Pulmonary surfactants and their role in pathophysiology of lung disorders

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Surfactant is an agent that decreases the surface tension between two media. The surface tension between gaseous-aqueous interphase in the lungs is decreased by the presence of a thin layer of fluid known as pulmonary surfactant. The pulmonary surfactant is produced by the alveolar type-II (AT-II) cells of the lungs. It is essential for efficient exchange of gases and for maintaining the structural integrity of alveoli. Surfactant is a secretory product, composed of lipids and proteins. Phosphatidylcholine and phosphatidylglycerol are the major lipid constituents and SP-A, SP-B, SP-C, SP-D are four types of surfactant associated proteins. The lipid and protein components are synthesized separately and are packaged into the lamellar bodies in the AT-II cells. Lamellar bodies are the main organelle for the synthesis and metabolism of surfactants. The synthesis, secretion and recycling of the surfactant lipids and proteins is regulated by complex genetic and metabolic mechanisms. The lipid-protein interaction is very important for the structural organization of surfactant monolayer and its functioning. Alterations in surfactant homeostasis or biophysical properties can result in surfactant insufficiency which may be responsible for diseases like respiratory distress syndrome, lung proteinosis, interstitial lung diseases and chronic lung diseases. The biochemical, physiological, developmental and clinical aspects of pulmonary surfactant are presented in this article to understand the pathophysiological mechanisms of these diseases.

Keywords: Adult respiratory distress syndrome, Hyaline membrane disease, Infant respiratory distress syndrome, Lamellar bodies, Lungs, Phosphatidylcholine, Surfactant associated proteins

Surfactant and surface tension

The agents that decrease surface tension are known as surface active-agents or surfactants. Surface tension can be defined as the cohesive force of attraction experienced by the molecules present at the interphase of two media. The surface tension may develop between solid-liquid, liquid-liquid or liquid-gas media. Surface tension tends to pull the molecules at the interphase inwards thereby reducing the interaction between two phases. The surfactants are amphipathic molecules that form a film between the two media in such a way that their interactions are thermodynamically stable and result in reduced surface tension.

In our body, lungs offer a large surface area where atmospheric air (gaseous media) comes in contact with body fluids (aqueous media) for gaseous exchange. The presence of pulmonary surfactant at the gaseous-aqueous interphase reduces the surface tension facilitating the diffusion of gases. Pulmonary surfactant is also important for maintaining the structural integrity (alveolar size), lung compliance, elasticity of lung tissue, preventing atelactasis, balancing hydrostatic pressure and keeping the alveoli dry. In addition, it plays important role in host defense.

History of surfactant

Von Neergard\(^2\) gave the concept of surface tension in lungs for the first time while performing experiments with porcine lungs. He demonstrated that surface tension existing between the air-water interphase of lungs is important factor for the recoil of lung and reduced surface tension facilitates respiration. He also suggested the relevance of surface tension with the first breath of new born. Subsequently, Thannhauser \textit{et al.}\(^3\) reported that lung tissue has remarkably high content of lecithin a lipid now called as dipalmitoylphosphatidylcholine. Afterwards, Gruenwald\(^4\) stated that surface tension offers resistance to aeration of the neonate lungs and surface-active agents reduce such resistance. Pattle\(^5\), while working with the nerve gases that damage the lungs, observed fairly stable air bubbles that were ineffective to anti-foaming agents. He postulated that
the bubbles might be lined by some surface active agents/substances and deficiency of such surface-active material might be responsible for infant respiratory distress syndrome (IRDS). Clements and Macklin also came to similar conclusions regarding the presence of surface active agents while working with nerve gases independently. They further showed that various lipid fractions especially those rich in dipalmitoyl phosphatidylcholine have surface tension reducing properties similar to the naturally occurring pulmonary surfactant. In late 1950’s, Avery and Mead demonstrated that IRDS or hyaline membrane disease of the new born is due to high surface tension or surfactant deficiency. Various studies have shown that phosphatidylcholine is an important component of pulmonary surfactant as it remarkably decreases the surface tension compared to other lipids like cholesterol, phosphatidylinositol, phosphatidy ethanolamine etc. Further, Hallman et al. showed that phosphatidylglycerol is important for spreading of phosphatidylcholine on the alveolar surface. Since then, studies have been undertaken to characterize the pulmonary surfactant, its physico-chemical properties and its role in the pathophysiology of various lung diseases.

Cells lining the airways

The respiratory tract comprises of nose, pharynx, larynx, trachea, bronchi, bronchioles, terminal bronchioles and alveoli. The first sixteen segments are called conducting airways (meant for the transport of air) and the 17-23 segments are called respiratory airways (meant for gaseous exchange). The respiratory tracts are hollow tubular structures lined with various epithelial cell types (from columnar cells to flat cells) to facilitate transport of air and to perform protective functions (Fig. 1).

The respiratory airways are lined by various types of cells originating from the basement membrane. They include ciliary cells, goblet cells, basal cells, Clara cells, serous cells, chemosensory cells, brush cells, alveolar pneumocytes and other epithelial cells. These cell types have different morphologies and functions. Ciliated cells are cuboidal cells with a layer of cilia present on the apical side. Basal cells are undifferentiated epithelial cells that can be differentiated into other cell types at the time of tissue injury. Clara cells, goblet cells, serous cells and alveolar epithelial cells secrete various immunoprotective proteins, mucous, phospholipids and surfactant that lines the epithelium. They also contain

Fig. 1— Schematic showing the alveolar pneumocyte type-II in lungs. (A) lungs showing the level of sectioning, (B) section of lungs taken at the dashed line, (C) gross features of the lung section at the level of alveoli and (D) AT-II cell and surfactant metabolism are shown. In B and C, alveolar macrophages, alveolar pneumocyte type-II, fibroblasts, capillaries with endothelial layer and RBCs and alveolar pneumocyte type I. In D, CB=composite body, endo=endocytosis; exo=exocytosis; GB=Golgi bodies; LB=lamellar bodies, mito=mitochondria, MVB=multivesicular bodies, N=nucleus of AT-II cell, TM=tubular myeline, SM=surfactant monolayer.
various channels (sodium channels, potassium channels, chloride channels, aquaporins) that help to regulate the fluid volume lining the pulmonary epithelium\textsuperscript{13}. The distribution of different cell types in the respiratory tract is variable with respect to their functions\textsuperscript{12-17}. For example, more number of the ciliated cells are present in the conducting airways whereas more of basal cells and secretory cells are present in the respiratory airways\textsuperscript{12}.

The alveolar pneumocytes—The epithelial cells covering the alveoli are called alveolar pneumocytes and are of two types viz.; alveolar type I (AT-I) and alveolar type II (AT-II; Fig.1). They can be distinguished morphologically and functionally. AT-I cells are flat squamous cells originating from the basement membrane covering 95\% of the alveolar surface area\textsuperscript{17,18}. They possess fewer cell organelles and are metabolically less active\textsuperscript{19}. On the other hand, AT-II cells are cuboidal cells covering only 5\% of the alveolar surface area\textsuperscript{17,18}. They possess large number of cell organelles and are metabolically active\textsuperscript{19,20}.

The AT-I cells reduce the tissue resistance and allow free diffusion of gases at the alveolar surface\textsuperscript{19}. They can efficiently transport ions, water and other macromolecules in or out of the pulmonary cells, and thus play an important role in maintaining the composition and volume of the pulmonary fluid lining\textsuperscript{20-24}.

AT-II cells are small cells positioned at the corners or thickenings of the alveoli so that their morphology does not hinder the gaseous exchange. These cells are rich in membrane bound organelles known as lamellar bodies which are the site of synthesis, storage and secretion of the pulmonary surfactant\textsuperscript{25,26}. AT-II cells are involved in intracellular as well as extracellular surfactant metabolism (Fig. 1).

AT-II cells are considered as stem cells for AT-I cells\textsuperscript{27}. In addition, they have role in alveolar fluid balance, cellular (epithelial) repair, removal of dead (apoptotic) cells, immuno-regulation and host defense. They communicate with various other cells of the alveoli directly (cell-cell contact) or indirectly (through signaling molecules) and integrate various components of the alveoli functionally\textsuperscript{25}.

Airway surface liquid and alveolar lining fluid—The cells covering the respiratory tract are lined by a film of fluid that forms a barrier between the cells and air. It contains mucous, immuno-protective proteins, phospholipids, surfactant etc. The composition of the airway surface liquid varies with the population of constituent cell types in different regions of the respiratory tract. Airway lining fluid of the upper respiratory tract contains more of mucous and immuno-protective proteins whereas the airway surface liquid lining the alveoli is rich in surfactant\textsuperscript{28,29}. The airway surface liquid present at the alveoli is called alveolar lining fluid and it protects the underlying cells from desiccation, pathogens, tissue damage and facilitates the diffusion of gases. The lining fluid volume is maintained by ion channels and water channels of various cells\textsuperscript{13}. Decreased surfactant with decreased volume of fluid lining results in lung atelactasis (collapse). On the other hand decreased surfactant with increased fluid produces pulmonary edema. Both of them decrease the gaseous exchange resulting in hypoxia and hypoxia-induced stress as seen in various lung disorders.

Pulmonary surfactant
Lungs offer a large surface area that comes directly in contact with air for gaseous exchange into the body fluids. The surface tension at the gaseous-aqueous interphase of lung is reduced by the presence of a pulmonary surfactant. It is a heterogenous mixture of lipids (90\%) and proteins (10\%) that forms a stable monolayer at the gaseous-aqueous interphase.

The presence of surfactant is important to maintain the surface tension at reduced levels to prevent collapse of lung at the end of expiration thus allowing proper exchange of gases. Surfactant increases the lung compliance (volume change to unit pressure change) facilitating proper ventilation. It also maintains the volume of fluid lining the alveoli and size of the alveoli in different phases of respiratory cycle. The lung volume is not constant; it continuously undergoes inflation and deflation during respiration. Accordingly, the surface area exposed to air keeps on changing. The interrelation between the surface tension (T), deflating pressure (P) and radius of the alveoli (r) obeys the Laplace law, $P = 2T/r$; according to which, the surface tension increases with increase in radius of the alveoli and vice versa\textsuperscript{7}. Therefore, the smaller alveoli develop more deflating pressure and may get emptied into the larger alveoli. The presence of surfactant prevents the emptying of smaller alveoli into the larger ones. Surfactant undergoes cyclical changes (with respect to its composition and structural conformation) to maintain low surface tension at the time of inspiration and to prevent the alveolar collapse at the time of expiration\textsuperscript{11}. Further, surfactant assists the elastic
recoil of lungs after each breath during health. In neonates, it is essential for the lung expansion against the hydrostatic forces at the time of first breath. In addition, surfactant also has important role in host defense and other immunological functions.

Pulmonary surfactant and compliance—The compliance of the lung is defined as the change in volume of lungs to unit change in pressure. This indicates the expansibility of the lungs. The lungs are subjected to continuous volume change in response to the pressure difference generated by the respiratory muscles. The pulmonary compliance can be measured by calculating the slope of pressure-volume curve. The main techniques used to measure pressure-volume curves in human subjects are super-syringe method, constant flow method and multiple occlusion method. In laboratory animals, compliance is measured by noting the pressure changes to a known volume of air introduced into lungs through a tracheal catheter. Also the volumes of lungs during deflation are greater than the inflation volumes. This difference is called hysteresis and is due to the higher surface tension present in lungs before inflation. As stated above, the pulmonary surfactant plays an important role in this context as it decreases the resistance against inflation and facilitates the simultaneous changes in volume of lungs with respect to pressure changes in different phases of respiration. Further, the compliance of the lung when actual movement of air takes place is called the dynamic lung compliance whereas the compliance in quiescent phase (when there is no air flow) is called static lung compliance. The dynamic compliance is greater than static compliance.

The pressure-volume curves are important in diagnosing pulmonary disorders (associated with or without surfactant deficiency). The curve shifts to right in adult respiratory distress syndrome (ARDS), interstitial lung diseases, pulmonary fibrosis, after abdominal surgery etc denoting decreased compliance. On the other hand, shifting of the curve to left denotes increased compliance as seen in cases of emphysema and chronic obstructive pulmonary disorders (COPD). The evaluation of pressure-volume curves have been extensively applied for customizing ventilator settings in case of ARDS. However, the methods applied to measure compliance have their own limitations and cannot detect the actual lung volumes, mechanics operating in the chest wall and the respective alveolar behaviour.

Composition of surfactant

The exact composition of the surfactant in vivo is not known but most of the studies are done using the endotracheal lavage fluid and in vitro lung preparations. Based on these studies, it has been demonstrated that surfactant is mainly composed of 90% lipids and 10% proteins. Phospholipids form the bulk of lipids present in the surfactant. Phosphatidylcholine (PC) is the main phospholipid with surface active properties that makes 70-80% of the total lipids. It may be present in unsaturated form (17%) or in saturated form (50%; with dipalmitoyl species). Phosphatidylglycerol (PG) accounts for 7% and is the next most abundant lipid which is important for even spreading of the surfactant monolayer on the surface of alveoli (PC as such has very less spreading properties). Apart from these two, traces of phosphatidylinositol (PI; 2%), phosphatidylethanolamine (PE; 5%), sphingomyeline (Sph; 2%), other phospholipid (PL; 3%) and other neutral lipids (in traces; 5%) constitute the lipid part of the surfactant.

The protein part of surfactant constitutes four types of surfactant-associated proteins viz., SP-A, SP-B, SP-C and SP-D. SP-A is the most abundant protein followed by SP-B, SP-C and SP-D. SP-A is the most abundant protein followed by SP-B, SP-C and SP-D. SP-A and SP-D are hypdophillic proteins whereas SP-B and SP-C are hydrophobic in nature. The hydrophobic proteins play a direct role in structural organization of the surfactant at the interphase whereas the hydrophilic proteins play a regulatory role in surfactant metabolism along with immunological functions. The interaction between lipids and proteins is important for the surface-active properties and homeostasis of the surfactant.
Surfactant lipid biosynthesis

The biosynthetic pathway for the surfactant lipids and the membrane lipids is the same. The surfactant is synthesized mainly in the AT-II cells and few other cells of the respiratory airways whereas membrane lipid biosynthesis as such is common for all cell types. The specific composition of lipids and proteins gives surfactant its characteristic properties. As compared to plasma membrane, saturated PC, PG and PI are present in larger amounts in the surfactant. Therefore, the biosynthesis of lipids constituting the surfactant is basically the biosynthetic pathway for PC, PG and PI biosynthesis from precursors like dihydroyacetone phosphate (DHAP), glyceraldehydes-3 phosphate, phosphatidic acid, choline and some acyl derivatives. The important enzymes involved in the PC biosynthesis are: choline kinase, choline phosphate cytidyl transferase, choline phosphotransferase, acyltransferase and the enzymes of fatty acid synthesis for synthesizing non-lipid precursors.

Regulation of lipid biosynthesis

Enzymatic regulation—The biosynthesis of PC is mediated by sequential actions of different enzymes. The reaction catalysed by phosphocholine cytidyl transferase (CT) is the rate limiting step for PC biosynthesis. Therefore, modulating CT serves as regulatory mechanism for the biosynthesis of PC. CT is an enzyme present as soluble (inactive) and membrane-bound (active) forms. Translocation of CT from soluble to membrane bound form is promoted by decreased PC or more amounts of fatty acids and diacylglycerols (DAG; precursors of PC) in the cell. Some CT binding proteins have also been implicated for the activation of the enzyme. The phosphorylation and dephosphorylation state of the enzyme is another factor for the activation of CT. The soluble form is highly phosphorylated and therefore less active and vice versa. The phosphorylation interferes with the interaction of enzyme with substrates/regulators (lipids) thereby decreasing its activity. Certain kinases and phosphatases might be involved in regulating the enzyme activity within the cell.

Hormonal regulation—The physiological regulation of phosphatidylcholine biosynthesis is primarily hormonal. Various hormones like glucocorticoids, thyroid hormone, estrogen and prolactin have been shown to increase the surfactant lipid biosynthesis. The biosynthesis can be increased by two mechanisms: either by increasing enzyme activity or by increasing enzyme mass. The hormones act via intracellular signaling molecules that specifically bind to certain enzymes and increase their activity or increase the amount of enzyme synthesis from mRNA/gene. The hormones can also modulate the cellular metabolism to increase the availability of precursors like DAG and other lipids for enzyme (increasing the substrate availability and enzyme activity indirectly). Therefore, hormonal regulation of surfactant biosynthesis assumes paramount importance for the development and maturation of lung.

Glucocorticoids: Glucocorticoids predominantly increase the enzymatic activity (not enzyme mass) to increase surfactant lipid synthesis. Glucocorticoids stimulate fatty acid biosynthesis resulting in increased availability of precursors that act as substrates for the enzymes involved in PC synthesis. This results in increased incorporation of precursors into surfactant lipids. Supporting this, the increased production of cortisol in 30-32 weeks of gestation has been associated with the fetal lung maturation and surfactant production.

Estrogen: The effect of estrogen is similar to glucocorticoids. It increases surfactant biosynthesis by increasing the activity of enzymes like phosphocholine cytidyltransferase and lysolecithinacyl transferase involved in PC biosynthesis. In vitro studies have shown that estrogen increases choline incorporation into PC. The increase in level of estrogen during pregnancy has been linked with the fetal lung maturation and surfactant production.

Prolactin: Prolactin is an anabolic hormone that is important for the fetal growth. Some studies correlate the increased prolactin levels in the last phase of pregnancy and lung maturation. They state that prolactin increases phospholipid content of the surfactant specifically PC and PG. However, some other studies report that there is no effect of prolactin on surfactant biosynthesis and lung maturation. Further studies are required to establish the relationship between prolactin and surfactant biosynthesis.

Thyroid hormone: Thyroid hormones increases surfactant production and number of lamellar bodies in the AT-II cells. Thyroxine has no effect on the activities of enzymes involved in PC biosynthesis. Therefore, thyroxine is thought to accelerate the translocation of lipids into the lamellar bodies and
increases the incorporation of precursors into PC and helps fetal lung maturation. Respiratory distress syndrome is shown to be associated with decreased thyroid hormone levels or its receptor abnormalities.

Insulin: Insulin has a dual role in surfactant biosynthesis. In vitro experiments have shown that very low doses of insulin increase surfactant biosynthesis whereas high doses inhibit surfactant biosynthesis, the later effect being pronounced in case of developing fetus. The fetuses of diabetic mothers experience hyperglycemia, hyperinsulinaemia and hyperlipidemia. All of these result in altered surfactant lipid metabolism. Such fetuses are prone to develop lung disorders like respiratory distress syndrome. An interesting interrelationship between respiratory distress syndrome (RDS; lung injury) and diabetes has been reported. Diabetes is associated with hyperglycemia, hyperglycemia-induced oxidative stress and release of various inflammatory mediators. These factors can exacerbate or ameliorate surfactant metabolism leading to RDS. However, insulin administration postnatally or in adults with surfactant deficiency improves the surfactant synthesis by improving the energy metabolism.

Surfactant proteins

Hydrophillic proteins—SP-A and SP-D are the two hydrophilic proteins present in the surfactant. They are large glycosylated proteins belonging to collectin family, characterized by the presence of collagen like domain and carbohydrate binding properties. These proteins are found in the multivesicular bodies of the AT-II cells. They regulate the surfactant secretion and reuptake by the AT-II cells. These proteins also play important role in host defense. They bind to specific sites (carbohydrate moieties) on the foreign pathogens and stimulate immunological reactions like opsonisation and phagocytosis.

SP-A: SP-A is the first surfactant protein to be purified and has been extensively studied. It is a large glycosylated protein present in a number of isoforms with molecular weight ranging from 28-36 kDa (depending upon the degree to which the isoforms are glycosylated). SP-A is a multimer of six collagen like helices with 18 polypeptide chains. It is localised at the corners of tubular myeline (an intermediate lattice structure of surfactant). It binds to phospholipids in a calcium-dependent manner and helps in formation of surfactant structure (tubular myeline) at the air-water interphase. It also provides feedback inhibition to AT-II cells for surfactant secretion. It is involved in signaling recycling and clearance of surfactant. In addition it has immunoprotective functions because of its structural similarity with C1q of the classical complement system. It also protects the surfactant from denaturation by plasma proteins.

SP-A is synthesized in AT-II cells and Clara cells from two genes called SP-A1 and SP-A2 present on chromosome 10 of humans. The expression of these genes is developmentally regulated corresponding to the phospholipid biosynthesis. After translation, the protein is targeted to the endoplasmic reticulum by means of a signal peptide at the amino terminal of the protein. In the endoplasmic reticulum the protein undergoes post-translational modifications and is directed to the Golgi bodies where it gets glycosylated. Then it is translocated to the lamellar bodies or to low density multivesicular bodies.

cAMP has been shown to increase the SP-A gene expression and promote lung maturation. In addition, various growth factors, prostaglandins, β-adrenergic agonists and oxygen also increase SP-A gene expression. On the other hand, insulin, TNF-α and transforming growth factor-β decrease SP-A gene expression. Interestingly, glucocorticoids have a dual role in mediating the expression of SP-A gene. They can stimulate or inhibit the SP-A gene transcription and translation depending upon the dose and developmental stage. At higher dose, glucocorticoids inhibit SP-A expression.

SP-D: SP-D is another member of collectin family with molecular weight of 43 kDa. It is a trimer with collagen like C-terminal and lectin like N-terminal. Its role solely as a surfactant protein is conflicting since similar protein has also been found in the gastric tissue. It specifically binds to PI in a calcium-dependent manner. It is encoded by a gene present at the adjacent locus to the SP-A gene on chromosome 10. It is synthesized in AT-II cells as well as Clara cells in a developmentally regulated manner. Some of the surfactant associated proteins and phospholipids are thought to stimulate the expression of SP-D gene and therefore, it is expressed in the late phase of gestation. It is involved in activation of alveolar macrophages and protection against pathogens.
Hydrophobic proteins—SP-B and SP-C are the two hydrophobic proteins present in the surfactant. They are synthesized by proteolytic cleavage of a precursor molecule and specific post translational modifications. They are found aggregated along with the phospholipids in the lamellar bodies and are important for the surface active properties of the phospholipids. These proteins help in the stabilization of tubular myelin and adsorption of lipids onto the surfactant monolayer.

SP-B: SP-B is a dimeric protein with molecular weight of 8 kDa. It has three intrachain disulfide bridges and is glycosylated at asparagine residues at the C-terminal. The gene coding SP-B is present on chromosome 2 of humans. SP-B is expressed in AT-II cells and some bronchiolar epithelial cells. The translated product of the SP-B gene undergoes specific proteolytic cleavage and glycosylation in the Golgi bodies and multivesicular bodies to form the functionally mature SP-B. The mature SP-B protein is transported to the lamellar bodies along with various other phospholipids. It is important for the aggregation of lipids into the lamellar body and facilitation of formation of surfactant monolayer at the interphase. In contrast to SP-A, cAMP has moderate effect on SP-B gene expression and glucocorticoids stimulate SP-B gene expression in a dose-dependent manner.

SP-C: SP-C is a small protein with molecular weight of 4 kDa. SP-C is coded by two genes present on chromosome 8 of humans. The expression of SP-C coding gene is specific to AT-II cells. Similar to SP-B, SP-C is also translated into a pre-SP-C protein that undergoes post translational modifications like cleavage of specific carboxy and amino terminal residues and palmitoylation in the terminal sacs of Golgi bodies. Further, modifications take place in the lamellar bodies to form a mature SP-C. cAMP has moderate effect on SP-C gene expression and glucocorticoids stimulate SP-C gene expression in a dose-dependent manner similar to SP-B.

Packaging of surfactant: Lamellar bodies

Alveolar type II cells possess characteristic lamellar bodies involved in surfactant metabolism. Lamellar bodies are lysosome like membrane bound organelles. Previously, lamellar bodies were considered as the site of surfactant synthesis. However, studies have shown that the actual surfactant synthesizing enzymes are not present in the lamellar bodies. Therefore, it can be expected that different components of the surfactant (proteins and lipids) are synthesized by cellular organelles like the endoplasmic reticulum and Golgi apparatus from where the components are packed into small vesicles (multivesicular bodies) and are transported to the pre-lamellar or composite body by various transporter proteins. In the pre-lamellar bodies different components of the surfactant get assembled to form the lamellar bodies. The lamellar bodies undergo exocytosis form AT-II cells to secrete the surfactant components into the extracellular matrix. The surfactant components get recycled in AT-II cells periodically. The details of the surfactant metabolism are given in Fig. 3.

Surfactant secretion

Various physical and chemical stimuli can induce surfactant secretion. The physical stimuli include mechanical stretch or contraction of the AT-II cells during breathing. The chemical agents include catecholamines (β-adrenocceptor agonists), purinoceptor agonists, cAMP, histamine, vasopressin, and some calcium ionophores. The surfactant lipids and proteins are secreted in different manner. The surfactant lipid secretion has been extensively studied but little is known about surfactant protein secretion. Upon stimulation the lamellar bodies are translocated to the apical side of the AT-II cell by cytoskeletal proteins (actin and microtubules) where they undergo exocytosis to release their contents into the extracellular matrix. Though the underlying mechanism is not exactly delineated, the stimuli directly or indirectly increase the intracellular cAMP and calcium levels that in turn activates protein kinases like PKA, PKC and CaMK. Phosphorylation of cellular proteins by various protein kinases is a crucial step for the surfactant lipid secretion.

The hydrophobic surfactant proteins (SP-B and SP-C localized within the lamellar bodies) are secreted in lamellar body-dependent manner along with the lipids while the hydrophilic proteins (SP-A and SP-D) are secreted in lamellar body-independent manner. These surfactant proteins help in the intracellular vesicular trafficking, fusion of lamellar body with cell membrane and exocytosis. The secreted form of surfactant as such is non-functional but after secretion it undergoes structural/conformational changes to give rise to an intermediary lattice structure known as tubular myeline. The exact structure of the tubular myeline...
has not been characterized; however it is known that the presence of surfactant associated proteins, phospholipids and calcium are important for the structural organization of tubular myeline. The tubular myeline then forms the surfactant monolayer by adsorbing the lipid components into the air-water interphase. The surfactant associated proteins also help in adsorption of the lipid moieties into the monolayer and stabilization of the monolayer. This monolayer is the functionally active form of surfactant and has the surface-active properties. Deficiency in surfactant associated proteins results in the aggregation of surfactant components and improper formation of tubular myelines thus impairs the surface-active properties of the surfactant.

**Surfactant recycling**

After secretion and incorporation into monolayer, surfactant undergoes dissociation into its components. Most of the components are recycled back through receptor mediated endocytosis into the alveolar cell while some components are cleared by the airway lining and alveolar macrophages. Recycled components are present in the apical side of AT-II cell in the form of light and dense multivesicular bodies which are nothing but early endosomes. These can fuse with the pre-lamellar bodies or may get degraded within the cell. The recycling helps to maintain the surfactant pool at the alveolar level and conserves energy required for synthesizing the components again. The signaling mechanism and details of recycling are not known but SP-A is considered important for signaling the uptake of surfactant (SP-A mediated endocytosis).

**Effect of physical parameters on the surfactant metabolism**

The interaction between the lipids and proteins is very critical for the functioning of surfactant. Environmental factors like temperature, pressure and...
hypoxia affect the physical state of the surfactant components, their structural stability and functioning directly or indirectly. Depending upon the temperature and hydrostatic pressure, composition of surfactant lipids and proteins is altered so as to maintain the optimal fluidity/rigidity of the surfactant film\(^{108}\). For example, more of unsaturated phospholipids and cholesterol are synthesized so as to retain surfactant fluidity at lower body temperatures and more of short chain fatty acids and surfactant associated proteins SP-B and SP-C are produced to increase the spreadability of surfactant at greater hydrostatic pressure (as seen in diving mammals)\(^{101,102}\).

Hypoxia is another physical factor that is important for lung development in fetus and lung functioning in adults. Hypoxia induces the expression of a transcription factor called hypoxia-inducible factor 2α, that is crucial for fetal lung (AT-II cells) development and production of phospholipids\(^{103}\). Increased sympathetic activity induced by hypoxia (via peripheral chemoreceptors) also increases the surfactant lipid biosynthesis\(^{44,46,104}\). Hypoxia triggers lung expansion and maturation of lungs at the time of birth. However, prolonged fetal hypoxia associated with maternal anemia, hypertension and placental infarction causes fetal growth retardation leading to impaired lung development\(^{100}\). Acute hypoxia as experienced at high altitude in adults alters the surfactant system. Hypoxia is associated with hemodynamic alterations, pulmonary hypertension and pulmonary edema. Edematous fluid is rich in plasma proteins and proteolytic/lipolytic enzymes that degrade the surfactant\(^{105,106}\). Since the surfactant lipid and proteins are altered, (due to lipolysis and proteolysis) the surfactant components form aggregates at the alveolar surface and fail to organize into stable monolayer resulting in surfactant insufficiency.

**Effect of inhaled toxicants on surfactant metabolism**

Toxicants like ozone, nitrogen dioxide, sulphur dioxide, chemical exhausts and dust damage the lung tissues and cause alterations in the surfactant system directly or indirectly. The damage to lung tissue and inflammation are the main outcome of exposure to these inhalants lead to metabolic alterations in the surfactant system\(^{107,44}\).

**Ozone**—In the biological systems, ozone induces excessive oxidative damage to the tissues. Surfactant alteration can be considered a consequence of tissue damage and inflammation induced by exposure to ozone. Ozone alters fatty acid composition of the surfactant phospholipids and decreases surfactant secretion\(^{108,109}\). It also causes ultrastructural alterations in the lamellar bodies and prevents the structural organization of lamellar body contents into tubular myeline\(^{110,111}\). Further, it impairs the activity of SP-A that is important for the formation of surfactant monolayer\(^{112}\).

**Nitrogen dioxide (NO\(_2\))**—Nitrogen dioxide is an air pollutant that can induce oxidative damage in biological systems similar to ozone. It is released from automobile exhausts can cause damage to the lung tissues. Nitrogen dioxide causes lipid auto-oxidation and alters the lipid composition of the surfactant\(^{113}\). Acute exposure of AT-II cells to nitrogen dioxide increases lipid synthesis and results in lipid accumulation whereas chronic exposure decreases the ability of the cells to synthesize lipids\(^{107}\).

**Sulphur dioxide (SO\(_2\))**—It is another common of air pollutant present in automobile exhausts. Sulphur dioxide interacts with water present in the fluid linings of the respiratory tract, eyes, throat etc and readily forms sulphuric acid which damages the epithelial layers of the respective tissues. It alters the structure and functions of AT-II cells and decreases the synthesis of pulmonary surfactant\(^{114}\). Further, it drastically affects the physico-chemical properties of the surfactant\(^{115}\).

**Aerosols**—Lacrimators, tear gases and other chemicals dispersed in aerosols can produce respiratory tract irritation. Commonly used lacrimators are 1-chloroacetophenone (CN), 2-chlorobenzylidene malononitrile (CS) and dibenz [b,f]-1,4-oxazepine (CR). Amongst these, CN is the most toxic lacrimator followed by CS and CR\(^{116}\). Exposure to these gases causes inflammation, damage to the alveolar capillary membrane, pulmonary edema and destruction of the pulmonary surfactant\(^{115}\). CN has been shown to decrease the total phospholipid and sphingomyelne content in rat lungs\(^{117}\). On the other hand, exposure to CR, decreases the phosphatidylycholine and ethanolamine synthesis and increases sphingomyelne synthesis leading to altered lipid constitution of the surfactant\(^{117}\).

Inhalation of detergent aerosols interferes with the surfactant activity at the air-water interphase. These detergents increase the surface tension and produces pulmonary edema by increasing the vascular permeability\(^{118,119}\).
Phospholipid and SP-A and SP-D content in the lung are harmful for the lungs. Silica increases the surfactant contents are produced in excess but fail to aggregate properly. Asbestos inhalation also leads to alveolar proteinosis like situation where AT-II cells injury altering the surfactant system.

Artificial ventilation—Artificial ventilation used for treatment in critical cases of ARDS or other diseases can be very harmful. Artificial ventilation decreases lung compliance and alters the surfactant homeostasis leading to lung dysfunction. Excess of oxygen induces the formation of free radicals that can damage various tissues and cellular constituents. In addition, there is alteration in the lipid content of the surfactant and therefore it fails to aggregate properly. The release of inflammatory mediators like TNFα, IL-6, IL-10 after hyperoxia might be interfering with the signaling pathways of surfactant packaging and secretion.

Hydrogen sulphide—It is a potent pulmonary irritant that lowers the surface-active properties of the surfactant. Its effect on surfactant mediated indirectly by inducing tissue injury, pulmonary edema and the release of surfactant denaturants like plasma proteins.

Smoke—Cigarette smoke or polyurethane smoke reduces the phospholipid and protein (SP-A and SP-D) content of the surfactant. Cigarette smoke especially decreases the phospholipid/protein ratio of the broncho-alveolar lavage fluid (BAL) obtained from humans. In addition, the surface-active properties of surfactant (monolayer) are decreased due to altered composition and interference by smoke particles. Long time exposure may cause tissue (AT-II cells) injury altering the surfactant system.

Dust—Inhalation of silica, cadmium or quartz dust is harmful for the lungs. Silica increases the surfactant phospholipid and SP-A and SP-D content in the BAL. Thus, dust stimulates surfactant production and leads to alveolar proteinosis like situation where the surfactant contents are produced in excess but fail to aggregate properly. Asbestos inhalation also leads to similar changes. Cadmium chloride inhibits surfactant secretion in AT-II cells thereby increasing the intracellular PC content.

Effect of inflammatory mediators and intracellular signaling molecules on surfactant metabolism

TNF-α, NO, IL-1, Interferon-γ and other inflammatory molecules play critical role in surfactant metabolism. They can increase or decrease the surfactant biosynthesis. TNF-α released due to edema, infection, oxidative damage, or any type of tissue injury cause alteration in the surfactant homeostasis. TNF-α decreases surfactant PC synthesis, increases PC turnover and alters the overall lipid content of the surfactant. It also down regulates the expression of surfactant associated proteins. Other cytokines like transforming growth factor, interferon-γ and IL-1 present in the amniotic fluid stimulate the production of surfactant (lipids as well as proteins) and helps fetal lung maturation.

Nitric oxide (NO) present at physiological concentration promotes surfactant secretion by increasing intracellular cGMP levels and activation of protein kinases. However, excessive production of NO due to inflammation inhibits the surfactant secretion.

Endothelin-1 increases the surfactant secretion by increasing intracellular calcium levels and activating PKC. Prostaglandins increase the surfactant biosynthesis and secretion. Increase in prostaglandins with gestational age has been considered important for fetal lung development and maturation. The prostaglandin levels also increase at the time of labour that is considered to facilitate surfactant release and breathing of the new born. In adult lungs, porstaglandins like PGE2 and PGF2α promote PC biosynthesis. PGE2 also promotes SP-A biosynthesis.

cAMP is an important cellular messenger involved in the surfactant metabolism. cAMP increases surfactant lipid biosynthesis by increasing the rate of incorporation of the precursor into phospholipids. Increased intracellular cAMP levels inhibit glycogen synthase providing more precursors for surfactant lipid synthesis. cAMP also increases the expression of the genes coding for surfactant proteins. In vitro experiments have shown that adenylate cyclase activators (aminophylline, forskolin), phosphodiesterase inhibitors (caffeine) and others that increase the intracellular cAMP levels promote surfactant synthesis. Moreover, cAMP is involved in the signaling pathway for surfactant secretion. Glucocorticoids stimulate the surfactant synthesis and secretion by means of cAMP. Further, β-adrenoceptor agonists, purinoceptor agonists, catecholamines, histamine and other pharmacological agents increase surfactant secretion by increasing intracellular cAMP levels that activates the downstream cascade of surfactant secretory pathway.
Clinical importance

The decrease in surfactant level/function is associated with a number of diseases like infant respiratory distress syndrome (IRDS), adult respiratory distress syndrome (ARDS), lung proteinosis, obstructive lung diseases, interstitial lung diseases and chronic lung disease. Number of direct or indirect mechanisms may be involved in the impairment of surfactant biosynthesis and functioning which can be broadly categorized into genetic, metabolic and inflammatory mechanisms.

Genetic factors include mutations in genes coding for surfactant proteins, transcription factors involved in surfactant protein biosynthesis and proteins involved in transport/translocation of surfactant components altering the surfactant homeostasis. Mutations in genes coding for SP-A, SP-D, SP-B, SP-C and surfactant transporting proteins (coded by ABCA3, TERT gene) are associated with respiratory distress syndrome and interstitial lung diseases of infants. Further, mutations in genes coding for SP-A and SP-D proteins are associated with immunological deficiencies and may lead to the development of respiratory infections.

Metabolic alterations include decreased secretion of surfactant, increased reuptake of surfactant, proteolytic/lipolytic cleavage of surfactant components or oxidative damage to the surfactant components. These factors can be considered secondary to the genetic abnormalities. In addition, pathophysiological mechanisms of other diseases and release of inflammatory mediators can affect the surfactant metabolism.

Pathophysiology of diseases associated with surfactant insufficiency

Respiratory distress syndrome is associated with surfactant deficiency. It is characterized by increased work of breathing, impaired gas exchange, decreased compliance, atelectasis, hypoxia, interstitial edema, pulmonary hypertension, hemodynamic alterations and other associated complications. It is very common in infants, where it is called infant respiratory distress syndrome (IRDS). In adults, it is called adult respiratory distress syndrome (ARDS) and can occur due to multiple factors leading to lung injury, dysfunction and decreased surfactant synthesis. In other diseases like obstructive lung diseases, chronic lung diseases and interstitial lung diseases, the surfactant deficiency is a secondary to inflammatory or metabolic abnormalities.

Infant respiratory distress syndrome (IRDS) — Infant respiratory distress syndrome is common in pre-mature babies. IRDS is characterized by the immature lungs and failure to produce sufficient amount of surfactant. Genetic or acquired factors are implicated for the development of IRDS. Genetic factors include the mutations in genes coding for the surfactant associated proteins and various other transporter proteins involved in surfactant packaging. Non-genetic factors include the gestational age, lung maturity, maternal health, hormones like glucocorticoids, estrogen, thyroxine, prolatin and cathecholamines that directly or indirectly affect the surfactant system.

In IRDS, the surfactant components are normal but they fail to form tubular myeline. This may be due to the deficiency of surfactant lipids and proteins that are essential for the adsorption into the functional monolayer. The BAL obtained from IRDS patients has reduced amounts of phospholipids and SP-A. In addition, mutations in genes coding for SP-A, SP-B, SP-C and lipid transporter protein (ABC3) might be associated with development of IRDS.

Adult respiratory distress syndrome (ARDS) — ARDS is a complex disorder which is characterized by surfactant deficiency. The major pathophysiological mechanism leading to surfactant deficiency is the accumulation of fluid in the alveolar space and/or decreased pulmonary fluid clearance resulting in pulmonary edema. The pulmonary edema is rich in plasma proteins like fibrinogen, fibrin monomers and proteolytic enzymes that degrade the surfactant proteins. In addition, the tissue damage and release of inflammatory mediators further damage surfactant lipids and proteins as discussed earlier. As a result the composition and functional properties of the surfactant are altered.

Phospholipid content of surfactant is decreased in case of ARDS. One of the reasons is the increased phospholipase A2 activity that causes hydrolysis of the surfactant phospholipids. There is decreased content of SP-A, in the BAL from ARDS patients. This may be due to proteolytic degradation of SP-A.

Obstructive lung diseases — Various obstructive lung diseases like chronic obstructive pulmonary disease (COPD), asthma and bronchiolitis are associated with decreased surfactant function. Obstructive lung diseases are characterized by airway inflammation, activation of alveolar macrophages and
massive release of various proteins, proteolytic enzymes, inflammatory mediators, reactive oxygen species and reactive nitrogen species. All these chemical agents decrease surfactant availability at the interphase by decreasing its synthesis or functional capacity. The content of phospholipids especially phosphatidyl choline, SP-A and SP-D are decreased in the BAL of subjects COPD. 

Pulmonary alveolar proteinosis—It is an idiopathic disease characterized by accumulation of the surfactant contents in the alveoli. There is increased amount of surfactant but there is impaired formation of tubular myeline. The content of SP-A, SP-B and SP-C are increased in BAL. There is alteration in the constituent lipids and therefore they fail to aggregate properly. 

Interstitial lung diseases—Sarcoidosis, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis are some of the interstitial lung diseases associated with decreased surfactant. They are characterized by respiratory distress, pulmonary infiltration and abnormal functioning of lungs. The total phospholipid content decreases in case of idiopathic pulmonary fibrosis whereas total surfactant pool is decreased in case of sarcoidosis. The changes in surfactant associated protein levels are reported to be variable in these conditions. Mutations in SP-B, SP-C and ABC3 genes are associated with the development of interstitial lung diseases.

Chronic lung diseases—Diseases like cystic fibrosis, pneumonia and HIV are associated with decreased surfactant levels. These diseases are characterized by lung infection, endobronchial inflammation, increased infiltration in the lungs and decreased immunity, respectively. The surfactant dysfunction is secondary outcome of these pathophysiological mechanisms. There is decreased synthesis of PC and other phospholipids. Inflammation induces the lipolysis of surfactant altering the fatty acid composition. The amounts of SP-A and SP-D are markedly reduced.

Therapeutic agents used in surfactant deficiency states

The therapeutic strategies used for decreased surfactant or surfactant function can be divided into exogenous and endogenous agents. The endogenous agents include hormones and other pharmacological substances that increase surfactant synthesis or secretion in the lungs whereas the exogenous agents include direct administration of surfactant or surfactant proteins.

Endogenous agents—Hormones like glucocorticoids, thyroxine and estrogen can increase the biosynthesis of surfactant. Glucocorticoids have been used in combination with other agents like salbutamol, purinoceptor agonists and calcium ionophores that increase the surfactant secretion. Maternal administration of these agents effectively increase surfactant synthesis and can be used antenatally in suspected cases of pre-term deliveries. However, these therapies are associated with growth abnormalities in the later life of the infants.

Insulin for RDS: Insulin administration has been beneficial in treating critical cases of respiratory distress syndrome (RDS). Insulin helps to regulate the energy metabolism thereby reducing the oxidative stress and tissue damage. The effect of insulin has been considered to be immunomodulatory in nature (independent of glucose homeostasis). Some studies have shown that other treatments used in diabetes are also useful in preventing mortality in ARDS patients. However, further studies are required to establish the usage of insulin in lung injury.

Exogenous agents—Administration of surfactant or its components has been successfully used in case of respiratory distress syndrome. Natural or synthetic surfactants are available with variable constitution and composition (shown in Table 1). Surfactant supplement prevents lung atelactasis, improves the ventilation and lung tissue maturation.

This is to note that, the pulmonary surfactant is known to have a detergent-like action in decreasing the surface tension at the interphase of two media. However, its composition (protein and lipid) as well as functions are very specific for maintaining the structural and functional integrity of lungs. Detergents on the other hand, are a mixture of organic compounds and strong alkalies that damage the biological membranes. Therefore, detergents cannot be used as replacement for the pulmonary surfactant.

**Table 1—Different types of surfactant preparations.** (This table has been taken and modified from Stevens and Sinkin.)

<table>
<thead>
<tr>
<th>Animal surfactants</th>
<th>Synthetic surfactants</th>
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<tbody>
<tr>
<td>Lung lavage: Alveofact, BLES, Infasurf</td>
<td>Protein-free: ALEC, Exosurf</td>
</tr>
<tr>
<td>Supplemented, processed animal lung tissue: Curosurf, Surfacten, Survanta</td>
<td>Peptide-containing: Surfaxin, Recombinant SP-C surfactant</td>
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lungs. They are organic surfactant extracts containing surfactant lipids with surfactant proteins SP-B and SP-C\textsuperscript{107}. Some of the commercially available natural surfactants are survanta, infasulf, alveofact and curosurf\textsuperscript{171}. They can be administered by nebulisation or instillation methods. In addition, some surfactants isolated from amniotic fluid are also available. The drawback with these surfactants is the availability and expensiveness besides their immunogenic property\textsuperscript{11,107}.

**Synthetic surfactants:** Synthetic surfactants are protein free mixtures of phospholipids like phosphatidylcholine and phosphatidyl glycerol in a definite proportion. Exosurf and ALEC are two examples of synthetic surfactants\textsuperscript{171}. However, these surfactants have been of moderate benefit in curing ARDS. At present, synthetic surfactants with definite composition of phospholipids and human SP-B, SP-C recombinant proteins have been developed that resemble natural surfactant eg. KL-4 and venticute\textsuperscript{107,171,172}. Surfactant treatment with a combination of hormones and pharmacological agents can be formulated for efficient treatment of complicated RDS.

**Summary and conclusion**

Pulmonary surfactant, a mixture of lipids and proteins, is very important for proper lung function. It decreases surface tension at the alveolar interphase, prevents collapse of alveoli during expiration and prevents the emptying of smaller alveoli into the larger ones. The maintenance of lung compliance and ventilatory capacity is also a function of the pulmonary surfactant. In addition, it plays an important role in host defense and other immunoprotective functions. The surfactant is synthesized and secreted from specific cuboidal cells present in the alveolar epithelium called type II pneumocytes (AT-II). AT-II cells contain membrane bound organelles called lamellar bodies. The components of the surfactant are synthesized separately and are packed into the lamellar bodies which are mainly involved in surfactant metabolism. The synthesis, secretion and recycling of surfactant is highly regulated. Though most of the underlying mechanisms are known, the specific signaling and regulatory pathways are yet to be identified. Any genetic or metabolic abnormalities in surfactant homeostasis can result in surfactant deficiency. Deficiency of surfactant is seen in diseases like respiratory distress syndrome, chronic lung diseases, interstitial lung diseases etc. A combination of surfactant (natural or synthetic) and hormones or other pharmacological agents associated with surfactant homeostasis are useful in treating these diseases. However, the exact formulation of combinational therapy is yet to be done. The detailed knowledge about surfactant with respect to the pathophysiological alterations in various lung diseases would be helpful in designing novel surfactant supplements.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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