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Pulmonary Surfactant Nanostructures and their Implications

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ABSTRACT

A surface active material that lines our lungs is referred to as pulmonary surfactant (PS) and consists of a self-assembled complex of nanostructures (NSs) rich in phospholipids (PLs) and proteins that lie at the air-liquid interface of the pulmonary alveoli. It serves a very critical function during respiration by dynamically modifying surface tension. It is responsible for the attainment of near zero surface tension at the end of expiration. PLs are assisted by surfactant proteins to form lamellar bodies of 500 nm to 1-2 μm in size, nanotubes of 2-5 nm height, monolayer films of domain heights of 0.8 to 5 nm and multilayered stacked reservoir phases during the surfactant life cycle. During respiration, the PL molecules present in the surfactant film undergo molecular rearrangement to alter surface tension and maintain high lung compliance. Destruction or absence of PS and/or the above mentioned NSs can occur due to genetic variations, direct or indirect lung injuries and lead to many respiratory diseases. Surfactant NSs, their composition and the packing of the surfactant monolayer are altered in diseased states. Transmission electron microscopy and atomic force microscopy are useful techniques to evaluate pulmonary NSs and confirm their alterations in diseased states. Drug-loaded nanoparticles (NPs), when delivered in the respiratory system, first interact with pulmonary surfactant. These interactions can alter drug release, residence time and cellular interactions of the NPs. Similarly, pulmonary surfactant also influences the cellular response and toxicity of respirable environmental fine particles. This review describes the various nanostructures formed by PS, the interactions NPs with PS and their implications.

Keywords: drug delivery, lamellar bodies, monolayer, nanostructures, pulmonary surfactant, tubular myelin

Abbreviations: **ABCA3**, ATP-binding cassette transporter A3; **AE2**, alveolar type II; **AFM**, atomic force microscopy; **CF**, cord factor; **DMPC**, dimyristoyl phosphatidylcholine; **DPPG**, dipalmitoyl phosphatidylglycerol; **DPPC**, dipalmitoyl phosphatidylcholine; **LA**, large aggregate; **LB**, lamellar body; **LC**, liquid condensed; **LE**, liquid expanded; **LE-LC**, liquid expanded-liquid condensed; **LPS**, lipopoly-saccharide; **MA**, mycolic acid; **NP**, nanoparticle; **NS**, nanostructure; **PAMAM**, poly-amidoamine; **PC**, phosphatidyl choline; **PE**, phosphatidylethanolamine; **PG**, phosphatidylglycerol; **PI**, phosphatidylinositol; **PLGA**, polylactic-co-glycolic acid; **PL**, phospholipid; **POPC**, palmitoyloleoyl phosphatidylcholine; **POPG**, palmitoyloleoyl phosphatidylglycerol; **PS**, pulmonary surfactant; **SA**, small aggregate; **SP**, surfactant protein; **TEM**, transmission electron microscopy; **TM**, tubular myelin

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INTRODUCTION

The alveolar epithelium in the lungs is covered with a liquid lining (Goerke 1974). This alveolar lining basically consists of an aqueous subphase covered by a film of pulmonary surfactant (PS). PS is a complex mixture of lipids (~90%) and proteins (~10%) secreted by the alveolar epithelial type II (AE2) cells into the alveolar space (Rooney *et al.* 1994; Frick *et al* 2001; Andreeva *et al.* 2007). PS forms many lipid–protein nanostructures (NSs) like the lamellar bodies

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Invited Review

Table 1 Functions of PS.

Surface tension related functions
Reduce work of breathing (Bakshi *et al*. 2008)
Near zero surface tension at expiration (Kaviratna and Banerjee 2009)
Maintain large surface area for gas exchange (Schurch *et al*. 1992)
Atelectasis prevention (Hamvas and Patterson 2002)
Maintain airway patency (Enhorning 2001)
Anti-oedematous factor (Veldhuizen *et al*. 1998)
Maintain pulmonary compliance (Bakshi *et al*. 2008)
Non surface tension related functions
First line of defence against inhaled pathogens (Wright 2003)
Immuno modulating action against invading pathogens (Wright 2003)

(LBs) (Ogaswara *et al.* 2009), tubular myelin (TM) and a phospholipid (PL)-rich film of single to few molecule thickness during its life cycle. These NSs are formed at different stages as a result of interaction between various surfactant proteins (SPs A, B, C and D) and the PLs of PS (Dietl and Haller 2005). Each nanostructure plays a crucial role in the surface tension modulation process during the breathing cycle. These NSs show interesting dynamic polymorphism ranging from formation of onion peel-like concentric condensed bilayer PL rings in the case of LBs (Schmitz and Muller 1991; Weaver *et al.* 2002; Ridsdale and Post 2004) to formation of highly ordered square-shaped, mesh-like interconnected tubular networks in TM and a mono- or multilayered film at the air-liquid interface which undergoes changes in composition and arrangement with changes in film area during respiration (Larsson *et al*. 2003).

The major function of the PS film is to dynamically modulate surface tension at the alveolar interface from 25 to 30 mN/m during inspiration to < 1 mN/m on expiration (Zuo *et al.* 2008a; Rugonyi *et al.* 2008). Lungs are a huge collection of interconnected alveoli of varying sizes. These hollow sphere-like alveoli, inspite of varying dimensions, are maintained at equal pressure by maintaining different surface tensions at the interface by PS thereby preventing collapse of the alveolar network (Hill 1999). Dynamic modulation of surface tension by PS film at alveolar interface reduces work of breathing resulting in improved respiratory compliance (Reifenrath 1975). Also by lowering the surface tension, PS reduces elastic recoil of the alveoli preventing alveolar collapse. An effective PS also helps in maintaining pulmonary vascular perfusion oxygenation by preventing narrowing of alveolar capillaries present in vicinity of alveolar epithelium (Iekagami *et al.* 2009). It also serves as an anti-oedema factor preventing fluids from flooding into the alveoli (Vazquez de Anda and Lachmann 2001). Apart from being present in alveoli, PS lines the terminal bronchioles and help in maintaining airway patency (Ellyett *et al.* 2006), reducing resistance to air flow in the terminal airways (Macklem *et al*. 1970; Sanderson *et al*. 1976; Enhorning *et al.* 1995; Enhorning 2001). Further, PS provides the first line of defense against respiratory infections (Fehrenbach 2001). The functions of PS are summarized in **Table 1**. This review focuses on the various surfactant NSs, their functional implications and their interactions with drug loaded and environmental nanoparticles (NPs).

PULMONARY SURFACTANT: COMPOSITION

PS comprises of approximately 85-90% PLs, 8-10% proteins and 5% neutral lipids (Veldhuizen *et al.* 1998). Four surfactant-specific proteins viz. SP-A, SP-B, SP-C and SP-D have been found in the PS system of which SP-A and SP-D are large, hydrophilic proteins and SP-B and SP-C are small hydrophobic proteins (Haagsman and Diemel 2001). A brief description and function of these surfactant proteins is discussed in a latter section. PL composition varies in various species (Postle *et al.* 2001; Schiller *et al.* 2001; Bernhard *et al.* 2004) indicating various adaptation measures taken by various species during the course of evolution. The dipalmitoyl phosphatidylcholine (DPPC) content of PS has been reported to vary from 36.5 to 56% in mammalian species (mouse and humans, respectively) (Postle *et al.* 2001). In comparison to the PC content there is more pronounced variation in terms of the amount of different molecular species in phosphatidylglycerol (PG) and phosphatidylinositol (PI) group. The PG fraction of PS in rat surfactant contains 26.7% PG 16:0/18:1, 16.9 PG 16:0/16:0, 11.2% PG 16:0/20:4 and 10.6% PG 16:0/22:6 (Schlame *et al.* 1986) whereas PG 16:0/16:0 (34.7%) is highest amongst the PG group in rabbit surfactant (Hayashi *et al.* 1990). On the other hand the human PG fraction is dominated by three mono unsaturated PG species with PG 16:0/16:0 constituting a very small fraction (Postel *et al.* 2001).

Lipids

The major portion of PLs (up to 60%) is comprised of saturated phosphatidylcholine (PC) (Veldhuizen *et al*. 2000a). Dipalmitoylphospatidycholine (DPPC) forms the major portion of saturated PC. DPPC is one of the most important components contributing to a surfactant's activity (Notter *et al*. 1997), however Lang *et al.* (2005) reported DPPC content as low as 2 mol% present in the marsupials of Tasmanian devil (*Sarcophilus harrisii*) indicating that surface activity of a surfactant may not necessarily be determined by one single PL. In addition, PS also has negatively charged PLs viz. PG and PI. PG comprises approximately 10% of the lipid pool and small amounts of lysophosphatidic acid and phosphatidylethanolamine (PE) are also found. Cholesterol is the second most abundant neutral lipid present in PS present around 10% weight in the surfactant pool (Orgeig and Daniels 2001; Keating *et al.* 2007). Unsaturated lipids play a crucial role in the formation of a lipid reservoir and hastening the adsorption of PS to the interface (Ingenito *et al.* 2000; Klenz *et al.* 2008).

Surfactant proteins

There are four surfactant associated proteins viz., large hydrophilic SP-A, SP-D and small hydrophobic SP-B and SP-C. SP-A belongs to the collectin (collagen-lectin) family having a collagen-like domain at the N-terminal and connected to a globular C-terminal domain (Khubchandani and Snyder 2001; Kishore *et al.* 2006). It is usually assembled in a 650 kDa octadecameric form having six trimeric C-terminal globular subunits of ~8 nm size connected to the collagen domain by ~20 nm-long six triple helical stems (Palaniyar *et al.* 2001; Salvador *et al*. 2007). The globular domain is involved in binding of SP-A to carbohydrate, calcium and PLs (especially DPPC). SP-A plays an important role in the formation of tubular myelin (TM) (Batenburg and Haagsman 1998), a transient surfactant structure found immediately following surfactant secretion in the alveolar hypophase. SP-A has also been shown to enhance the effects of SP-B and help in PL aggregation and adsorption (Veldhuizen *et al.* 2000a). SP-A helps in maintaining the homeostasis between intracellular and extracellular surfactant pools and in the defence mechanisms of lungs (Kingma and Whitsett 2006). SP-A plays a role in modulating the opsonisation of microorganisms and directly inhibiting the growth of some pathogens (Sano *et al*. 2005). By interacting directly with macrophages, SP-A stimulates the production of reactive oxygen species and facilitates Fc receptor or CR-1-mediated phagocytosis (Crowther *et al.* 2004; Kuroki *et al.* 2007; Wofford and Wright 2007).

SP-D has been thought to play a role in the development of innate immunity along with SP-A (Sorensen *et al.* 2007; Wang *et al.* 2007). SP-D is a hydrophilic 43 kDa collectin belonging to the superfamily of collagen-containing C-type lectins, and is structurally similar to SP-A (Pastva *et al.* 2007). SP-D plays an important role in the innate immune system by binding to specific carbohydrate and lipid structures on the surface of bacteria, viral particles, fungi

Table 2 Surfactant proteins.

SP type	Mol wt (in kDa)	Nature	SP gene in humans	Gene location	Quaternary structure	Phospholipid binding selectivity	Isoelectric point (pI)	Function
SP-A	26-38	Hydrophilic	Gene has four exons in about 4.6 kb of DNA	long arm of human chromosome 10	Hexadecamer	DPPC	4-5	Formation of tubular myelin (TM) Plays a role in the immune function Hastens adsorption of surfactant Protects film from protein induced inhibition
SP-B	17	Hydrophobic	Gene has 10 exons in about 9.5 kb of DNA	short arm of human chromosome 2	Dimer	Anionic phospholipids	8-9	Binds anionic lipids Aids respreading and adsorption Membrane fusion and binding Formation of tubular myelin Reuptake of surfactant by type II cells
SP-C	4.2	Hydrophobic	Gene has 6 exons in about 3.5 kb of DNA	short arm of human chromosome 8	Monomer	Anionic phospholipids	8-9	Binds anionic lipids Aids respreading and adsorption Reuptake of surfactant by type II cells Stablization of the monolayer lipid film
SP-D	43	Hydrophilic	Gene has 8 exons in about 11 kb of DNA	long arm of human chromosome 10	Dodecamer	Phosphatidyl ionositol (PI)	4-5	Plays a role in the immune system Regulates surfactant balance

Sources: Jesus and Keough 1998; Veldhuizen and Haagsman 2000

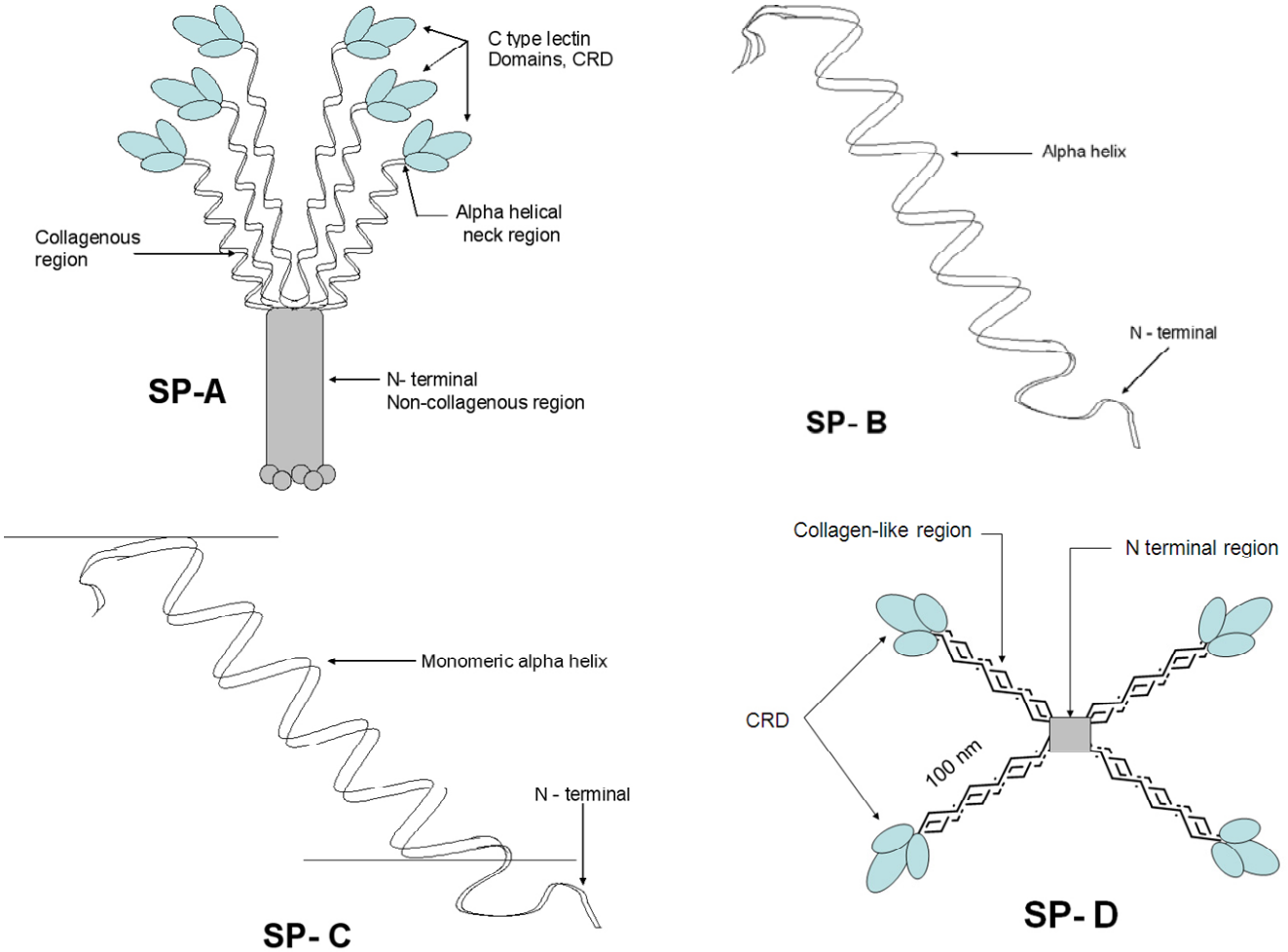
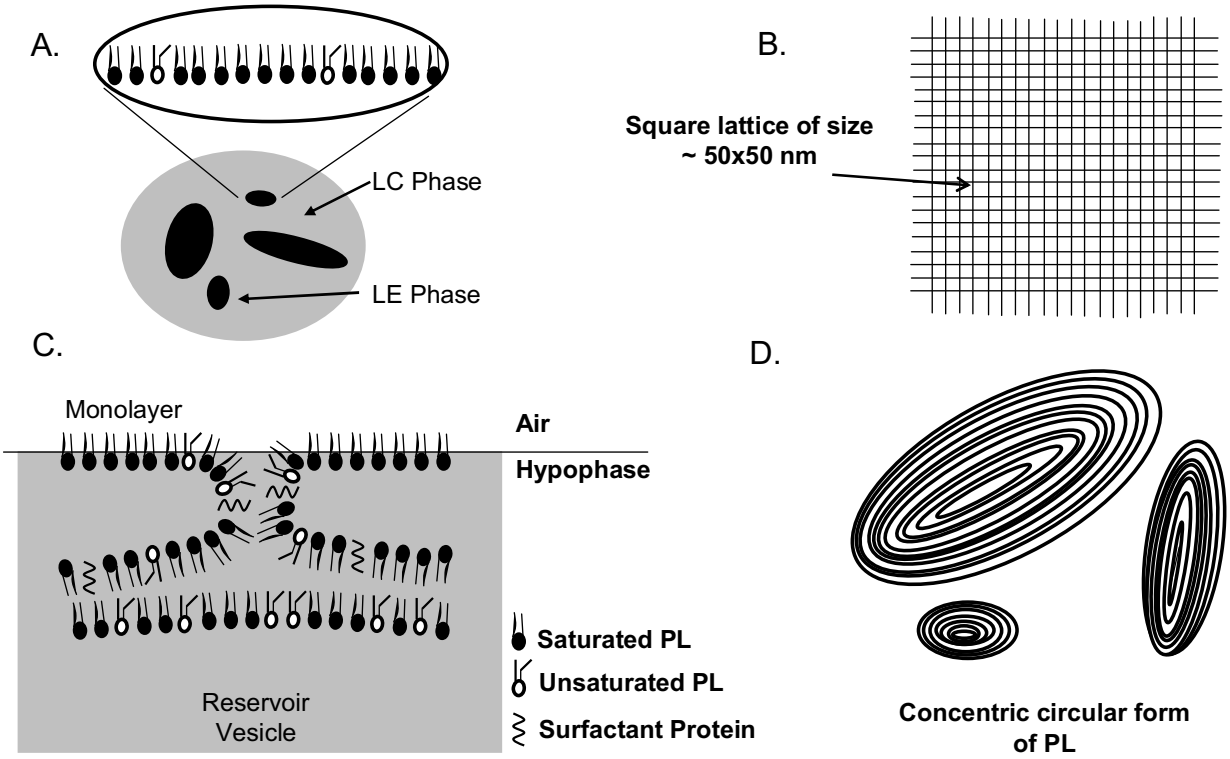


Fig. 1 Surfactant proteins. (**A**) SP-A is a hydrophilic protein. It is a hexadecamer. N-terminal region is non-collagenous with cross linking monomeric subunits. The collagen-like region activates the immune system and has spacing subunits. Carbohydrate recognition domain (CRD) binds carbohydrate in a calcium dependent manner. The neck region is alpha helical. (**B**) SP-B is a hydrophobic protein having two subunits. It is a dimer. Each subunit has three disulfide bridges and the dimer. (**C**) SP-C is a hydrophobic and transmembrane protein. It is a monomer with alpha helix. Its palmitoyl groups are covalently linked to two cysteine residues in a NH_2-terminal domain. (**D**) SP-D is a hydrophilic protein. It is a dodecamer. The N-terminal region is non-collagenous with cross linking monomeric subunits. Carbohydrate recognition domain (CRD) binds carbohydrate in a calcium-dependent manner.

and protozoa through a calcium-dependent interaction (Kishore *et al.* 2006). It has also been thought to play a role in the control of lung inflammation (Kingma and Whitesett 2006; Haczku 2008) and in preventing hypoxia-mediated lung injury by modulating proinflammatory cytokines (Jain *et al.* 2008).

SP-B is a 17.4 kDa homodimeric and hydrophobic protein having two subunits with 79 amino acids each. Each



PULMONARY NANOSTRUCTURES

Fig. 2 Pulmonary surfactant nanostructures. (**A**) PS exhibits LE and LC phases during compression and expansion cycle. LC phase consists mostly of saturated PLs which are closely packed, on the contrary LE phase consists of mixture of saturated and unsaturated PLs with comparatively loose packing. (**B**) Tubular myelin is a highly organized square lattice type intermediate assembly of PS components formed from lamellar bodies. (**C**) Surfactant reservoir attaches itself to PS monolayer by forming an inwards fold where the fold necks are stabilized by SPs. Size 40-60 nm and usually ~2-3 monolayer thick (**D**) PS is stored inside type II pneumocytes in form of concentric circular assemblies known as lamellar bodies. Size ~1-2 μm.

subunit contains three disulfide bridges and the dimer (held together by a disulfide bond) may be responsible for its ability to cross-link different lipid membranes. It helps in the formation of TM in the presence of SP-A, PLs and calcium and is also critical for LB formation. The interaction between polar head groups and SP-B enhance the stability of the PL monolayer. For normal lung function, the surfactant system must possess the following important properties viz. the ability to rapidly form a film by adsorption of surface active material from the subphase, reach near zero surface tension upon compression, achieve a low film compressibility to reach low surface tensions with minimal change in surface area, form a stable film and effectively respread to the interface upon film expansion. Pure DPPC bilayer adsorbs very slowly, the adsorption rate of DPPC molecules is improved to certain extent by the presence of unsaturated PC and unsaturated PG but maximally by the presence of SP-B and SP-C (Veldhuizen *et al.* 2000b).

SP-C is a 35 amino acid 4.2kDa hydrophobic and transmembrane protein (Johansson 1998; Plasencia et *al.* 2005). The palmitoyl groups of SP-C are covalently linked to two cysteine residues in a NH_2-terminal domain. SP-C and SP-B remain associated with the PS film and form multilayered structures during compression (Putz *et al.* 1999) which serve as a depot of surfactant molecules that can get adsorbed quickly upon film relaxation (Amrein *et al*. 1997). Surfactant proteins C along with SP-B not only help in interfacial monolayer formation (Veldhuizen *et al.* 2000b) but also assist in the refinement, stabilization and respreading of the surface film and recycling of the surfactant (Veldhuizen *et al*. 1998; Weaver *et al.* 2001). All the four surfactant proteins have been summarized in **Table 2**. **Fig. 1A-D** is a schematic diagram of the different surfactant protein structures.

PS NANOSTRUCTURES: FORMATION, FUNCTION AND IMPLICATIONS

PS is secreted by AE2 cells in the form of LB into the hypophase which then unwinds to form short-lived structures composed of a lattice of highly ordered tubules called TM (Fehrenbach *et al*. 1998). Surfactant molecules are rapidly adsorbed from TM at the interface and form an insoluble monolayer film (Dietl and Haller 2005). Schematics representation of PS NSs is shown **Fig. 2**. The details of the steps of formation these NSs and their function are explained below.

Lamellar bodies (LBs)

PS is synthesized in the endosomes of AE2 cells and assembled as LBs before being exocytosized into the hypophase (Rooney *et al.* 1994). SP-B is thought to be essential for the surfactant membrane accumulation and packing in the LBs. ATP-binding cassette transporter A3 (ABCA3) protein in conjugation with SP-B and SP-C play an important role in the biogenesis of surfactant and the formation of LBs. Fitzgerald *et al.* (2007) reported the loss of pulmonary surfactant and reduction in PG and development of respiratory distress in mice with inactivated ABCA3 expression indicating the crucial role of the protein in surfactant biogenesis. ABCA3 is present in the limiting membrane of LBs inside the AE2 cells (Ban *et al.* 2007; Cheong *et al.* 2007). It is a membrane transporter protein which actively transports saturated PLs from the endosomal compartment to form the LBs (Yamano *et al.* 2001). As a result of active accumulation of the surfactant molecules, especially PCs and PGs, further addition of the surfactant is facilitated either by membrane folding giving rise to LBs having parallel packed surfactant material or budding and vesiculation of the sur-

factant bilayer forming LBs with a series of concentric rings arrangement of lipid molecules (Pérez-Gil 2008). As the accumulation and packing of the surfactant molecules continues, LBs are intracellularly moved towards the apical plasma membrane, probably with the help of actin (Tsilibary and Williams 1983). Fusion of LB endosomal membrane with the plasma membrane of AE2 cells mediated by GTP-bound protein of Rab family in the presence of calcium ions causes membrane swelling and finally releases the lamellar bodies into the alveolar hypophase (Mason and Voelker 1998). In the presence of secreted SP-A in the hypophase, the released LBs then unwind to form an intricate mesh network of TM. Alternately, the packed LB may reach the air-liquid interface and immediately release tightly packed lipid-protein content into the interface (Dietl and Haller 2005). This property of the LB to immediately release the tightly packed surfactant content at the interface may help to rapidly replenish surfactant during the respiration process.

Nanotubes of tubular myelin

On secretion into the subphase, LBs usually unfold to form an intricate mesh-like network of nanotubes, the TM which consists of DPPC, unsaturated PG, SP-A, SP-B and calcium ions (Stratton 1977a). SP-A is important for the formation of TM and genetic knock out studies of SP-A also suggest the absence of TM in the lungs (Korfhagen *et al.* 1998). The schematic representation of a TM is shown in **Fig. 3**. TMs are formed as a result of the interaction of PLs and proteins. TMs form distinct square-like lattices prior to monolayer formation (Stratton 1977b). Depending on the concentration of calcium ions, SP-A undergoes self association and causes aggregation of PL vesicles by hydrophobically binding to DPPC vesicles or DPPC-rich bilayers (Ruano *et al*. 1996, 2000).

Atomic force microscopy (AFM) imaging of TM shows lateral spacing between the openings of tubular myelin to be 40-50 nm with heights of 2-5 nm (Nag *et al*. 1999). The square shape of TM also shows crosshatched structures suggesting the probable presence of proteinaceous material, mostly SP-A (Larsson *et al.* 2003). SP-B and unsaturated palmitoyloleoyl phosphatidylglycerol (POPG) are thought to be present at the corner of the square lattice making up the non-bilayer structures at the intersection and helping in fusion of bilayers to form the intricate mesh-like network (Casals and Ignacio García-Verdugo 2005). The distance between the centers of the nanotubes of tubular myelin in TEM has been reported to be 30 to 40 nm (Stratton 1977a, 1977b) whereas in AFM studies, the distance is recorded as 35-75 nm (Nag *et al*. 1999). On removal of the lipid bilayer, skeletons of the nanotubes show a more rectangular shape. This may be due to the removal of the lipids or may be an artefact due to the LR-White resin used in such studies.

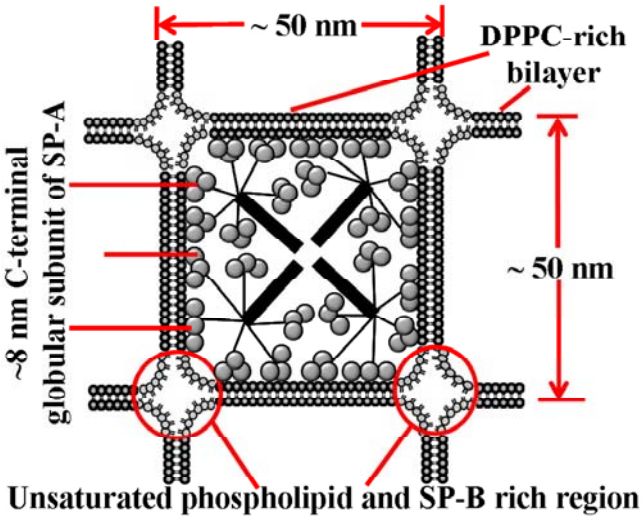


Fig. 3 Tubular myelin. Organization of lipids and proteins in tubular myelin are depicted. The square lattices are ~50 × 50 nm tubular myelin (TM) cell. Each side of highly ordered TM has high content of DPPC molecules (dark phospholipids molecules). Unsaturated phospholipids along with SP-B proteins make up the corner (circled) of each square shaped structure of TM. The cross hatched centre seen in TEM images of TM is thought to be due to extensive accumulation of SP-A proteins having ~ 8 nm C-terminal globular proteins.

Alternatively, it is thought that all the globular domains of SP-A proteins may interact with PL membranes resulting into corrugation of the planar PL bilayers (Palaniyar *et al*. 1999; Pérez-Gil 2008). Corrugation results in the creation of boundaries between the ordered rigid membrane phase and the much more fluid deformed membrane region. This in turn causes the membrane to show hill- and valley-like undulations at regular intervals. Each such corrugated membrane may merge its valleys with the corresponding valleys of another membrane through interactions between the N-terminal of the collagen terminals of the SP-A proteins present in the valley region. Such arrangement and coupling of SP-A proteins will also bring the respective hills of the two membranes close together causing fused structures by SP-B–SP-B interactions. SP-D is also said to mediate the formation of more atypical TM-like structures which are circular to hexagonal in cross section with ~90 nm in diameter showing central target-like electron density of the protein (Poulain *et al*. 1999).

At the alveolar interface, TM releases PLs into the interface thereby replenishing the surfactant molecules lost during film compression on expiration. As the surfactant material is released from TM, SP-A present in the TM also gets transferred to the subsequent multilayers of the film. This high concentration of SP-A in close proximity to the alveolar interface may provide protection against bacterial insult (McCormack *et al.* 2002). Thus TM may serve dual roles of providing protective covering to alveoli against bacterial insult and acting as a surfactant replenishing reservoir.

PS film

The surfactant film formed at the interface must be capable of reducing the surface tension to low values (< 1 mN/m) on film compression (Hills 1999). Also upon relaxation of the film, as during inspiration, it should be able to increase the surface tension at the interface with increase in the surface area to balance the pressures in different sized alveoli (Hill 1999). This whole process is complex and involves the simultaneous interaction of the lipids, surfactant proteins typically SP-B, SP-C and to some extent SP-A along with neutral molecules like cholesterol (Keating *et al*. 2007).

Paradoxically, although a DPPC-rich monolayer is able to attain near zero surface tension upon film compression, it adsorbs very slowly at the air liquid interface (Zasadzinski *et al*. 2001). Perturbation in the monolayer is necessary for the effective insertion of the surfactant into the existing monolayer. The surfactant proteins SP-B and SP-C are involved in this process. SP-B and SP-C help in improving the spreading of the surfactant at the interface (Wüstneck *et al.* 2005). SP-A plays an important role in the formation of TM and augments adsorption of DPPC at the interface in the presence of SP-B.

Incorporation of the surfactant with the surface and bilayer-monolayer contacts

The insertion of the PLs from the aggregates into the bilayer surface film is assisted by the surfactant proteins SP-B and SP-C present in the monolayer (Schürch *et al*. 1995). The positive charge of SP-B and SP-C are important for the interfacial adsorption of the PLs into the interface. Other PLs such as PG mediate the bilayer-monolayer contact via electrostatic interaction (Ingenito *et al*. 2000; Cruz *et al*. 2004).

Enrichment of the DPPC in monolayer

During expiration the surface area of the interface decreases reducing the area occupied per molecule in the surfactant monolayer. The traditional view suggests that surface film needs to become enriched in DPPC content so as to reduce the surface tension to near zero value (Hills 1999). The enrichment of the monolayer in DPPC content has been thought to occur by two processes: (1) Selective removal of the non-DPPC lipids from the surface film by squeeze out on compression (Pastrana-Rios *et al.* 1994) and (2) Selective adsorption of DPPC into the monolayer. SP-B and SP-C play a major role in the process of selective adsorption of the DPPC molecules at the interface (Veldhuizen *et al.* 2000b). Another recent view which does not require enrichment of DPPC in the monolayer is explained in the subsequent section "Supercooled surfactant".

Surface film reservoir

Cyclic compression and relaxation of PS film in a captive bubble surfactometer has revealed formation of PL multilayers. These multilayers are a result of removal of surfactant molecules along with SP-C, from the film during compression, which stay attached to the monolayer, forming protrusions or multilayer stacks (Schürch *et al.* 1995, 1998). Von Nahmen *et al.* (1997) carried out scanning force microscopy of Langmuir films of DPPC and DPPG-containing SP-C and found formation of similar multilayer protrusions of 55-60 Å in height, which were slightly higher than the height of the DPPC bilayer in the gel phase. Frequently, there are an odd number of laminae in alveolar surface films, but at some sites the film facing the air phase appears to be a bilayer or to have an even number of lamellae (Follows *et al.* 2007).

Reorganisation of the surfactant film upon expansion

As the film starts to expand a newer surface is created transiently as a result of the incomplete expansion of the compressed film. Surfactant thus starts getting readsorbed on this interface. The adsorption of the surfactant takes place with the movement of the lipid from the subphase along with addition of lipid from the surface film reservoir and stretching of the compressed film (Pérez-Gil and Keough 1998). The composition of the surfactant aggregate that migrates from the hypophase toward the interface is different from the composition of the collapsed phase. SP-B and SP-C play an important role in the respreading of the surface film. That portion of SP-B or SP-C, which appears to remain in the surface reservoir film (Nag *et al.* 1997) and also in the selectively excluded phase, can enhance the rate of redistribution of material in the interface upon expansion. The exclusion of the protein occurring during compression can be reversed on expansion since the material excluded from the interface seems to be closely associated with the monolayer (Schürch *et al*. 1995).

Recycling of surfactant material

During the compression of the PS film, small surfactant particles get excluded permanently from the monolayer and form small subphase structures that have reduced tensoactive properties. This phenomenon reduces the total functional surfactant aggregates available for the adsorption process and is thought to be dependant on the activity of the enzyme 'Convertase' (Oulton *et al.* 1999). SP-A and Ca^{+2}, which play a key role in the formation of the TM and production of extensive functional aggregates of surfactant, inhibit this surfactant conversion during cycling. The small surfactant aggregates are either phagocytosed by alveolar macrophages or subjected to reuptake by AE2 cells for reprocessing and addition to the surfactant synthesized *de novo* (Rooney *et al.* 1994).

PHASE TRANSITIONS IN DPPC MONOLAYER AND PS FILMS

DPPC, one of the major constituents of PS, forms a film at the interface which shows many phases during compression. At the start of compression, the molecules are loosely scattered throughout the interface and are considered to be in a gaseous state. As the film is compressed the area per molecule starts decreasing bringing the molecules closer and the surface pressure slowly rises. This stage is called liquid expanded (LE) in which the molecules start getting closer and orient in one direction, however they are still loosely packed and continue to remain in LE till around 3 mN/m. As the lateral compression of the film is continued the DPPC molecules start condensing into small nuclei of DPPC-enriched domains (at 3.8-3.9 mN/m surface pressure) which gradually grow in size as the film is further compressed (McConlogue *et al*. 1998). Further film compression to surface pressure of 11-30 mN/m results in the formation of a closely-packed structure called the Liquid Condensed (LC) phase where the DPPC molecules arrange themselves in a highly ordered manner with very small spacing between adjacent DPPC molecules. LC domains show shape transformation from star-shaped nuclei to formation of a bean-shaped domain to formation of "S" domains to formation of a tri-lobed shape as the film is compressed (Weidemann and Vollhardt 1995; McConlogue *et al*. 1997).

In between the LE and LC phase, the DPPC molecules continue to rearrange and orient themselves to closely packed DPPC domains resulting in the formation of coexisting LC and LE phases called the LE-LC phase, which is usually detected in the surface pressure-area isotherm as a plateau after the LE phase and found in the region of 4 to 10 mN/m surface pressure (Klopfer and Vanderlick 1996). AFM analysis of the DPPC monolayer in the LE-LC phase at a surface pressure of 6.7 mN/m shows the presence of a loosely scattered 2.25 nm-thick star-shaped LC phase DPPC domains of radius 6.3 μm and 131.5 μm^2 areas coexisting with the LE phase of DPPC 1.2 nm thick (Lucero Caro *et al*. 2009) indicating a height difference of 1.05 nm between the LC and LE phase. Zuo *et al.* (2008b) reported a similar kind of height difference of 0.6-1 nm between the LC and LE domain phases for bovine lipid extract surfactant (BLES) monolayers deposited on mica sheets at a surface pressure of 20 mN/m.

A solid packed film of DPPC molecules is formed on further compression of the film in the LC phase. The lipid molecules here form rigid and highly ordered structures at the interface and this phase is also known as the gel phase. The DPPC monolayer in the gel phase has a very tight packing arrangement with virtually no free space between two adjacent molecules. Further lateral compression of the film results in a fracture of the film and leads to the collapse of the monolayer of the DPPC film (Scheif *et al*. 2003). The surface pressure corresponding to this is around 70 mN/m.

Alonso *et al.* (2004) reported similar phase transitions in Survanta™, a commercially available animal-derived erogenous surfactant. Below a surface pressure of ~10 mN/m Survanta™ formed a film having high compressibility indicating that the surfactant molecules are loosely packed, as in the LE phase. Further compression of the film till around 38 mN/m resulted in an increase in the slope of the isotherm indicating the formation of a less compressible film as a result of gradual transition of DPPC from the LE to the LC phase with formation of more DPPC condensed domains. AFM studies at 15 mN/m corresponding to the LE-LC phase revealed the presence of a fluid phase ~30 nm thicker than the condensed domain. Further compression of the surfactant film resulted in a plateau region in the isotherm wherein surfactant proteins and unsaturated phospholipids got squeezed out of the film resulting in removal of the LE phase and formation of a DPPC-rich monolayer, which, on further compression further showed a steep rise in the slope of the isotherm indicative of formation of highly incom-

pressible film that is able to sustain high surface pressure of 70 mN/m.

Lipid raft model

Transitions of PS films can be imaged as phase segregated domains of one of the components coexisting in a background of another phase. Further, the film itself is not a monolayer at all places and shows localised stacking of bi- and multilayers and tubular outgrowths. Domains of condensed or gel phase lipids like DPPC may phase separate out at body temperature in mixed lung surfactant monolayers like BLES during phase transitions (Nag *et al.* 2002). Hence, it is proposed that surfactant films may show similar lipid rafts as those in cell membranes. Lipid rafts are localised lateral regions in cell membranes which occur due to the preferential packing of sphingolipids and cholesterol and are said to be associated with protein sorting and signalling (Simons and Ikonen 1997; Silvius 2003). Similarly, condensed phases of lung surfactant may play special roles in the function and pre-sorting of lung surfactant films making them behave similar to lipid rafts that play specialised roles in cell membranes (Nag *et al*. 2005). X ray diffraction studies also suggest that such phase separations may be in terms of cholesterol-rich and -poor regions which may play a role in the complex functions of cholesterol in lung surfactant function, which are poorly understood (Worthman 1997).

Supercooled surfactant, a two dimensional alloy

Recent studies of surfactant film biophysics and NSs suggest that surfactant films may behave as two dimensional analogs of alloys where the mixed monolayer has distinctly different properties than those of the individual components. Surfactant monolayers close to near zero surface tension show 30% coverage of DPPC-rich phases whereas the majority of the film appears to be in a fluid phase (Piknova *et al*. 2002). Further, DMPC a fluid phase PL, when compressed at high rates in a captive bubble surfactometer, was found to sustain low surface tensions of 5 mN/m (Scheif *et al.* 2003). Hence, the LC phase domains within a LE phase give PS its unique properties which differ from that of the individual components or phases. It is possible that the arrangement of the domains play a major role in the attainment of the low surface tensions.

Zuo *et al*. (2008b, 2008c) showed the presence of small discrete ~90 nm size nano condensed DPPC domains in the LE region having the same height as that of the LC phase. Cruz *et al.* (2004) studied LE and LC phase domain details in a DPPC monolayer deposited at 11 mN/m surface pressure using scanning force microscopy with epifluorescence microscopy and showed that both phases have some heterogeneity. The LC domains had many pores and holes in it of 25-150 nm whereas the LE phase had numerous nanodomains condensed phases of 0.1-1 μm in length. This may explain a fluid PL reaching low surface tensions and the fact that low surface tension films of lung surfactant still contain fluid phases. AFM of BLES (Bovine Lipid Extract Surfactant) with and without SP-A showed distinct changes. BLES with SP-A showed the presence of larger nanodomains (about 80 nm in size). The ratio of nanodomains to microdomains at 40 mN/m was increased to about 18: 1, whereas in the absence of SP-A, this ratio was about 7: 1.

It has also been proposed that surfactant films may act as though they are in a supercooled glassy state which may explain their unique properties which differ both from fluid and solid phase materials (Nag *et al*. 2005). However, the exact nanostructure organisation–function correlation of lung surfactant films has not yet been completely understood.

ALTERATIONS IN NSs AND PHASES OF PS IN DISEASED STATES

Various direct and indirect lung injuries can lead to inactivation of PS activity (Notter and Wang 1997). The alteration in PS monolayers can occur as a result of change in composition, or due to its dysfunction. Dysfunction of PS occurs in presence of blood proteins like albumin (Larsson *et al*. 2006; Rachana and Banerjee 2006; Zuo *et al*. 2008c) and serum, on aspiration of acids from the environment (Kaviratna and Banerjee 2009), or on interaction with endotoxins (Fehrenbach *et al.* 1998) and environmental pollutants (Kanishtha and Banerjee 2006). The structural alterations in PS by meconium (Ochs *et al*. 2006) and taurocholic acid (TA) have also been reported (Gross *et al*. 2006). These alterations can lead to inactivation of PS.

Chimote and Banerjee (2008b) studied the AFM images of DPPC films in the absence and presence of mycolic acid (MA), a prominent lipid in the cell wall of mycobacterium tuberculosis. The surface topography of the DPPC monolayer film was smooth and homogeneous. The height difference between the DPPC monolayer domains was 0.87 nm as measured by section analysis. Surface topography of DPPC with MA films exhibited a height difference of about 52 nm between the domains. This height difference could be due to alteration in monolayer composition or tilt of the hydrocarbon chains in the monolayer or could be due to the presence of multilayered structures of MA aggregates within DPPC film.

Similarly, they also evaluated the interactions between DPPC monolayer and cord factor (CF) (Chimote and Banerjee 2008b). The DPPC monolayer reached a surface pressure of about 61 mN/m while cord factor reached a lower surface pressure of about 36.6 mN/m. With an increase in the molar concentrations of cord factor in the mixed monolayers, the isotherms reached lower surface pressures ranging from 40 to 55 mN/m, almost similar to the control isotherm for cord factor. AFM of DPPC films exhibited a height difference of 0.87 nm and mean surface roughness of 9.24 nm/μm^2 whereas the DPPC-CF films exhibited a height difference of 31-33 nm and mean surface roughness of 83.18 nm/μm^2, which is much higher than DPPC only. The physical state of DPPC film in the presence of cord factor changed from a liquid-condensed to a liquid-expanded phase and with the appearance of clusters and aggregates altering the surface topography.

Another study by the same authors (Chimote and Banerjee 2008c) confirmed the inhibitory action of mycobacterial cell wall lipids, mycolic acid and cord factor on Curosurf™. AFM of Curosurf™ films alone exhibited a height difference of 6-8 nm, which increased to 20-30 nm in the presence of MA. A similar effect was observed for CF. In a recent study, Chimote and Banerjee (2009a) also found similar effects on Survanta™ due to mycobacterial lipids.

Zuo *et al*. (2008c) studied PS films in the presence and absence of albumin. The height of BLES-albumin at 30 and 45 mN/m was 0.76 ± 0.3 and 4.5 to 5 nm, respectively. At 20 mN/m, pure BLES monolayers showed a clear separation of micrometer sized tilted condensed (TC) and LE phases. However, TC domains were not observed in the presence of albumin at 20 mN/m. At 30–40 mN/m, there was a decrease in domain area as microdomains appeared to be destabilized on compression of pure BLES monolayers. There was a decrease in microsized domains but an increase in nanometer sized TC domains. Albumin molecules were found to partition into the LE phase of the BLES monolayers. Albumin penetrates the BLES monolayer at low surface pressure (about 20 mN/m) and this penetration depends on the phase of the monolayer (Zuo *et al*. 2008c).

Ochs *et al*. (2006) reported that in acute lung injury and related conditions, the inactivation of PS is due to increased conversion of surface active large aggregates (LA) to small aggregates (SA) with poor surface activity. These different surfactant aggregates can be distinguished morphologically using an electron microscope. Three morphologically dif-

ferent subtypes observed in Curosurf™ are lamellar body-like forms, multilamellar vesicles, and unilamellar vesicles. For example, meconium altered the ultrastructural composition of the natural modified surfactant Curosurf ™ as observed by TEM. Incubation of Curosurf ™ with increasing concentrations of meconium resulted in an increase in the volume fraction of unilamellar vesicles and a decrease in the volume fraction of multilamellar vesicles (resembling an increase in the SA/LA ratio). This ultrastructural finding is paralleled by an increase in minimum surface tension. Such structural alterations may explain the surfactant dysfunction seen in meconium aspiration syndrome.

Fehrenbach *et al.* (1998) studied the effect of endotoxins on lung surfactant. Four groups were evaluated based on different time periods of ventilation and perfusion and exposure to lipopolysaccharide (LPS). Group 1 was the untreated rat lungs which were isolated, perfused and ventilated for 150 min. Group 2 was the LPS treated rat lungs isolated, perfused and ventilated for 150 min. Group 3 consisted of untreated rat lungs which were isolated, perfused and ventilated for 10 min and group 4 were the LPS treated lungs which were isolated, perfused and ventilated for 10 min. Giant lamellar bodies were present in type II cells of LPS treated lungs from groups 2 and 4. The volume density of the nucleus and mitochondria was significantly higher in lungs ventilated and perfused for 150 min than in lungs ventilated and perfused for only 10 min. The increase in the duration of perfusion and ventilation from 10 to 150 min resulted in a decrease in the volume density of intracellular surfactant structures by 55%. However, LPS treatment increased the volume density of intracellular surfactant by 27% compared to the LPS untreated groups. Multilamellar structures with densely coiled lamellae were abundant in LPS treated lungs and those ventilated for 150 min.

Larsson *et al*. (2006) studied the effect of bovine serum albumin (BSA) on the alteration of BLES, a commercial surfactant of bovine origin. On X-Ray diffraction, it was found that BSA significantly reduced the thickness of the BLES bilayers. The thickness of the bilayer of only BLES in saline solution was 44.3Å whereas when both BSA and BLES were associated in the bilayers the thickness was reduced to 42.3Å. However, in presence of BSA in the aqueous layer and BLES forming the bilayer, the bilayer thickness was found to be further reduced to 40.25Å. The presence of BSA decreased the bilayer thickness affecting the structure of BLES. This reduction in the bilayer thickness could be due to increased fluidity and more disordered lipid chains in the bilayers.

Rachana and Banerjee (2006) studied the effect of BSA and erythrocyte membrane components on PLs of PS. Albumin was found to be more inhibitory to DPPC monolayers in the presence of other PLs like PE, PG and palmitoyl-oleoyl phosphatidylcholine (POPC) than DPPC alone. Serum and erythrocyte membranes were found to be more inhibitory to PL monolayers than albumin.

Gross *et al*. (2006) studied the structural alterations in Curosurf™ in the presence and absence of taurocholic acid and meconium by using cryo-TEM. The vesicles of Curosurf ™ were multilamellar and unilamellar with a diameter ranging from 200 to 800 nm. In the presence of 1.9 mM taurocholic acid, these vesicles were elongated, with broken and damaged membranes. On addition of 0.25 mg/ml meconium to 0.5 mg/ml Curosurf™, the Curosurf™ components were found to be open and damaged.

Kaviratna and Banerjee (2009) studied the effect of hydrochloric, nitric and sulphuric acids on DPPC and found that the presence of acid in the subphase caused a change in the shape of DPPC isotherms. Under neutral subphase pH 7.4, the lift-off area obtained with DPPC was 58 $Å^2$ whereas the lift off area at pH 1 with nitric acid was 105 $Å^2$. Similarly, the lift off area at pH 0.4 with sulphuric acid was 100 $Å^2$ and the lift off area at pH 0.8 with hydrochloric acid was 75 $Å^2$. The larger lift off area under acidic condition suggests that the molecules probably occupied a larger surface area at the same surface pressure resulting in a greater expanded state of the monolayer. The phase changes in the DPPC monolayer could be due to protonation or interaction of the anions of each acid with DPPC head groups even at the same pH. These findings may have implications in acid lung injury.

PS INTERACTIONS WITH NPs

The alveolar interface, lined by a thin surfactant layer, is the first level of contact between exogenous substances that reach the alveoli, be they respiratory gases, pollutants, microspheres or NPs intended as drug carriers to the lungs. This surfactant layer performs the function of maintaining alveolar patency by a very sophisticated, multi-step mechanism (Rugonyi *et al*. 2008). External particles bring about alterations in dynamics of the PS layer, usually by adsorbing components from PS (Kendall 2007; Salvador-Morales *et al*. 2007; Bakshi *et al*. 2008). Even the slightest of physical, chemical or structural changes, which may involve changes in surfactant monolayer composition or orientation, etc., by any incoming agent can significantly change the dynamics of the processes at the tissue-air interface. This subsequently affects the minimum surface tension achieved and the spreading of lung surfactant film and has a direct bearing on PS function. Whilst drug and its carriers can affect PS function, PS can also bring about changes in drug release, efficacy and residence time in lungs. The factors that primarily affect these interactions are physical, chemical and surface properties of drug and carrier, particle size, coating on particle and charge (Hadinoto *et al*. 2007; Tomoda and Makino 2007; Ku *et al*. 2008). The interactions between NPs of environmental origin and drug-loaded NPs with PS structures are described in the next two sections.

Interactions with environmental fine particulates

Respirable particulate matter from the environment can enter the lungs system at the time of inspiration. Particles having aerodynamic diameter less than 2.5 to 3 μm can reach the bronchiolar and alveolar regions, while larger particles get trapped in the upper airways (Hoet *et al*. 2004). On reaching the alveolar region, the particles come in direct contact with the PS system. These particles alter lipid and peptide structure and packing and can translocate the surfactant film (Choe *et al*. 2008). Nanoparticles of 235 nm in size have been found to have maximum affinity for the DPPC monolayer, which simulates the surfactant layer in lungs (Ku *et al*. 2008). Kanishtha *et al*. (2006) studied the inhibition of lung surfactant monolayers due to the direct effects of particulate matter of size < 2.5 μm from biofuel emissions. The particles increased the minimum surface tension by causing destabilization of the spread films by NPs-surfactant interactions.

Stenger *et al*. (2009) studied the alteration in Curosurf™ and Survanta™ adsorption onto air-water interface due to exposure to environmental tobacco smoke (ETS). ETS changed the morphology of Curosurf™ films from large condensed domains (8–12 μm) to smaller domains (~2 μm) which were more monodispersed and condensed. The large, solid phase domains were more in number in Curosurf™ during expansion than ETS-exposed Curosurf™. AFM images of Curosurf™ and ETS-exposed Curosurf™ at 40 and 60 mN/m showed continuous LE and LC phases. The surface-associated reservoir was found to be predominantly associated with the LE phase as the thickness of LE phase was more than the LC phase. The height of LE domains in Curosurf™ (~126 nm) was greater than ETS-exposed Curosurf™ (~14 nm). This indicated the presence of a larger surface-associated reservoir under the monolayer. Few large LC phase domains but many smaller LC domains were observed after ETS exposure. The LC phase domains after ETS exposure were monodispersed with a size range of 0.2 to 1 μm. Survanta™ has also shown some structural changes in the presence of ETS but appeared to be less susceptible to ETS-induced changes than Curosurf™ (Sten-

ger *et al*. 2009).

Bringezu *et al*. (2003) also studied the effects of ETS on primary lipids of lung surfactant using AFM. ETS exposure altered both LE and LC lipid domains and caused extrication of the unsaturated lipids such as POPG. This change affected the collapse of structures of the lipid films, leading to higher minimum surface tension. Thus it is evident that a small change in nanoscale organisation of the surfactant films due to environmental particulates can lead to significant changes in surfactant properties.

Similarly, Bakshi *et al*. (2008) studied the effect of gold NPs on PS function. The average diameter of the studied gold NPs was around 15 nm. Using a captive bubble tensiometer and DPPC (POPG)/SP-B (70: 30: 1 w/w/w) as surfactant, the authors reported that in the presence of gold NPs higher surface tensions were achieved during dynamic film compression and respreading of surfactant was also adversely affected during expansion. It was also found that the presence of SP-B resulted in the formation of highly aggregated gold NPs, which may partially explain the adverse effects of gold NPs on PS function. TEM characterization showed NP aggregated in the form of long threads or strands. Since gold NPs were not surface active, physical adsorption of surfactant on NPs may be responsible for the changes observed.

Another study by Erickson *et al*. (2008) evaluated the effect of model NPs namely poly-amidoamine (PAMAM) dendrimers on Survanta™ monolayers. AFM was used to characterize the changes taking place in a Survanta™ bilayer, deposited on clean mica substrate, on addition of PAMAM dendrimers. Information from the images was suggestive of attachment of dendrimers on the top of the bilayer or partially inside the hydrophilic region of the bilayer. More important was the finding that PAMAM caused disruption of lipids from the bilayer. PAMAM, after getting adsorbed onto the surface, interacted strongly with the hydrophilic head of PLs causing their removal. Removal of PLs from alveolar layer may alter the surface tension lowering properties if similar situations occur in the alveoli.

Interactions with drug-loaded particles

The presence of PS in alveoli modifies the properties of administered drug-carrier complexes, by virtue of its surface active properties (Evora *et al*. 1998; Jones *et al*. 2002). Surface active self assembled NSs in PS like the PL monolayer or SPs may adsorb onto the carrier and modify the drug release and phagocytosis of the administered particles. Tomoda and Makino (2007) examined the effect of SURFACTEN®, a bovine derived surfactant, on *in-vitro* rifampicin release from polylactic-co-glycolic acid (PLGA) microspheres. Results showed that microspheres with adsorbed SURFACTEN® exhibited an increased release of drug and quicker degradation of the microspheres. Two independent groups of researchers (Evora *et al*. 1998; Jones *et al*. 2002) showed that phagocytosis of PLGA microspheres by macrophages was inhibited by addition of DPPC in the matrix or on the surface of the particle. These observations also draw attention towards modification of the possible interactions between macrophages and microspheres due to changes in the surface composition of the NPs/microspheres. The results of these studies highlight that interactions between PS and drug-carriers can be modulated to design pulmonary drug delivery system with varying residence time and release.

Chimote and Banerjee (2005) studied the *in vitro* effect of anti-tubercular drugs on the surface activity of DPPC, the major component of PS. The drugs evaluated were isoniazid (INH), rifampicin (RFM) and ethambutol (ETH). DPPC mixtures with antitubercular drugs in certain ratios led to attainment of lower values of minimum surface tension and hastened the adsorption of the vesicles suggesting an overall improvement in surfactant functioning. The authors also found that adsorption of DPPC was enhanced by the liposomal systems INH-DPPC (1: 1, w/w), triple drug-DPPC mixture 1: 1 and 1: 2 (w/w). Such liposomal systems may be promising for pulmonary drug delivery of anti-tuberculosis drugs by acting as drug-loaded surfactants. A recent study by the same authors (Chimote and Banerjee 2009b) also explored the concept of NP aerosols of antitubercular drug-loaded surfactant vesicles.

PS enhances the wettability of drug particles (Wiedmann *et al*. 2000) and wetting of particles is a necessary condition for solubilisation of drugs. PS can enhance the solubility of a drug. Enhanced solubility is directly linked to enhanced absorption i.e. higher bioavailability. A surfactant's general spreading properties can help in effective drug distribution at the lung surface (Vermehren *et al*. 2006). A larger surface area of the lung, along with well distributed drug particles, can increase both the rate and extent of absorption of drugs. Surfactant re-expands atelectatic areas of lungs which are possibly the sites of infection. So, a surfactant as an anti-microbial drug carrier may serve an added function of enhancing the therapy's efficacy (Haitsma *et al*. 2001). Yet another significant fact that strengthens the idea that surfactant molecules can be used as carriers is that surfactant has the ability to form nanostructured lamellar bodies by self assembly. Thus, a surfactant can be directed to form liposomes in which a drug can be incorporated.

Modulation of the interactions between drug loaded nano or microparticles and lung surfactant monolayers may allow optimisation of cellular internalisation. Further, surfactant nanovesicles may themselves act as carriers for drugs in the respiratory system.

FUTURE PERSPECTIVES

The intricate NSs of PS may be utilised as templates for the development of other NSs. The tubular myelin assembly provides a self assembled square lattice of regular 40-50 nm spacing. TM may play similar roles as that of S layers which are self assembled protein monomolecular arrays on the cell envelopes of bacteria which have been used as templates for development of inorganic NSs (Pum and Sleytr 1999).

Individual biological self assembled nanotubes have been used as templates for fabricating conducting nanowires which find applications in the nano/microelectronics industry (Zhang 2003). Lipid nanotubes can act as templates for metallisation and on removal the pure conducting nanowire can be obtained. Lvov *et al*. (2000) have fabricated nano and microwires by coating various lipid nanotubules with silica and gold nanocrystals. Similar possibilities can be explored using the lipoprotein nanotubes of reconstituted lung surfactants. However, a deeper understanding of the surfactant NSs and their stability will be required prior to their efficient manipulation as nanotechnology templates.

The functional relevance of the nanodomains of PS films is another important area of investigation. It will be interesting to specifically fingerprint the alteration of nanodomains in the PS films in various diseases and in the presence of drugs. Another related and fascinating question is the effect of NSs on internalisation, recycling and exocytosis by the AE2 cells. The ability to modulate nanodomains in PS films and optimise recycling of NSs by the AE2 cells may have implications for the development of newer types of surfactant replacements.

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