacial area. The force along a curved surface, such as in the alveoli, translates directly into a difference in pressures across the interface. For a spherical interface with radius R, mechanical equilibrium between surface tension, σ, and the difference in pressures, $p - p_o$, occurs (Figure 2) when

$$\pi R^2 \cdot (p - p_o) = 2\pi R\sigma$$

which leads directly to the law of Young and LaPlace for a sphere,

$$\Delta p = 2\sigma / R$$

Inflation of lungs with air therefore requires higher pressure to overcome interfacial forces that are absent during inflation with saline.

Several methods have been used to determine surface tension in the lungs. The difference in P-V curves between air- and saline-filled lungs can provide surface tensions, either with simple assumptions concerning alveolar geometries and tissue forces, or with an energetic analysis that makes those assumptions unnecessary. Rinsing the lungs with a series of detergents or liquids provides an air/liquid interface with constant known surface tensions, and the intersection of P-V curves from these and normal lungs indicates points of common surface tension. Fluorocarbon droplets deposited on the interfacial film in peripheral alveoli spread according to the relative surface tensions of the fluorocarbon and film, and the shape of droplets containing different materials has provided the most direct estimates of surface tensions in situ. These different approaches have produced remarkably consistent results. During excursions through large volumes, surface tension shows a major hysteresis between inflation and deflation that explains the hysteresis of the P-V mechanics. Deflation from total lung capacity to functional residual capacity lowers surface tension from ~30 mN/m to < 5 mN/m. Over tidal volumes, surface tension varies between 10 mN/m and values as low as 1 mN/m.

These surface tensions are well below the values for a clean air/water interface, which would be 70 mN/m, and indicate the presence of a surfactant. Surfactants are the general class of compounds that have higher concentrations at the surface than in the aqueous medium. They are amphipathic, with distinct hydrophilic and hydrophobic regions of the molecule, and best satisfy the energetics of both portions by orientation across an interface. Once trapped within the two-dimensional surface, surfactants tend to spread, resulting in a force that expands the interface and opposes surface tension. Surfactant films with higher densities produce larger reductions in surface tension. The very low surface tensions in the lungs indicate films with particularly high densities.

Surfactants can lower surface tension by adsorbing to an interface only to a limited extent. Adsorbed surfactants reach a maximum density, above which further constituents form a three-dimensional bulk phase at the interface rather than adding to the two-dimensional film. Surface tensions in the lungs reach values well below this minimum equilibrium value, and they also vary with volume. These observations indicate that the low surface tensions and high densities of the films result not from insertion of more constituents, but from a decrease in surface area during deflation.

The low surface tensions reached during deflation are impressively stable in static lungs for prolonged periods. The low magnitude and particularly the stability of the surface tensions indicate that the compressed films have specific characteristics. When compressed above the maximum equilibrium density, films that can flow from the surface will collapse to form the three-dimensional bulk phase, thereby reestablishing

equilibrium surface tensions. Only films that are solid, defined by their inability to flow, can resist the tendency to collapse and demonstrate the behavior observed in the lungs.

When compressed sufficiently, even solid films collapse. An interface without surface tension has no basis for existence, and at sufficiently high densities, solid films must also rupture. The hysteresis of surface tension, and of hydrostatic pressures, between deflation and inflation over large volumes reflects at least partially material lost from the interface during collapse.

Lavaging the lungs produces the increase in recoil pressures expected from removal of a surfactant and a resulting increase in surface tension. Material recovered from the lungs can form films with characteristics indicated by the in situ measurements. Material obtained from alveolar foam, when suspended in saline, can form small bubbles that persist for prolonged periods, indicating that the pressure-difference across their surface, which would tend to dissolve the gas in the surrounding liquid, must be low, and that according to the law of Young and Laplace, surface tension must also be small. When compressed in vitro by changing their surface area, films formed from lavaged material can reach and sustain surface tension below the minimum equilibrium value. Material purified from lavaged material can also restore the P-V mechanics of the original lungs. These observations provide the basis for the identification of the surfactant in the lungs.

Material recovered by lavage contains two sets of phospholipid vesicles that differ in size. The larger form has the same morphological appearance as a subcellular organelle,

the lamellar body, of the type II pneumocytes (Figure 3). Organic extracts of these larger particles can restore the P–V mechanics of lavaged lungs, which perhaps best defines these vesicles as "pulmonary surfactant". The smaller particles may represent material excluded from the interface during compression to very low surface tensions. Vesicles of both sizes contain the same set of phospholipids with small amounts of cholesterol. The larger particles are much more capable of lowering surface tension in vitro than the smaller vesicles. Four proteins copurify with the larger particles but are absent from the smaller forms. The organic extracts of the large particles, which function well in lavaged lungs, lack surfactant proteins SP-A and SP-D, and based on a variety of assays, their primary function appears related to processes other than the lowering of surface tension. SP-B and SP-C, which are sufficiently hydrophobic to extract with the lipids into organic solvents, determine the difference in surface activity between the large and small particles.

NORMAL PHYSIOLOGICAL PROCESSES

To achieve the surface tensions observed in the lungs, pulmonary surfactant must satisfy conflicting constraints. Vesicles must first adsorb rapidly to form the interfacial film. Pulmonary mechanics become normal during the first few breaths following the initial air-inflation of fluid-filled lungs, suggesting that adsorption to the newly created air/water interface forms a film having the equilibrium density within seconds. The low surface tensions reached during deflation indicate that the compressed film avoids the reverse process of desorption to reestablish the equilibrium density. Replicating in vitro the full

performance of films in the lungs has been difficult, and the mechanisms by which pulmonary surfactant functions in the alveoli remain the subject of active investigation.

Adsorption

The adsorption of pulmonary surfactant is fundamentally different from the process for other more common surfactants, which insert into the interface as individual molecules. Surfactants share the general characteristic that they exist in solution as individual monomers only up to a certain concentration, above which they aggregate into structures such as micelles and bilayers (Figure 4), the nature of which depends on the effective shape of the particular molecule. The "critical micelle concentration" at which phospholipids aggregate is approximately 10^{-9} M. Constituents of pulmonary surfactant therefore exist in the alveolar lining exclusively as vesicles, which insert as collective units into the interface. Because the vesicles themselves are stable in aqueous medium, an energy barrier limits adsorption, which occurs quite slowly for lipid vesicles without the hydrophobic proteins.

In the alveolar lining, lamellar bodies secreted by the type II pneumocytes unravel to from a distinct intermediate structure known as tubular myelin that apparently represents the direct precursor of the interfacial film (Figure 3). SP-A is required in vitro for the reconstruction of tubular myelin, which is absent from extracted surfactants and transgenic animals that lack SP-A. Extracted surfactants, however, function well in surfactant-deficient lungs, and mice without SP-A have normal pulmonary mechanics. The functional significance of tubular myelin for adsorption is therefore unclear. The

absence of SP-B, whether in transgenic animals or in patients with genetic abnormalities, does produce abnormal pulmonary function, consistent with the crucial role suggested by in vitro studies of this protein for adsorption.

Stability of compressed film:

Under equilibrium conditions, attempts to increase the density of surfactant monolayers above a maximum density, either by adding more constituents or by decreasing interfacial area, instead forms a three-dimensional bulk phase that coexists with the two-dimensional film. The bulk phase of phospholipids is a liquid-crystal, in which layers of material stack in the regularly repeating manner characteristic of a crystal, but with each layer having the disordered structure that is characteristic of liquids. Slowly compressed monolayers of pulmonary surfactant in vitro flow into these stacked structures (Figure 5), and their ability to flow indicates that the films are fluid. In the lungs, the prolonged low surface tensions, well below the minimum equilibrium values, indicate films that have the defining characteristic of a solid that they resist flow. The structure of two-dimensional solid films could be either highly ordered, analogous to a three-dimensional crystal, or amorphous, like a glass.

At physiological temperatures, a single constituent of pulmonary surfactant, dipalmitoyl phosphatidylcholine (DPPC), can form highly ordered films that approach the structure of a two-dimensional crystal. DPPC has the unusual characteristic relative to other biological phospholipids that both acyl chains are fully saturated and that it constitutes an

unusually large amount, 30-50%, of pulmonary surfactant. A widely held view contends that the functional film in the lung consists of essentially pure DPPC.

The difference in composition between the secreted vesicles and the hypothetical functional film of DPPC could result either from selective adsorption of DPPC or from selective collapse of other constituents. Both process are difficult to reconcile with current understandings of how adsorption and collapse occur. One prediction of the model is that P-V curves should change abruptly over a narrow range of temperatures. Films of DPPC, like three-dimensional crystals, melt from solid to fluid structures at specific temperatures. At surface tensions below the minimum equilibrium value, rates of collapse increase when solid films melt, resulting in increased surface tensions that would produce higher recoil pressures. Measurements of the temperature-dependence for P-V curves have yielded conflicting results, and the presence of a highly ordered film remains unconfirmed. Although films containing only DPPC would explain the stability of low surface tensions in the lungs, experimental evidence to support that possibility is limited.

The solid films that sustain low surface tensions in situ could also have a structure that resembles a two-dimensional glass. Three-dimensional liquids, if cooled fast enough and far enough below their freezing temperatures, retain their disordered structure but become frozen in place, forming amorphous solids, or glasses. Two-dimensional fluid films, defined by their ability to flow into collapsed structures, similarly become jammed into a form that resists collapse if supercompressed to sufficiently low surface tensions. These

supercompressed films retain their solid behavior and slow rates of collapse when expanded, even when returned to the surface tensions at which they originally collapsed. If they reach low surface tensions in the lungs during a single exhalation, the films would be transformed, and their ability to avoid collapse could persist through multiple cycles of tidal breathing. To achieve low surface tensions, however, the initially fluid films must be compressed faster than they can collapse. The required rates may occur during normal breathing, but they are faster than rates in quasi-static experiments with excised lungs. The supercompressed films, which would require no compositional change, could explain surface tensions observed in the lungs, but like the films of pure DPPC, the process by which they would form remains unclear.

Original views concerning surface tension in the lungs considered an interfacial film with the thickness of one molecule. Although perhaps difficult to explain how they might form, monolayers that have the characteristics of films in the lungs are well described, and more complicated structures were unnecessary to explain the observed behavior. Electron microcopy, however, has demonstrated that in situ, at least parts of the interface are occupied by films that are multilayered. Whether these structures are formed during adsorption or collapse, and the extent to which the additional material might affect the mechanical characteristics of the film, are both unknown.

PHYSIOLOGICAL PROCESSES IN RESPIRATORY DISEASES

The disorder in which an abnormality of pulmonary surfactant most clearly represents a major pathogenic factor is the Respiratory Distress Syndrome (RDS) that occurs in

premature babies. Ventilation of immature lungs that lack adequate amounts of surfactant injures the alveolocapillary barrier, resulting in pulmonary edema and respiratory failure. Two mechanisms, both involving shear stresses, could explain how a deficiency of pulmonary surfactant would produce the injury. First, elevated surface tension would produce an increased tendency for small alveoli to collapse, and the shear stresses involved in reopening the closed air-spaces could produce the injury. Second, the meniscus of any fluid column in the small airways would have an increased surface tension, and the greater pressure-difference across the interface could rupture the epithelial cells over which it passes. Elevated surface tensions would also lower interstitial and pericapillary pressures, resulting in a greater transmural pressure-difference that would increase the flow of fluid across the alveolocapillary membrane. The most direct evidence that deficient surfactant causes RDS comes from manipulation of surfactant levels. Subsequent to removing surfactant by lavage, ventilation of animals produces an injury that replicates RDS. Conversely, giving exogenous surfactant to premature babies at risk for RDS prevents or reverses the disorder.

The acute respiratory distress syndrome (ARDS) was originally called adult RDS to point out the clinical similarities between adults with injured lungs and the infants with RDS, and to suggest that the common presentation resulting from a variety of insults might reflect an abnormality of surfactant acting as the final common pathway. Although the primary defect in ARDS is an inflammatory process, abnormal surfactant might perpetuate the initial injury by the same processes that result from an elevated surface tension in RDS.

Surfactant function could be altered in ARDS by either deficiency or inhibition. Injured lungs have reduced levels of the large active surfactant vesicles, suggesting a deficiency. Pulmonary surfactant could also be inhibited by the large number of extraneous compounds, such as plasma proteins and membrane lipids, that reach the alveolus in injured lungs and that can act as surfactants. Direct evidence for elevated surface tensions in ARDS, however, is limited. The presence of edema complicates the interpretation of P-V mechanics, and the distinction of small lungs, caused by fluid-filled air spaces that occur with any pulmonary edema, from stiff lungs, caused by increased surface tension, has been difficult. Evidence that exogenous surfactants can mitigate ARDS is also lacking. The larger doses required to treat adults with ARDS relative to premature babies with RDS has limited the therapeutic agents that can be used. Initial trials with surfactants that lack SP-B have produced no improvement, just as early attempts to treat babies with RDS using aerosolized DPPC had no benefit. The role of therapeutic surfactants in ARDS, and of abnormal surfactant in its pathogenesis, therefore remains unresolved.

FIGURE LEGENDS:

1. Origin of surface tension: Molecules in the bulk water experience an attractive force towards neighboring molecules that is equal in all directions, resulting in no net force. Within a few molecular diameters of the interface, however, the absence of neighbors towards the surface results in a net inward pull that tends to shrink the interfacial area. A film of surfactant at the interface tends to spread, resulting in an opposing force that lowers surface tension.

2. Relationship of surface tension to hydrostatic pressure across a spherical surface. Mechanical equilibrium occurs when the inward recoil force of surface tension (σ) and the opposing force of hydrostatic pressure ($p - p_o$) are equal. At the midsection of a spherical bubble with radius R, surface tension will produce a force of (length$\cdot\sigma$) = $2\pi R\cdot\sigma$, and the force from hydrostatic pressure will be [area$\cdot(p - p_o)$] = $\pi R^2\cdot(p - p_o)$. The equality leads directly to the law of Young and Laplace that $\Delta p = 2\sigma/R$.

3. Schematic of pulmonary surfactant in the alveolus. Constituents of pulmonary surfactant are synthesized in type II pneumocytes and assembled into lamellar bodies, which are secreted into a thin liquid layer that lines the alveolus. The vesicles unravel and first form tubular myelin, which appears to represent the immediate precursor of a film at the air/water interface. Lavage of the lungs recovers both the large multilamellar vesicles, which contain mostly phospholipids with small amounts of cholesterol and proteins, and small unilamellar vesicles, which contain only the lipids.

4. Aggregation of surfactants. Above a certain concentration, the hydrophobic portion of surfactants makes them insoluble as individual molecules, and causes their aggregation into structures that expose their hydrophilic components to the aqueous medium while sequestering the hydrophobic segments. The particular form of the aggregate depends on the effective shape of the specific compounds. Single chain surfactants tend to form micelles. Biological phospholipids form bilayers that may stack into the concentric layers of a multilamellar vesicle.

5. Liquid-crystalline collapse. Under equilibrium conditions, surfactant films reach a minimum surface tension at which they undergo a phase transition to form a three-dimensional bulk phase. Phospholipids form bulk smectic liquid crystals, and compression of fluid phospholipid films at the minimum surface tension can cause the film to flow into the stacked structure. Solid films, which can resist flow and remain at the interface below the minimum equilibrium surface tension, eventually also collapse from the interface at very low surface tensions, although probably by a process more like fracture.

FURTHER READING :

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SEE ALSO

MS Code	Entry
4	Acute lung injury
5	Acute respiratory distress syndrome
24	Alveolar hemorrhage
26	Alveolar wall micromechanics
42	Breathing (Breathing in the newborn)
44	Breathing (Fetal lung liquid)
45	Breathing (First breath)
51	Bronchoalveolar lavage
71	Chest wall mechanics
116	Drug-induced pulmonary disease
139	Epithelial cells (Type I)
140	Epithelial cells (Type II)
157	Fluid balance in the lung
186	Infant respiratory distress syndrome
220	Lung anatomy
226	Lung Imaging
376	Surfactant (Overview)
377	Surfactant (SP-A)
378	Surfactant (SP-B)
379	Surfactant (SP-C)

380 Surfactant (SP-D)

KEYWORDS (10 - 15): alveolar liquid lining; collapse; DPPC; film; hysteresis; air/water interface; lung; mechanics; metastability; phospholipids; pulmonary surfactant; recoil; surfactant proteins; subphase; surface tension.

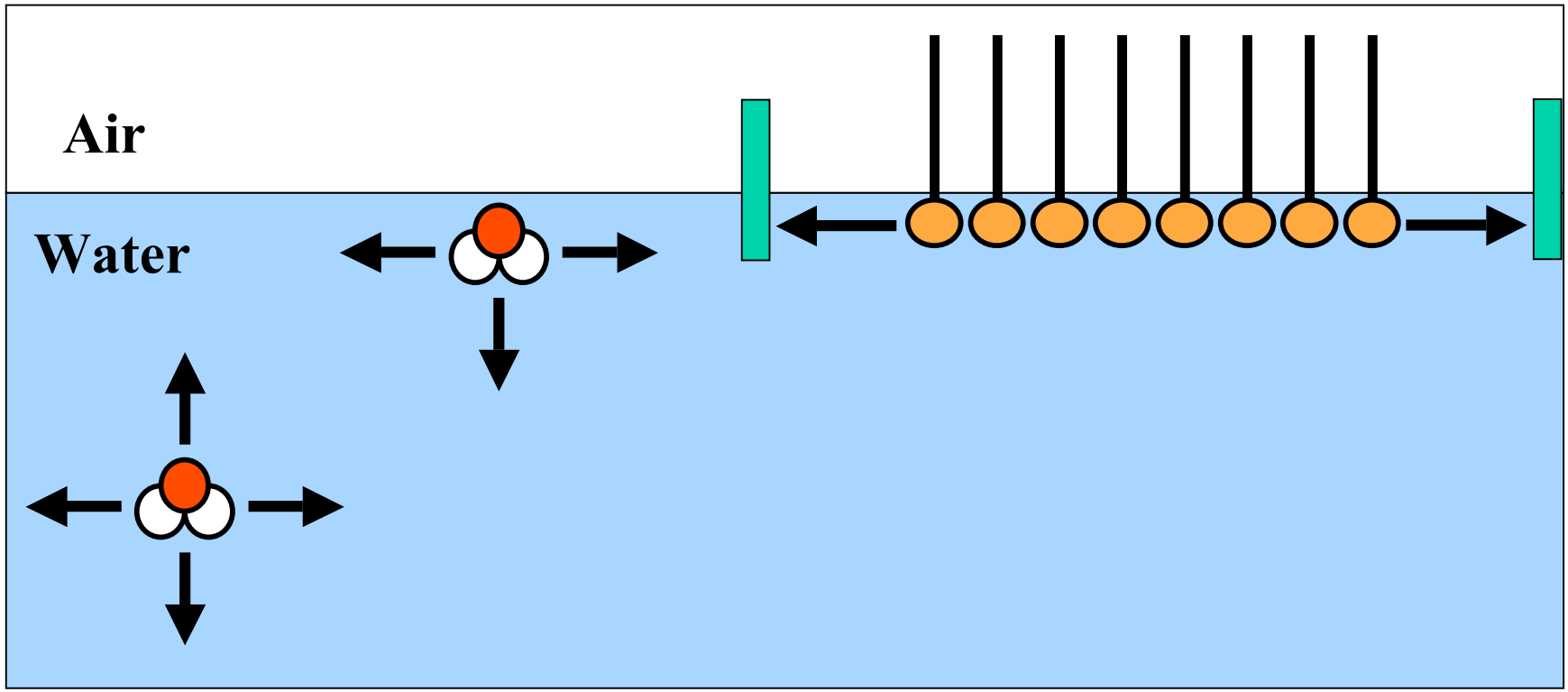


Figure 1

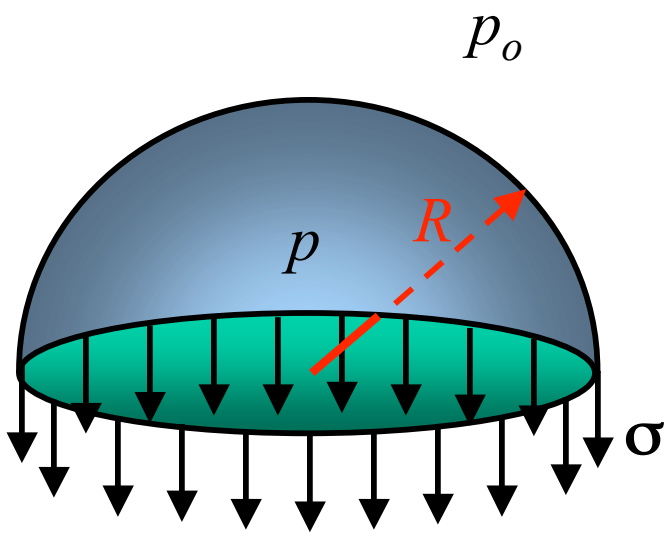


Figure 2

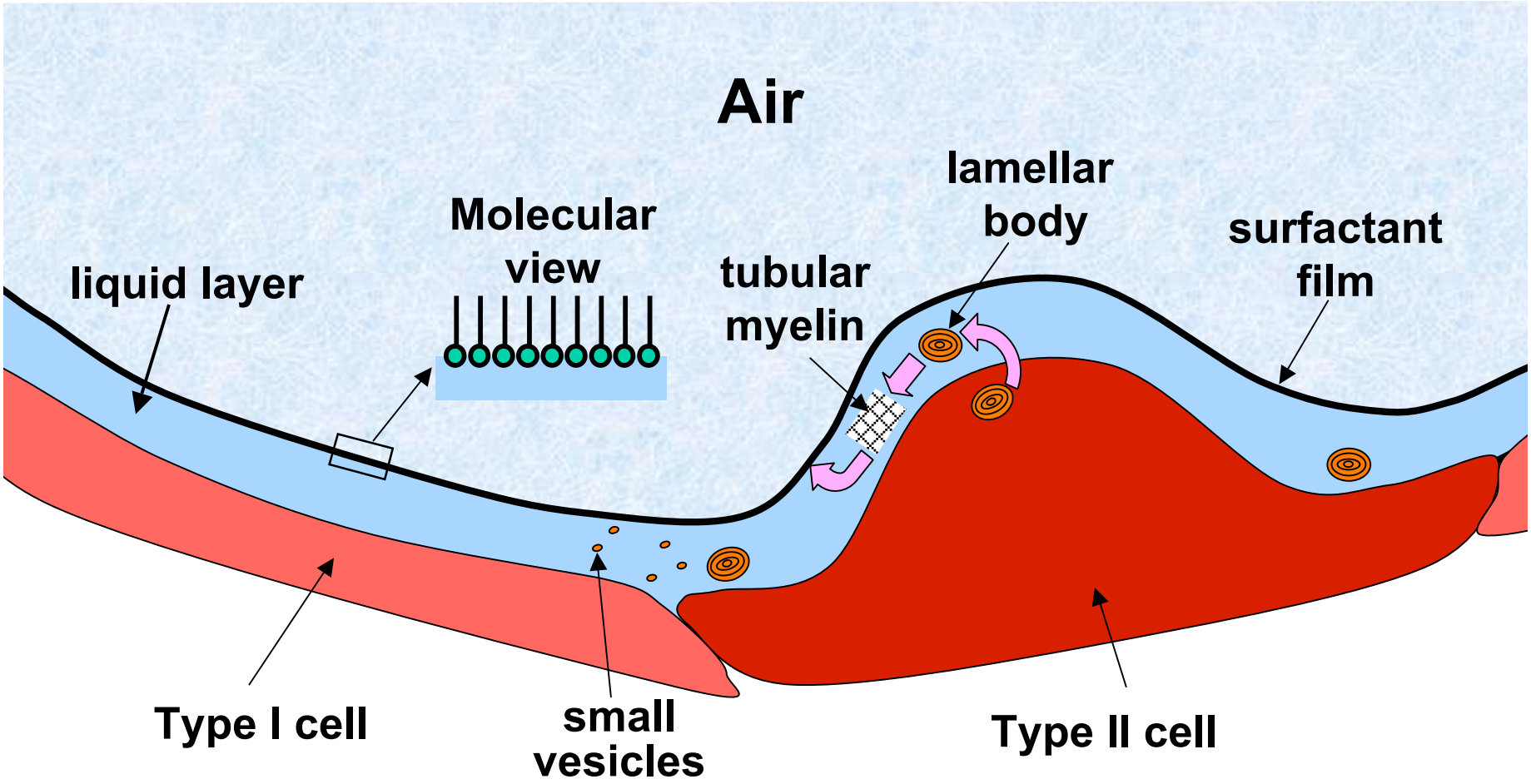
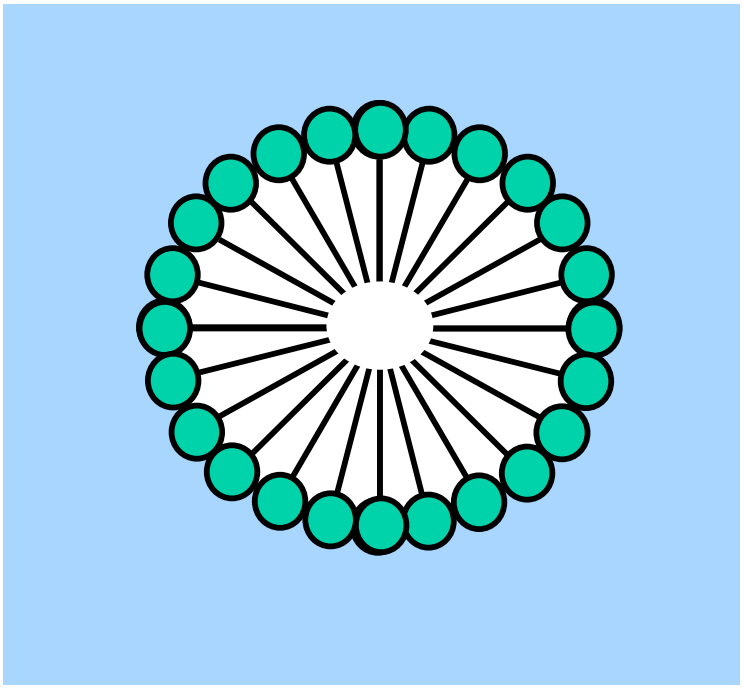

Air
lamellar body
Molecular view
surfactant film
liquid layer
tubular myelin
Type I cell
small vesicles
Type II cell

Figure 3



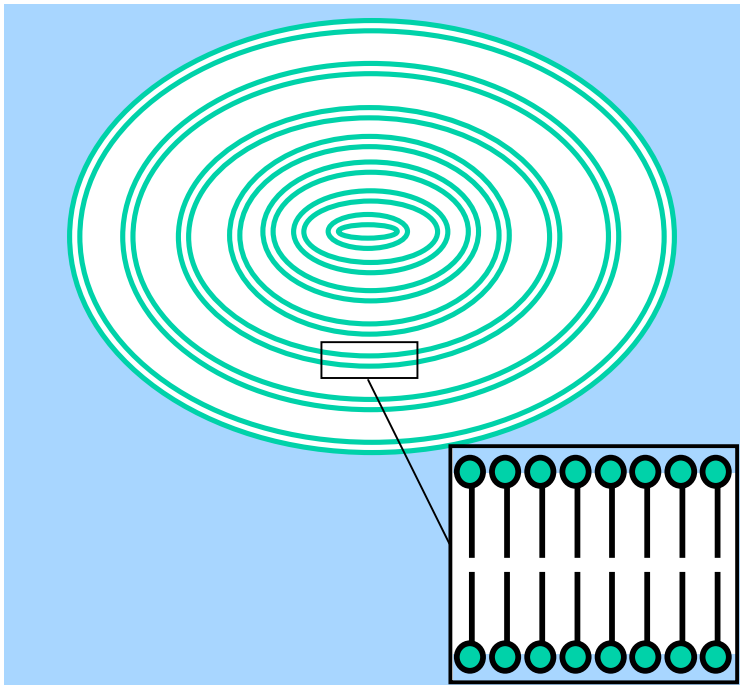


Figure 4

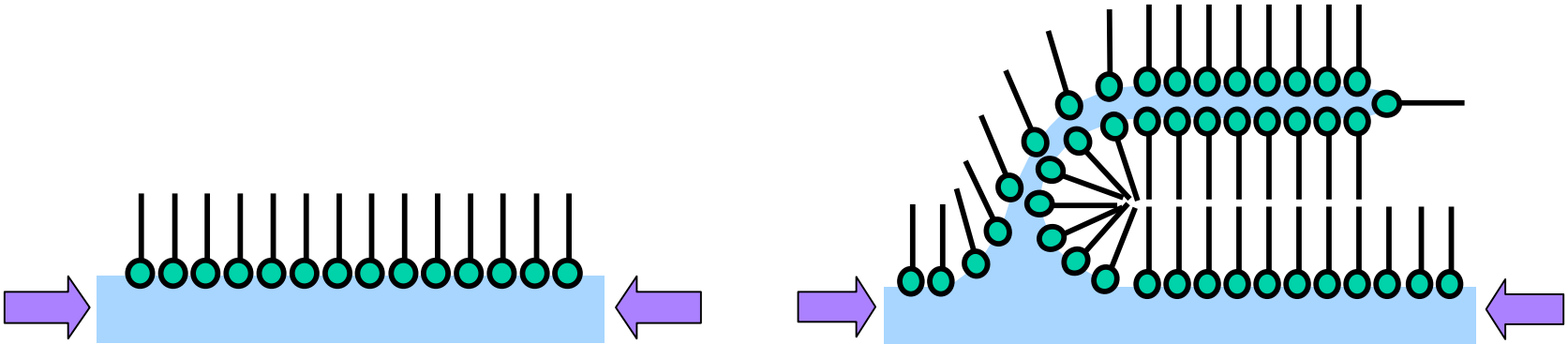


Figure 5