Journal of
Nanomedicine & Nanotechnology

Azam, J Nanomed Nanotechnol 2018, 9:3
DOI: 10.4172/2157-7439.1000504

Research Article Open Access

Investigation of Interaction of Soft Nanoparticles based Vesicles with Lung Surfactant via Langmuir-Blodgett Trough and Quartz Crystal Microbalance Study

Uzma Azam*

Department of Biological Sciences, School of Sciences, Birkbeck University of London, London, UK

Abstract

Major aim of this research study is to comprehend the interaction of soft nanoparticle-based vesicles with model lung surfactant monolayer. Vertebrate lung surfactant plays a pivotal role in gaseous exchange plus breathing. As pulmonary lung surfactant is the one which directly comes in contact to inhaled air so it also acts as defensive agent against air-borne pollutants (any foreign particle). In this enquiry, several different types of lipids namely 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, 1,2-dimitrystoyl-sn-glycero-3-phosphorylcholine, 1-palmitoyl-2-oleoyl-sn-glyceero-3-phosphocholine are used as lipid monolayer and vesicles in different combinations while using Langmuir-Blodgett trough. The different lipids are treated with chloroform and buffer solution to make vesicles and then extruded. Nanoparticles present in vesicles interact with lipid monolayer when processed in Langmuir-Blodgett trough and mimics the behavior of natural pulmonary lung surfactant system. In Quartz Crystal with gold mounted on it as thin layer, different combinations of lipid bilayer and vesicles are used from the same list of lipids. The results obtained from this investigation are quite informative in depicting the interaction of soft nanoparticle based vesicles with lung surfactant system, also this work would pave the way to explore still more on interaction of soft nanoparticles based vesicles with lung surfactant, as very little work is done on soft nanoparticles. This novelty of this work is to plan to develop some alternatives of lung surfactant because we cannot perform the experiment directly in living souls as some of these has lethal effects.

Keywords: Soft nanoparticles; Quartz crystal; Lung surfactant; Quartz crystal microbalance

Introduction

Vertebrate lung surfactant plays a pivotal role in gaseous exchange plus breathing. As pulmonary lung surfactant is the one which directly comes in contact to inhaled air so it also acts as defensive agent against air-borne pollutants (any foreign particle). A lot of research is made to understand the chemical composition of Lung Surfactant (LS). LS systems possess a highly complex structure. Lot of research studies resulted in development of Langmuir-Blodgett monolayer model system which mimics the pulmonary lung surfactant system *in vitro*. Advancement in nanotechnology urged upon to study interaction of nanoparticles with LS, because NPs are not only used as drug delivery but also straight away targeting LS. Pulmonary LS is a lipid-protein complex. Several investigations were carried out to understand the chemistry of LS of vertebrates [1]. All researchers end up with same conclusion that a big portion of LS is lipids (almost 90%). Main components spotted in LS are as; Phosphatidylglycerol, Phosphatidylcholine, Fatty acids, Cholesterol, and Unsaturated phospholipids [2]. Table 1 summarizes the relative abundance of some of the essential components of natural pulmonary LS system of vertebrates [3].

A look into various parts of vertebrate lungs (Figure 1), an important part of respiratory system [4], after inhaling air enters trachea from where it goes to bronchus (trachea divides into two bronchi, and then to bronchioles). Each bronchiole ends up in air sac called alveoli where gaseous exchange takes place (Figure 2).

Major tasks attributed by lipid portion of LS system are as it makes the LS resilient to withstand elevated pressure and sustains low surface tension [5]. Major tasks attributed by protein portion of LS system are as necessary for the formation of pulmonary surfactant film and is inevitable for stabilization of the pulmonary surfactant film [6].

Introduction to Nanoparticles

The name nanoparticles (NPs), is given to class of particles that bear size of 1 and 100 nm [7]. NPs can be characterized depending on their Size, Charge, and Shape etc. According to nature of nanoparticles, they are classified [8] as;

- Hard NPs
- Soft NPs.

Hard NPs (Figures 3-5) maintain their original size and shape during all the processes (physical and chemical). Hard NPs include inorganic materials.

Sr. No.	Component	Approximate relative abundance noticed
1	DPPC	41%
2	Phosphatidylglycerol	9%
3	Unsaturated Phosphatidylcholine	25%
4	Neutral lipids	5%
5	Other phospholipids	2%
6	Cholesterol	8%
7	Proteins	10%

Table 1: Different essential components of LS system.

***Corresponding author:** Uzma Azam, Department of Biological Sciences, School of Sciences, Birkbeck University of London, London, UK, Tel: 923056576561; E-mail: ahmad.rao517@gmail.com

Received: May 15, 2018; **Accepted:** May 28, 2018; **Published:** June 04, 2018

Citation: Azam U (2018) Investigation of Interaction of Soft Nanoparticles based Vesicles with Lung Surfactant via Langmuir-Blodgett Trough and Quartz Crystal Microbalance Study. J Nanomed Nanotechnol 9: 504. doi: 10.4172/2157-7439.1000504

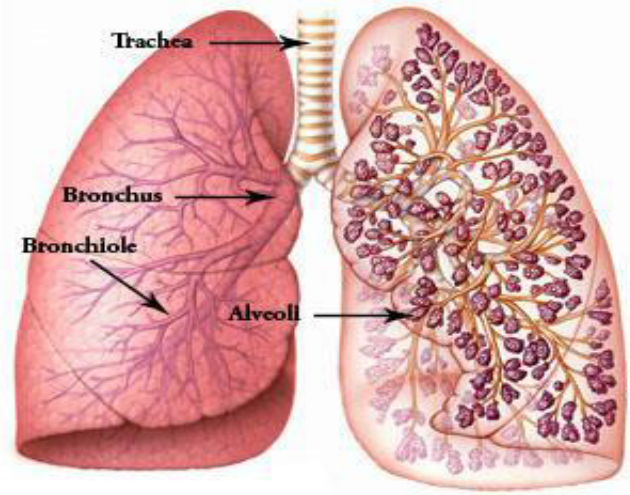

Figure 1: Chemistry of LS.

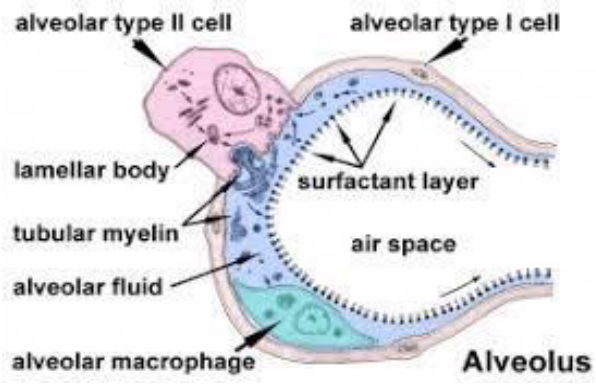

Figure 2: Internal cross section of LS.

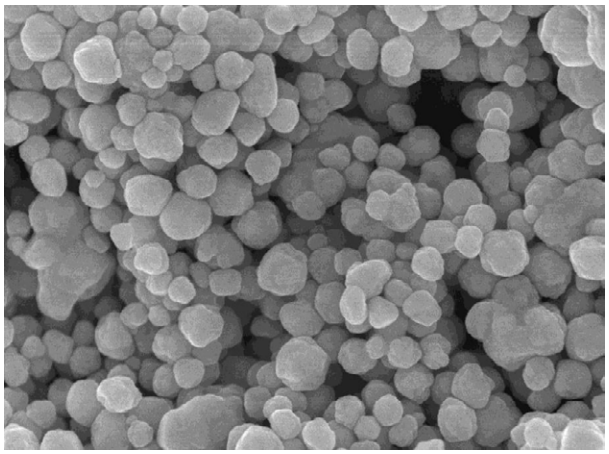

Figure 3: Hard NPs (Silver NPs).

Soft NPs are made by organic materials and they can accept change in original size and shape when subjected to different biological condition listed as; Temperature, pH, Pressure, Ionic strength [9] etc. Soft NPs can be produced from a number of organic materials. Some salient examples are; Liposomes (Figure 6), Biodegradable polymers, Non-biodegradable polymers, and Cyclodextrin based polymers.

Nanotechnology is prevailing in almost every field of life. In recent years, nanotechnology is wide spreading in electronics, cosmetics, medicines especially the use of nanotechnology in effective drug

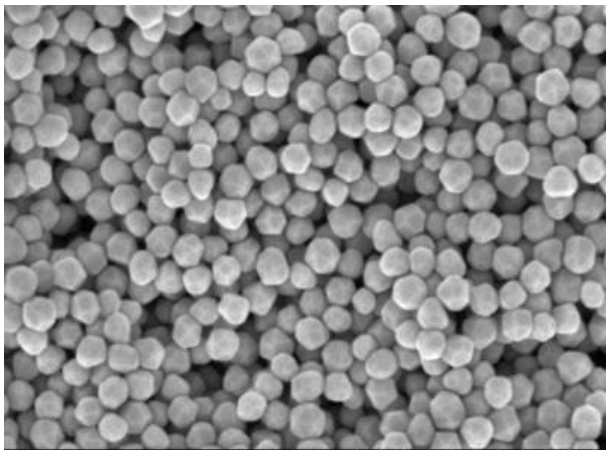

Figure 4: Hard NPs (Gold NPs).

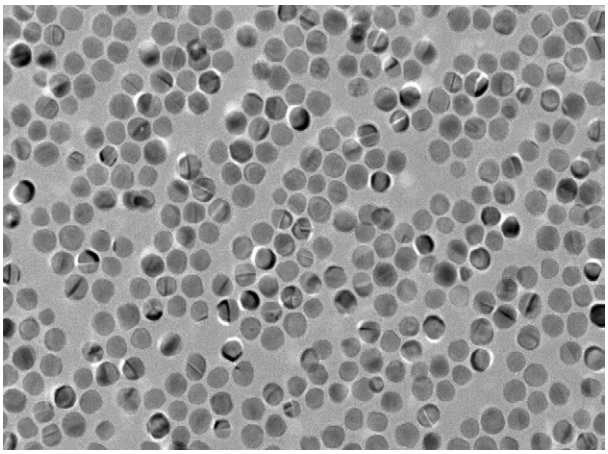

Figure 5: Nickle Catalyst Hard NPs.

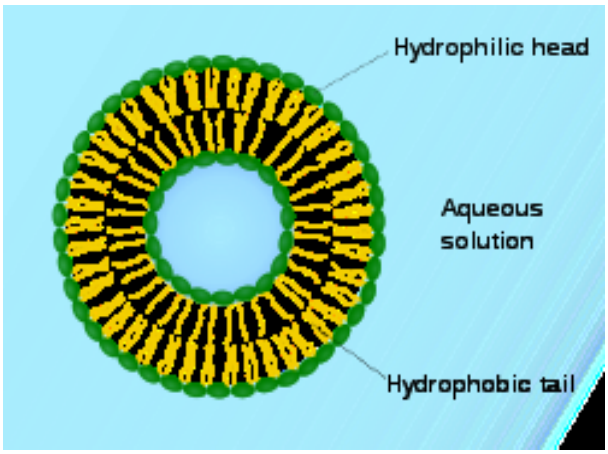

Figure 6: Liposome formed by phospholipid in an aqueous solution.

delivery. Almost all fields of life are receiving too much influence from NPs but biomedical section is the one with greater implications. Here a brief list is given to examine how NPs are becoming an essential part of every research;

NPs are actively used as an efficient drug carrier [10]. Several different characteristics of NPs made them an active agent to carry the drugs in right place at right time. For this purpose, timed released of drug (with NPs) is a best strategy which is effective in controlling toxicity of drug, unless the NPs bind to the target cell and drug is released. Nanotechnology has overcome this obstacle, as NPs possess very small size and uniform structure so they precisely effect the desired area in a given time. This is due to the fact NPs bear very small size so easily reachable to small areas. The surface properties of NPs can be altered for the effective delivery of drugs, NPs loaded in proteins and small bio-molecules are not detected by immune system so they effectively reach the targeted area [11].

A group of scientists in China used gold NPs to detect carcinoembryonic antigen (CEA) and alpha foetal protein (AFP) -important biomarkers in diagnosis of various types of cancer (lung, liver and breast cancer). Gold NPs are conjugated to antibodies [12]. For example, two different types of NPs were used for CEA and each nanoparticle is for different antibody. When CEA biomarker was subjected to the mixture with both nanoparticles, it interacted with both the NPs in mixture. This change is measured by noticing the decrease in photon number burst as they pass through the focused laser beam. The decrease in number of NPs is directly proportional to the amount of CEA, if it increases there is a significant decrease in NPs. The use of Gold NPS as detector is evident from their particular set of characteristics; They do not damage after continuous exposure to light and are easy to make and process [13]. Scientists also used NPs to diagnosis various types of ovarian cancer in mice. So, it is convenient for scientist to do further advancement in treatment of various types of cancer. Still a lot of work is required before it is used for trial on human beings.

NPs can play an important part in enhancing the stem cell therapy action. In a report published on October 5, in Proceedings of the National Academy of Sciences, it was reported that chemical engineers used NPs to increase the efficiency of stem cells, stem cells then regenerate affected vascular tissue and decrease muscle degeneration in mice. They investigated when stem cells are implanted in a living body, cells are not enough active to keep the tissue alive for longer periods. To increase the activity of cells performance, enhancing genes are used which serve the purpose. Researchers at Warwick university noticed that small NPs can stick to the interface of non-mixing liquids two times better. In a paper "Interaction of nanoparticles with ideal liquid-liquid interfaces" published in Physical Review Letters, the University of Warwick's researchers studied the interaction between a liquid-liquid interface and non-charged NPs. They noticed when NPs of size 1-2 nm were used a significant amount of energy is required to dislodge the particles from liquid-liquid interfaces that was not expected according to standard model. As the size of particles increased it acts according to standard model [14]. The researchers concluded that failure of previous models was due to ignoring of interaction of NPs at liquid to liquid interfaces. It is concluded if stem cells are used in contribution to NPs, then the activity of stem cells can be more vigilant. Stem cells incorporated with NPs tend to remain for longer period of time and play an effective role in regeneration of damaged or effective vascular tissue.

NPs are present almost everywhere around us, so, we come into direct contact with NPs intentionally or unintentionally. The easiest and direct route is air passage, as we inhale air, NPs made their way to lungs where they got opportunity to interact with lung surfactant [15]. Various techniques were used by previous researchers to investigate what NPs do as they come in to contact with lung surfactant system. One of the best instrument that is proved best in mimicking natural vertebrate lung system is Langmuir-Blodgett trough.

Langmuir-Blodgett Trough [14] is a versatile instrument normally used to compress the monolayer (a single layer of closely packed atom or molecules) of biomolecules deposited on a sub-phase (water or buffer solution). To form an insoluble monolayer (Figures 7 and 8), hydrocarbon chain should be long (n>12 i.e $(CH_2)n$), if the carbon chain is less than 12 then there are chances of formation of micelle which are soluble in water (Figure 9).

Langmuir monolayer, a name given to the insoluble, single, compact layer of organic molecules which spread onto water or buffer solution (sub-phase) used in Langmuir-Blodgett Trough.

Dr. Irving Langmuir was the first who came with the idea that single water-interface can show some interaction with solid substrate and thus the concept of Langmuir-Blodgett film was proved for the first time in 1917. Further advancement was made by Dr. Katherine Blodgett, who introduced the multilayer film model. Now a days LB trough are extensively applied in scientific research. Previously Brass LB trough were introduced but they were not durable as strong interaction of biomolecules with metal [16]. This issue is overcome by introducing Teflon coated LB troughs, which are resilient to almost all sort of possible hazards like interaction with biomolecules. Moreover, it is chemically inert.

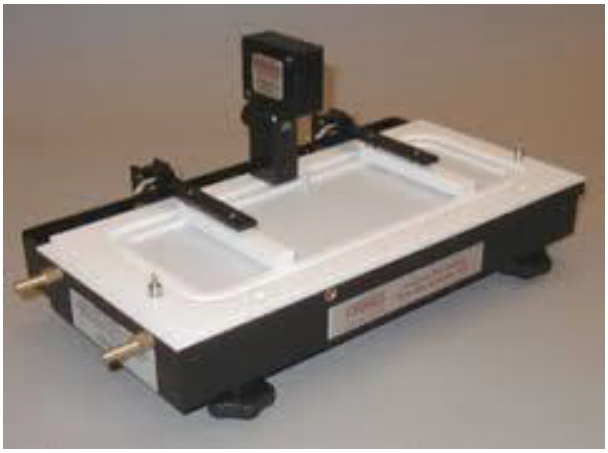

Figure 7: Langmuir-Blodgett trough.

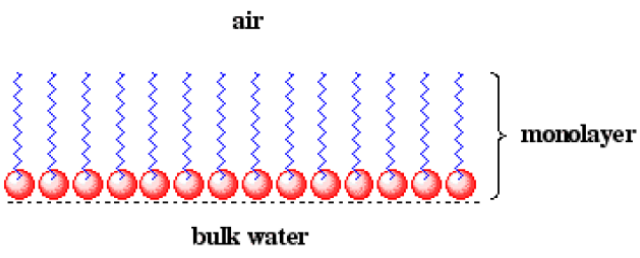

Figure 8: An insoluble monolayer formed at the water-interface by a surfactant.

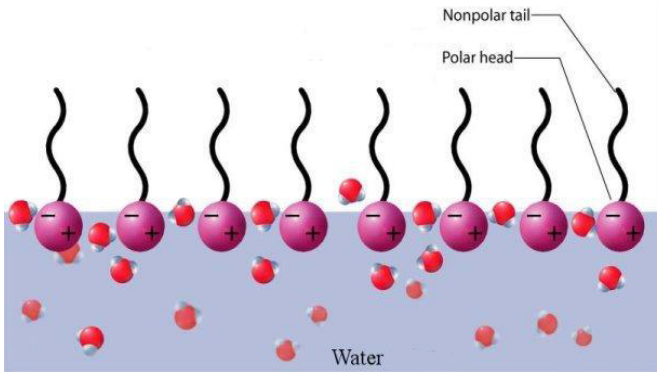

Figure 9: Surfactant molecule arranged on an air-water interface in Langmuir Trough.

Experimental Section

Authors dealt with (a) Langmuir-Blodgett Trough (Langmuir Small Microscopy Trough, Nima, UK), to study the interaction of soft NPs with lipid monolayers at air-water interface, and (b). QCM (Open QCM [17]), to study the interaction of soft NPs with lipid bilayers.

2.33961 g monobasic sodium phosphate (0.0195 moles) and 4.3298 g sodium phosphate dibasic (0.0305 moles) were mixed together in a 1 L volumetric flask by adding 2/3 of deionised water, mixed well and then filled the volumetric flak up to the mark with deionised water. Then checked the buffer solution for pH using pH paper, the pH was correctly 7.

Materials used

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC); $C_{40}H_{80}NO_8P$ (Figure 10), 1,2-Dimitrystoyl-sn-glycero-3-phosphorylcholine (DMPC); $C_{36}H_{72}NO_8P$ (Figure 11), 1-palmitoyl-2-oleoyl-sn-glyceero-3-phosphocholine (POPC): $C_{42}H_{82}NO_8P$ (Figure 12). All of them are purchased from a supplier; Avanti Polar Lipids, Inc.

Phase transition temperature

Phase transition also called phase change; defined as: "when different states of matter (solid, liquid, gas and in rare cases plasma) change from one state to another is called *phase transition* (Figure 13) and the temperature at which this transition takes place is called *phase transition temperature* [18].

Phase transition in lipids

Phase transition in Lipids means "When gel (ordered) phase of a lipid is changed to liquid crystalline (disordered) phase, is called phase transition (2D Transition) and the temperature at which this transition happens is called phase transition temperature for that lipid."

Phase transition temperature is of great concern, because when we will do extrusion we have to made lipid solution temperature above the phase transition temperature. Below phase transition temperature lipid would be in gel-like/solid phase, but for extrusion we need fluid phase which happens above the phase transition temperature.

Sample preparation of lipid solution

For the very first steps, approximately 10-20 mg of lipid in any recommended organic solvent (commonly chloroform) is mixed. Organic solvent is used because it plays an important role in; making clear solutions (a clear solution shows no lumps of lipids present and there is a complete mixing), and making homogenous mixtures of lipids [19].

Weighed 4 mg of DPPC lipid in weighing balance and added 4 ml chloroform to it (1 mg lipid in 1 ml chloroform). This solution is used to inject as surface monolayer in Langmuir Trough. While to make vesicles added 0.5 mg lipid in 1 ml chloroform. This lipid solution in chloroform is processed to make vesicles.

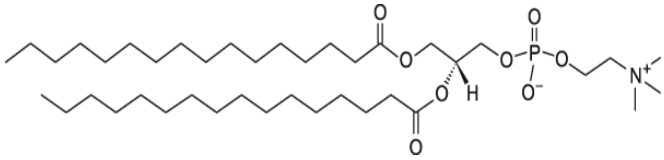

Figure 10: Structure of DPPC.

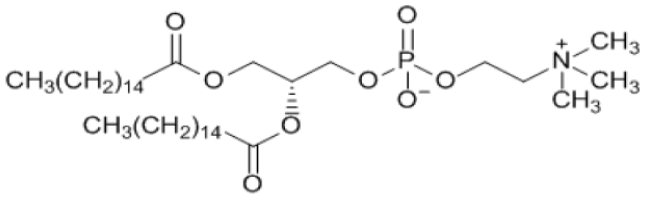

Figure 11: Structure of DMPC.

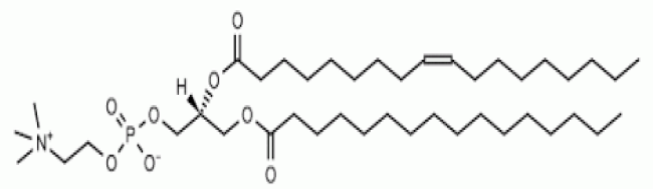

Figure 12: Structure of POPC.

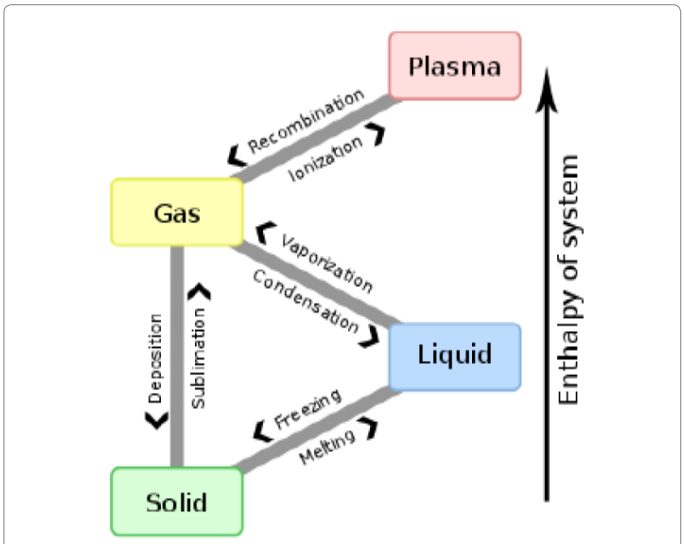

Figure 13: A schematic route showing the phase transition of different states of matter.

Composition of vesicles

In order to have better understanding of interaction of soft NPs with lung surfactant, lipid vesicles are prepared [20]. Vesicles consist of a tiny structure containing fluid and a lipid bilayer encapsulate that fluid. There are several different types of vesicles, to study the interaction of nanoparticles with lung liposomes were prepared. Liposomes are spherical vesicles having lipid bilayer. These are prepared usually as a result of sonication. In sonication, enough sonic energy is provided to disrupt the biological membrane to yield liposomes. Major types of liposomes include; Multilamellar vesicles (MLV), (size ≥ 0.5 μm), Small unilamellar vesicles (SUV), (size=20-100 nm), Large unilamellar vesicles (LUV), (size ≥ 100 μm).

Making of vesicles

Transferred the lipid solution (4 mg DPPC in 8 ml chloroform) to a new vial. Fixed the vial in a stand in fume hood and applied dry Nitrogen (N_2) to evaporate all the liquid. Usually it took 2-3 hours to completely evaporate it. Small volumes of lipid solution (<1 mL) are evaporated using by the use of dry nitrogen. Unlikely large volume of solutions 41 is evaporated using Rotary evaporation method. When fully evaporated, removed nitrogen supply. The vial is left with the dry film of lipid on the surface of glassware. This lipid dry film is then hydrated by using a variety of different hydrating media namely; Buffer solution, Distilled water, and Saline.

Formation of vesicles (liposomes)

Then solution (lipid film in 8 ml buffer) is processed to decrease the size of lipid vesicles. Various modes used to decrease the size of vesicles, are listed as; Sonication, and Extrusion.

Sonication

Sonication is a process that uses sound energy (sonic energy) to decrease the size of vesicles. A wide variety of instrumentation is used to sonicate the vesicle solution but the most commonly used are; Bath sonicator, and Probe-tip sonicator (Figure 14).

To decrease the size of particles in the solution in vial (containing vesicles+buffer), it is sonicated using probe-tip sonicator. First washed the tip of sonicator with deionised water. Immersed the probe-tip into vial and pressed the on button. Sonication usually performed for 3-4 mins.

Extrusion

Extrusion is a manual process to downsize the vesicle particles [21]. Extrusion is done by using an instrument; Avanti mini extruder (Figure 15), which uses polycarbonate filter and two syringes.in first syringe vesicle solution is filled and then manually propelled in second syringe (Cross section of Avanti mini extruder (Figure 16). This transfer is done for odd numbers usually 7 times and the resulting extruded vesicle solution is obtained in second syringe. Before performing extrusion, temperature of vesicle solution is made up to transition temperature, because below the transition temperature, lipid tends to be rigid (gel/solid) and for extrusion we need fluid phase which can be achieved above the transition temperature.

Filter size of 50 nm (pore size) was used in extruder. All the set up made in described way and then in first syringe (Hamilton) filled the 1000 μl vesicle solution, inserted inside the extruder on one side and the second Hamilton syringe on the other side. Slowly pushed all the vesicle solution from the first syringe into the second syringe and then from the second syringe to first one. Repeated this step 7 times and extruded vesicle solution got in second syringe. Transferred the extruded solution into a new vial and labelled it as; DPPC, which is vesicles sonicated and extruded.

Quartz crystal microbalance (QCM) study

To study the interaction of lipid vesicles with deposited lipid bilayer following steps were observed; 1. Cleaned the crystal with chloroform carefully, as crystal is very delicate it can break very easily if grasped tightly. After rinsing with chloroform let it to be dry in air 2. Then set the QCM frequency in air that should be less than 10 MHz which is 10017180.1 Hz. 3. Slowly injected 1.5 ml of water and noted drop in frequency. There is a decrease in frequency which is noted after being waited for 5 min which is 10015161.0 Hz. 4. Then very slowly injected 1.5 ml of isopropanol solution. Solution is injected very slowly because this instrument is really very sensitive so great care must be taken while injecting the solution. Waited for 5 min at which the frequency is 10014466.2 Hz. 5. Then very slowly and carefully injected 1.5 ml of DPPC in isopropanol (0.5 mg/ml), waited for 5 min at which the frequency is 10014234.7 Hz. 6. Then very slowly injected 1.5 ml of water resulted in the development of lipid bilayer and an increase in frequency but it was noticed that this increase in frequency is still lower than the frequency noticed at point. Waited for 5 min at which the frequency is 10014872.1 Hz. 7. Then again 1.5 ml of water was injected really very slowly and waited for 5 min at which the frequency=114352.8 Hz (*some suspected bubbles were noticed at this point) 8. Then very slowly injected 1.5 ml DPPC vesicle solution, waited for 5 min at which the frequency is 10015420.4 Hz 9. Then very slowly injected 1.5 ml of water and waited for 5 min at which the frequency is 10014988.6 Hz 10. Then again 1.5 ml of water was injected really very slowly and waited for 5 min at which the frequency is 10014992.8 Hz. 11. Then again 1.5 ml of water was injected really very slowly and waited for 5 min at which the frequency is 10014998.9 Hz.

Results and Discussion

Quantitative analysis

DPPC vesicles (sonicated+extruded) were then quantitatively analyzed to measure the size range of vesicles prepared. For this purpose, Dynamic Light Scattering was used and the instrument was Punk Unchained machine, in Biophysics Centre. DPPC vesicle solution sonicated for 4 min, extruded 7 times was taken to Biophysics lab. Set up the instrument with DPPC sonicated-extruded and loaded 5 μl of vesicle solution sample onto the blade cell then loaded the Blade cell.

Figure 14: Simple probe-tip Sonicator.

Figure 15: Avanti mini extruder.

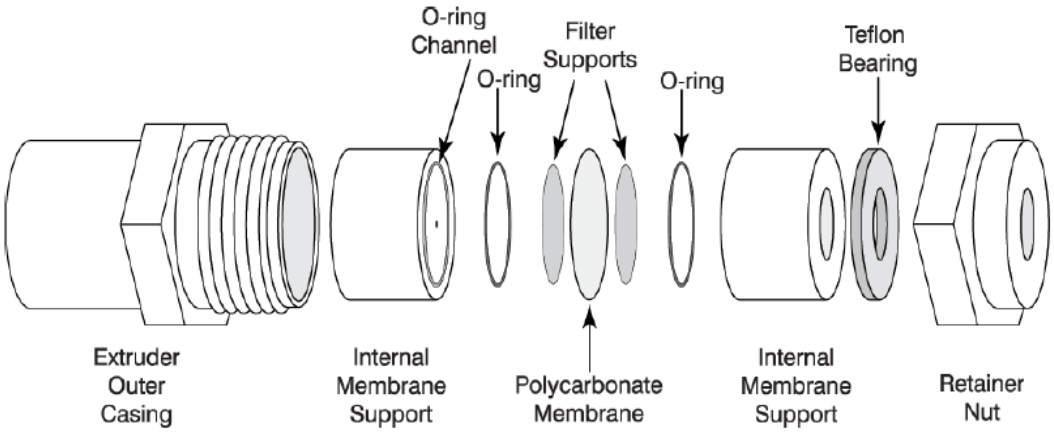

Figure 16: Internal cross-section of Avanti mini extruder.

Experiment was run and the range of size of vesicles in solution was noticed (all these steps were performed under the supervision of Dr Katherine Thompson). Size of the DPPC vesicles is 50 nm.The DPPC vesicle solutions ready to use in Langmuir-Blodgett trough (Figure 17).

Working on langmuir small microscopy nima, UK

Before each experimental use, trough is cleaned. For this purpose, one drop of washing liquid (Decon) in 100 ml graduated cylinder and filled the cylinder with deionized water up to 50 ml, poured all the water on the surface of trough and then evacuated it with the fitted evacuator, repeated it for seven times. Also, laboratory wipes with chloroform can also be used to wipe the trough surface. When cleaning is complete calibration is done for trough. In this case; Minimum area=21 cm^2 (standard), Maximum area=70.1 cm^2 (standard).

First, added 7 µl of DPPC lipid solution in chloroform as surface monolayer to the buffer and compressed the monolayer to surface pressure 25 mnm^{-1} (Area=50.3 cm^2), then waited for some time to become surface pressure steady and added 500 µl DPPC vesicle solution step by step at intervals of 10 minutes and noted the change in surface pressure. Then expanded the barrier to maximum area and noted the surface pressure. At end recompressed the barrier to the area 50.3 cm^2 and noted the change in pressure. When the saved data for this working was processed following results were obtained;

DPPC (monolayer) DPPC (vesicle), PiA (25 mN/m): Table 2 is showing the changes in surface pressure (mNm^{-1}) and area (cm^2) when DPPC (7 µl) added as monolayer, DPPC vesicles (500 µl) added, monolayer expanded to maximum area and then re-compressed to 50.3 cm^2.

DPPC (monolayer)-DMPC (vesicle), PiA (25 mN/m): Table 3 is showing the changes in surface pressure (mNm-1) and area (cm2) when DPPC (7 µl) added as monolayer, DMPC vesicles (500 µl) added, monolayer expanded to maximum area and then re-compressed to 50.3 cm^2.

DPPC (monolayer)-POPC (vesicle), PiA (25 mN/m): Table 4 is showing the changes in surface pressure (mNm-1) and area (cm2) when DPPC (7 µl) added as monolayer, POPC vesicles (500 µl) added, monolayer expanded to maximum area and then re-compressed to 30.4 cm^2.

DPPC (monolayer)-DPPC (vesicle), PiA (15 mN/m): Table 5 is showing the changes in surface pressure (mNm-1) and area (cm2) when DPPC (5 µl) added as monolayer, DPPC vesicles (500 µl) added, monolayer expanded to maximum area and then re-compressed to 28.4 cm^2.

DMPC (monolayer) DMPC (vesicle), PiA (15 mN/m): Table 6 is showing the changes in surface pressure (mNm^{-1}) and area (cm^2) when DMPC (5 µl) added as monolayer, DMPC vesicles (500 µl) added,

Figure 17: Langmuir Small Microscopy trough Nima, UK.

Sr. No.	Condition (Any addition made on Langmuir trough)	Area/cm²	Surface pressure/mNm⁻¹
1	DPPC monolayer only at maximum area	70	0.1
2	DPPC monolayer only after compression	50.3	25
3	10 minutes after DPPC vesicles injected below monolayer	50.3	17.8
4	Monolayer expanded to maximum area	70	9.2
5	Re-compressed	50.3	26.1
When DPPC lipid (about 7 µl) is added as surface monolayer to the buffer solution spread on Langmuir small microscopy trough and surface pressure is fixed at 25 mNm^{-1}, then DPPC vesicles are injected (500 µl) underneath the barriers. It is noticed when lipid vesicles come to surface and interact with monolayer there is an increase in pressure. In this case the increase in pressure noticed is from 25 mNm^{-1} to 28.4 mNm^{-1}. This increase is pressure is attributed to the interaction of nanoparticles with model lung surfactant film.			

Table 2: Changes in Surface pressure and area for DPPC (monolayer).

Sr. No.	Condition (Any addition made on Langmuir trough)	Area/cm²	Surface pressure/mNm⁻¹
1	DPPC monolayer only at maximum area	70	1.3
2	DPPC monolayer only after compression	30.5	25
3	10 minutes after DPPC vesicles injected below monolayer	30.5	9.9
4	Monolayer expanded to maximum area	70	-10
5	Re-compressed	30.5	27.44
DPPC lipid (about 7 µl) is added as surface monolayer to the buffer solution spread on the Langmuir trough pressure is again adjusted at 25 mNm^{-1}. Then DMPC vesicles (500 µl) are injected. When vesicles come to the surface and interact with surface monolayer there is an increase in surface pressure, in this case the surface pressure is almost 27.4 mNm^{-1}			

Table 3: Changes in surface pressure and area for DPPS (monolayer)-DMPC (vesicle).

Sr. No.	Condition (Any addition made on Langmuir trough)	Area/cm²	Surface pressure/mNm⁻¹
1	DPPC monolayer only at maximum area	70.1	0.5
2	DPPC monolayer only after compression	30.4	25
3	10 minutes after DPPC vesicles injected below monolayer	30.4	18.2
4	Monolayer expanded to maximum area	70	-0.6
5	Re-compressed	30.4	28.6
DPPC (about 7 µl) is added as surface monolayer to the buffer solution spread on the Langmuir trough and pressure is again adjusted at 25 mNm^{-1}. Then POPC vesicles (500 µl) are injected. When vesicles come to the surface and interact with surface monolayer there is an increase in surface pressure, in this case the surface pressure is approximately 28.6 mNm^{-1}.			
Note: when surface pressure was set at 15 mNm^{-1} the amount of lipid as surface monolayer is about 5 µl. The reason for this change was that when 7 µl was set then surface pressure cannot be set at 15 mNm^{-1} so, the amount of lipid is decreased to 5 µl.			

Table 4: Changes in surface pressure and area for DPPC (monolayer)-POPC (vesicle).

monolayer expanded to maximum area and then re-compressed to 29.3 cm^2.

DMPC (monolayer) POPC (vesicle), PiA (15 mN/m)

Table 7 is showing the changes in surface pressure (mNm^{-1}) and area (cm^2) when DMPC (5 µl) added as monolayer, POPC vesicles (500 µl) added, monolayer expanded to maximum area and then re-compressed to 33.0 cm^2.

DMPC (monolayer) DPPC (vesicle) PiA (15 mN/m)

Table 8 is showing the changes in surface pressure (mNm^{-1}) and area (cm^2) when DMPC 5 µl) added as monolayer, DPPC vesicles

Sr. No.	Condition (Any addition made on Langmuir trough)	Area/cm²	Surface pressure/mNm⁻¹
1	DPPC monolayer only at maximum area	70.1	0.3
2	DPPC monolayer only after compression	28.4	15
3	10 minutes after DPPC vesicles injected below monolayer	28.4	1.2
4	Monolayer expanded to maximum area	70	0.6
5	Re-compressed	28.4	13.8
DPPC (about 5 µl) is added as surface monolayer to the buffer solution spread on the Langmuir trough and pressure is adjusted at 15 mNm^{-1}. Then DPPC vesicles (500 µl) are injected. When vesicles come to the surface and interact with surface monolayer there is an increase in pressure but in this case the pressure is approximately 13.8 mNm^{-1}.			

Table 5: Changes in Surface pressure and area for DPPC (monolayer)-DPPC (vesicle).

Sr. No.	Condition (Any addition made on Langmuir trough)	Area/cm²	Surface pressure/mNm⁻¹
1	DPPC monolayer only at maximum area	70.1	0.1
2	DPPC monolayer only after compression	29.1	15
3	10 minutes after DPPC vesicles injected below monolayer	29.1	1.7
4	Monolayer expanded to maximum area	70.1	1.1
5	Re-compressed	29.1	12.74
DMPC (about 5 µl) is added as surface monolayer to the buffer solution spread on the Langmuir trough and surface pressure is again adjusted at 15 mNm^{-1}. Then DMPC vesicles (500 µl) are injected. When vesicles come to the surface and interact with surface monolayer there is an increase in pressure but in this case the surface pressure is approximately 12.74 mNm^{-1}.			

Table 6: Changes in surface pressure and area for DMPC (monolayer)-DMPC (vesicle).

Sr. No.	Condition (Any addition made on Langmuir trough)	Area/cm²	Surface pressure/mNm⁻¹
1	DPPC monolayer only at maximum area	70.1	0.9
2	DPPC monolayer only after compression	33.0	15.1
3	10 minutes after DPPC vesicles injected below monolayer	33.0	11.4
4	Monolayer expanded to maximum area	70.1	-2.1
5	Re-compressed	33.0	13.0
DMPC lipid (about 5 µl) is added as surface monolayer to the buffer solution spread on the Langmuir trough and surface pressure is again adjusted at 15 mNm^{-1}. Then POPC vesicles (500 µl) are injected. When vesicles come to the surface and interact with surface monolayer there is an increase in pressure and in this case the surface pressure is approximately 13 mNm^{-1}.			

Table 7: Changes in Surface Pressure and area for DMPC (monolayer)-POPC (vesicle).

Sr. No.	Condition (Any addition made on Langmuir trough)	Area/cm²	Surface pressure/mNm⁻¹
1	DPPC monolayer only at maximum area	70.2	1.0
2	DPPC monolayer only after compression	54.3	15.0
3	10 minutes after DPPC vesicles injected below monolayer	54.1	5.5
4	Monolayer expanded to maximum area	70.2	0.9
5	Re-compressed	54.3	10.9
DMPC lipid (about 5 µl) is added as surface monolayer to the buffer solution spread on the Langmuir trough and surface pressure is again adjusted at 15 mNm^{-1}. Then DPPC vesicles (500 µl) are injected. When vesicles come to the surface and interact with surface monolayer there is an increase in surface pressure and in this case the surface pressure is approximately 10.9 mNm^{-1}.			

Table 8: Changes in Surface pressure and area for DMPC (monolayer)-DPPC (vesicle).

(500 µl) added, monolayer expanded to maximum area and then re-compressed to 54.3 cm^2.

DPPC (monolayer) DMPC (vesicle), PiA (15 mN/m)

Table 9 is showing the changes in surface pressure (mNm^{-1}) and area (cm^2) when DPPC (5 µl) added as monolayer, DMPC vesicles (500 µl) added, monolayer expanded to maximum area and then re-compressed to 20.9 cm^2.

DPPC (monolayer) POPC (vesicle), PiA (20 mN/m)

Table 10 is showing the changes in surface pressure (mNm^{-1}) and area (cm^2) when DPPC (5 µl) added as monolayer, POPC vesicles (500 µl) added, monolayer expanded to maximum area and then re-compressed to 56.1 cm^2.

DPPC (monolayer) POPC (vesicle), PiA (15 m N/m): Table 11 is showing the changes in surface pressure (mNm^{-1}) and area (cm^2) when DPPC (5 µl) added as monolayer, POPC vesicles (500 µl) added, monolayer expanded to maximum area and then re-compressed to 55.5 cm^2.

Quartz crystal microbalance (QCM) results: When data was computed from information obtained from the above working following results (Table 12) were obtained; In QCM, when DPPC forms bilayer on gold surface and rinsed with water there was an increase in frequency but lower than the frequency recorded in water only. When DPPC vesicles in water added there was an increase in frequency and then as it was rinsed three 3 times with water until no change in frequency noticed.

Making of vesicles: When lipid film is hydrated it is noticed some lipids give clear solutions while others not. This behavior of different lipid solutions owes to their interaction to hydrating media (Table 13).

Formation of vesicles (Liposomes): When enough suitable energy is supplied to the large vesicle aggregates, they undergo to form unilamellar bilayer vesicles. This energy disrupts the biological membrane of vesicle aggregates and can be supplied in a variety of ways but the most commonly used are; Sonication, and Extrusion.

Sr. No.	Condition (Any addition made on Langmuir trough)	Area/cm²	Surface pressure/mNm⁻¹
1	DPPC monolayer only at maximum area	70.1	0.3
2	DPPC monolayer only after compression	20.9	15.0
3	10 minutes after DPPC vesicles injected below monolayer	20.9	10.3
4	Monolayer expanded to maximum area	70.1	0.1
5	Re-compressed	20.9	19.5
DPPC lipid (about 5 µl) is added as surface monolayer to the buffer solution spread on the Langmuir trough and surface pressure is again adjusted at 15 mNm^{-1}. Then DMPC vesicles (500 µl) are injected. When vesicles come to the surface and interact with surface monolayer there is an increase in surface pressure and in this case the surface pressure is approximately 19.5 mNm^{-1}.			

Table 9: Changes in Surface pressure and area for DPPC (monolayer)-DMPC (vesicle).

Sr. No.	Condition (Any addition made on Langmuir trough)	Area/cm²	Surface pressure/mNm⁻¹
1	DPPC monolayer only at maximum area	70	0.5
2	DPPC monolayer only after compression	56.1	20.1
3	10 minutes after DPPC vesicles injected below monolayer	56.1	18.4
4	Monolayer expanded to maximum area	70	10.9
5	Re-compressed	56.1	20.2
When DPPC lipid (about 5 µl) is added as surface monolayer to the buffer solution spread on the Langmuir trough and surface pressure is adjusted at 20 mNm^{-1}. Then POPC vesicles (500 µl) are injected. When vesicles come to the surface and interact with surface monolayer there is an increase in surface pressure and in this case the surface pressure is approximately 20.2 mNm^{-1}.			

Table 10: Changes in Surface pressure and area for DPPC (monolayer)-POPC (vesicle).

Sr. No.	Condition (Any addition made on Langmuir trough)	Area/cm²	Surface pressure/mNm⁻¹
1	DPPC monolayer only at maximum area	70	0.5
2	DPPC monolayer only after compression	55.5	15
3	10 minutes after DPPC vesicles injected below monolayer	55.5	7.2
4	Monolayer expanded to maximum area	70	1.8
5	Re-compressed	55.5	17.1
When DPPC lipid (about 5 µl) is added as surface monolayer to the buffer solution spread on the Langmuir trough and surface pressure is adjusted at 15 mNm^{-1}. Then POPC vesicles (500 µl) are injected. When vesicles come to the surface and interact with surface monolayer there is an increase in surface pressure and in this case the surface pressure is approximately 17.1 mNm^{-1}.			

Table 11: Changes in Surface pressure and area for DPPC (monolayer)-POPC (vesicle).

Components	Frequency (Hz)
Water only	10015161
Water+bilayer (DPPC)	10014872.1
Delta f/Hz for bilayer	288.9
Vesicles DPPC in water	10015420.4
Bilayer with vesicles after 1 rinse	10014988.6
Delta f/Hz on adding vesicles and rinsing	431.8

Table 12: Data obtained when DPPC is used as bilayer and DMPC vesicle solution.

Type of lipid	Hydrating Medium	Response
Highly charged	Low ionic potential	Viscous gel
Less hydrating		Aggregate

Table 13: General behaviour of different types of lipids in hydration media.

Sonication: The resulting vesicle solution was cloudy it is due to coarse particles, so sonicated the solution for more 3 mins. Freshly sonicated vesicle solution extruded to get finest particle size. Ultrasonic tip was used to sonicate the vesicle solution sample.

Sonication usually end up with particle size up to 12-50 nm.

Extrusion: Filter size of 50 nm (pore size) was used in extruder. All the set up made in described way and then in first syringe (Hamilton) filled the 1000 µl vesicle solution, inserted inside the extruder on one side and the second Hamilton syringe on the other side. Slowly pushed all the vesicle solution from the first syringe into the second syringe and then from the second syringe to first one. Repeated this step 7 times and extruded vesicle solution got in second syringe. Transferred the extruded solution into a new vial and labelled it as; DPPC, which is vesicles sonicated and extruded.

Conclusion

The results obtained from this research work using Langmuir trough, show a clear picture of interaction of soft NPs based vesicles with LS. NPs have tendency to make a relation with protein and lipid component of LS system. Lipid monolayer is set on surface, when lipid vesicle solution is injected underneath the barrier and waited for some time, they come up to surface and make an interaction with the surface monolayer [22]. Then the barriers were made open wider, so when the barriers were made open wide the monolayer is fully stretched and this surface film is compressed by moving the barriers close. Pressure-area isotherm is noticed when the barriers are compressed. As in this research work the intention was to study the interaction of nanoparticles with model LS system. It was decided to study the interaction of NPs based vesicles with LS at pressure; π=25 mNm^{-1}, π=15 mNm^{-1}. Using different

combinations of lipid monolayer and vesicle solution of different lipids, it was noticed that there is an increase in surface pressure, when after injecting vesicles, monolayer is re-compressed [23]. This result was in line with the previous information gathered on the study of interaction of NPs based vesicles with model LS film. A general trend is observed from results obtained on working of Langmuir trough, that is, there is an increase in surface pressure when surface monolayer of lipid solution is compressed by compressing the barriers [6]. In this case when lipid vesicles are injected underneath the barriers, vesicle solution come up to the surface develop an interaction with monolayer so it results in an increase in surface pressure. This trend is noticed in almost all the lipid solutions used in this research work. This increase in pressure is more pronounced when surface pressure of monolayer is adjusted at 25 mNm^{-1}. At high surface pressure, all the injected vesicles interacted with the surface lipid monolayer and as a result an increase in surface pressure is noticed. Unlikely when the surface pressure is fixed at 15 mNm^{-1}, no significant rise in surface pressure is noticed. One explanation to this question is at high surface pressure (25 mNm^{-1}), larger quantity of lipid was used while at lower surface pressure (15 mNm^{-1}), lower amount of lipid in chloroform was added to surface as monolayer. Moreover, it can be explained by providing the justification that the major task of lipid component of LS is to withstand higher surface pressure, when vesicles added to the surface monolayer (model LS), it interacted with this lipid monolayer, so arise in surface pressure is noticed.

When lipid vesicles are injected to the gold crystal containing gold NPs there is decrease in frequency. This decrease in frequency response is due to interaction of lipid vesicles with deposited bilayers [24]. It is assumed when vesicles are injected to supported lipid bilayer, they tend to graft themselves on the top of that bilayer and remain intact. Rinsing with water would remove some of the vesicles. As we keep on rinsing the QCM crystal, there is no more change in frequency. The reason is water rinsed the deposited bilayer and vesicles so no more interaction between them and frequency is stabilized [25]. In this work when different lipids are added to the QCM crystal drop in frequency is noticed. When lipid vesicles are added to the deposited lipid bilayer different responses were noticed. DPPC and DMPC vesicles brought a further decrease in frequency when added to DPPC bilayer [26]. POPC vesicles when added to DOPC bilayer a significant decrease in frequency is noticed. DOPC vesicles when injected to DMPC and POPC bilayer, decrease in frequency observed in both the cases but this decrease is more pronounced when bilayer is DMPC [22]. DMPC vesicles when injected to DOPC bilayer the decrease in frequency was very high. Interaction between DPPC vesicles and DOPC bilayer also followed the same trend of decrease in frequency [27].

Acknowledgement

Current Research work is carried out to investigate the interaction of soft NPs based vesicles with LS System, and the results declared are quite informative. Authors declared no funding or any financial assistance was taken from University or any other Research Centre during this work and authors have no conflicts of interest to disclose.

References

1. Baoukina S, Monticelli L, Amrein M, Tieleman DP (2007) The molecular mechanism of monolayer-bilayer transformations of lung surfactant from molecular dynamics simulations. Biophys J 93: 3775-3782.
2. Postle AD, Heeley EL, Wilton DC (2001) A comparison of the molecular species compositions of mammalian lung surfactant phospholipids. Comp Biochem Physiol A Mol Integr Physiol 129: 65-73.
3. Guzmán E, Liggieri L, Santini E, Ferrari M, Ravera F (2011) Effect of hydrophilic and hydrophobic nanoparticles on the surface pressure response of DPPC monolayers. J Phys Chem C 115: 21715-21722.
4. Raesch SS, Tenzer S, Storck W, Rurainski A, Selzer D, et al. (2015) Proteomic and lipidomic analysis of nanoparticle corona upon contact with lung surfactant reveals differences in protein, but not lipid composition. ACS Nano 9: 11872-11885.
5. Klenz U, Saleem M, Meyer MC, Galla HJ (2008) Influence of lipid saturation grade and headgroup charge: a refined lung surfactant adsorption model. Biophys J 95: 699-709.
6. Discher BM, Maloney KM, Schief WR, Grainger DW, Vogel V, et al. (1996) Lateral phase separation in interfacial films of pulmonary surfactant. Biophys J 71: 2583-2590.
7. Dwivedi MV, Harishchandra RK, Koshkina O, Maskos M, Galla HJ, et al. Size influences the effect of hydrophobic nanoparticles on lung surfactant model systems. Biophys J 106: 289-298.
8. Díaz-Moscoso A (2012) Soft versus hard nanoparticles in the delivery of aromatic macrocycles for photodynamic therapy of cancer. IJMBR 1: 12-23.
9. Sachan AK, Harishchandra RK, Bantz C, Maskos M, Reichelt R, et al. (2012) High-resolution investigation of nanoparticle interaction with a model pulmonary surfactant monolayer. ACS Nano 6: 1677-1687.
10. Liang H (2013) Non-labelled surface sensitive techniques as platforms for pharmaceutical nanotechnology research.
11. Kondej D, Sosnowski TR (2016) Effect of clay nanoparticles on model lung surfactant: a potential marker of hazard from nanoaerosol inhalation. Environ Sci Pollut Res Int 23: 4660-4669.
12. Mukherjee D (2015) Computational modeling of nanoparticle distribution and toxicity in biological systems. Rutgers the State University of New Jersey-New Brunswick.
13. Bourdos N, Kollmer F, Benninghoven A, Ross M, Sieber M, et al. (2000) Analysis of lung surfactant model systems with time-of-flight secondary ion mass spectrometry. Biophys J 79: 357-369.
14. Bolisetty VP (2013) Novel Approach of Using Polyvinylidene Fluoride Langmuir-Schaefer Film on Graphene-Polyaniline Nanocomposite for Supercapacitor Applications. University of South Florida.
15. Oosterlaken-Dijksterhuis MA, Haagsman HP, van Golde LM, Demel RA (1991) Characterization of lipid insertion into monomolecular layers mediated by lung surfactant proteins SP-B and SP-C. Biochemistry 30: 10965-10971.
16. Briand E, Zäch M, Svedhem S, Kasemo B, Petronis S (2010) Combined QCM-D and EIS study of supported lipid bilayer formation and interaction with pore-forming peptides. Analyst 135: 343-350.
17. Deakin MR, Buttry DA (1989) Electrochemical applications of the quartz crystal microbalance. Analytical Chemistry 61: 1147A-1154A.
18. Ross M, Krol S, Janshoff A, Galla HJ (2002) Kinetics of phospholipid insertion into monolayers containing the lung surfactant proteins SP-B or SP-C. European Biophysics Journal 31: 52-61.
19. von Nahmen A, Post A, Galla HJ, Sieber M (1997) The phase behavior of lipid monolayers containing pulmonary surfactant protein C studied by fluorescence light microscopy. Eur Biophys J 26: 359-369.
20. Dwivedi M, Sachan AK, Galla HJ (2014) Interaction of nanoparticles with lipid monolayers and lung surfactant films in Measuring Biological Impacts of Nanomaterials, Springer.
21. Yun HJ, Choi YW, Kim NJ, Sohn DW (2003) Physicochemical properties of phosphatidylcholine (PC) monolayers with different alkyl chains, at the air/water interface. Bulletin-Korean Chemical Society 24: 377-383.
22. Farnoud AM, Fiegel J (2012) Low concentrations of negatively charged sub-micron particles alter the microstructure of DPPC at the air-water interface. Colloids and Surfaces A: Physicochemical and Engineering Aspects 415: 320-327.
23. Kim JH, Kim HS, Lee JH, Choi SW, Cho YJ, et al. (2009) Hexagonally close packed langmuir-blodgett films from monodispersed silica nanoparticles. J Nanosci Nanotechnol 9: 7007-7011.
24. Hohner AO, David MPC, Rädler JO (2015) Controlled solvent-exchange deposition of phospholipid membranes onto solid surfaces. Biointerphases 5: 1-8.
25. Liu G, Zhang G (2013) QCM-D Studies on polymer behaviour at interfaces. Springer.

26. Klopfer K, Vanderlick T (1996) Isotherms of dipalmitoylphosphatidylcholine (DPPC) monolayers: features revealed and features obscured. Journal of colloid and interface science 182: 220-229.

27. Johannsmann D (2008) Viscoelastic, mechanical, and dielectric measurements on complex samples with the quartz crystal microbalance. Physical Chemistry Chemical Physics 10: 4516-4534.