The effect of two substances used in consumer spray products, Benzalkonuim chloride and Acudyne™DHR, on *in vitro* lung surfactant function

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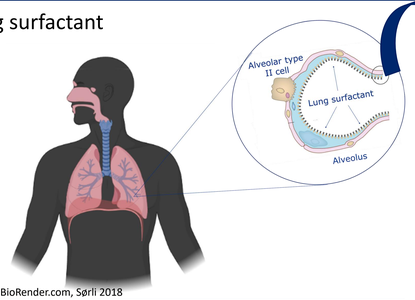
Key Question: Would the inhalation of certain surfactants and film forming polymers inhibit in vitro lung surfactant function if used in consumer spray products?

1. Investigate lung surfactant function inhibition; *in vitro* lung surfactant bioassay and Fourier Transform method.

a. Lung surfactant



Created by BioRender.com, Sørli 2018

Figure 1a: Lung surfactant (LS)
- A **thin film** at the air-liquid interface of the fluid lining the alveolar surface.
- Composed of a complex mixture of phospholipids (90%) and proteins (10%) that **regulates surface tension** at the air-liquid interface during respiration allowing **effortless breathing cycles**.
- Forms the **first point of contact** of inhaled particulates and chemicals in the air, this interaction may or may not interfere with **LS function**.
- If the interaction inhibits LS function, it can lead to alveolar collapse resulting in difficulty in breathing[1].

b. *In vitro* lung surfactant bioassay

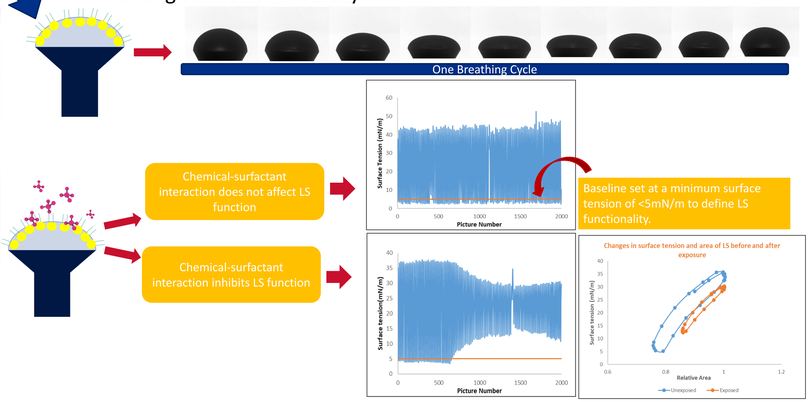


Figure 1b: *In vitro* lung surfactant bioassay to measure LS function:
The LS is placed on a pedestal which is enclosed in an exposure chamber, and cycled to an extent and frequency as that of the breathing of lungs. To simulate inhalation of chemicals, test substances are aerosolized into the chamber. If the interaction between the chemical and surfactant has no effect on surfactant function, **the minimum surface tension remains < 5mN/m**, however, if the interaction results in **disruption of surfactant function**, the regulation of surface tension is disrupted resulting **in high minimum surface tension** this can lead to alveolar collapse. LS images are constantly taken by a camera connected to the chamber, and analyzed by axisymmetric drop shape analysis (ADSA) software to calculate surface tension and surface area. The data is then used to plot the surface tension obtained from each image. Changes in the surface area of the drop results in changes in the surface tension as depicted by the surface tension as a function of the relative area graph [2-3].

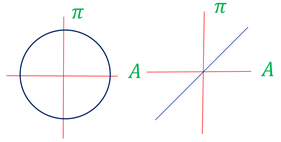
c. Fourier Transform Tensiometry Method

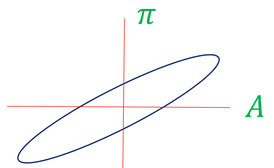
Periodic oscillation of droplet area $A(t) = A_0 sin\ \omega t$ and surface pressure $\pi(t) = E' \ln A_0\ sin\ \omega t + E'' \ln A_0\ sin\ \omega t$

where ω is mode of oscillation of the droplet size. Our method and E′ and E′′ are the surfactant monolayer storage and loss moduli respectively. The complex modulus is given by $E^* = E' + i\,E''$. The values of E' and E'' determine the viscoelastic properties of the surfactant monolayer (see right).

Data Processing Procedure

1. Dataset from times before and after exposure selected.
2. Largest mode of oscillation ω determined in pre- and post-exposure dataset using a Discrete Fourier Transform of $A(t)$ and $\pi(t)$ for pre- and post-exposure datasets.
3. Fourier Coefficients used to determine E^*_{pre} and E^*_{post}, the pre-exposure and post-exposure complex moduli respectively.
4. Surfactant inhibition determined from normalised change in complex moduli $\widetilde{\Delta E} = \frac{|E^*_{pre} - E^*_{post}|}{|E^*_{pre} + E^*_{post}|}$





- The current *in vitro* LS bioassay mimics the dynamic conditions of a breathing lung. It is capable of investigating changes in its surface-tension lowering property and compressibility of LS when exposed to a chemical.
- Previous studies extensively conducted to investigate the underlying premise of the molecular and biophysical disruption of LS when exposed to polymers indicate that LS monolayer becomes more viscoelastic in nature [4].
- Therefore, in this study we explore LS inhibition by studying the changes in its rheological properties by employing the Fourier Mode Dynamic Tensiometry method along with our established method of analysis

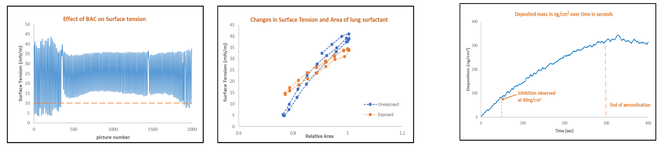
Advantages of comparing results with this model:
- It allows the investigation of rheological properties of a dynamic system, an essential part of biophysical understanding of the system.
- The current system deems a chemical inhibitory to LS function when the minimum surface tension >10mN/m, whereas the novel method uses changes in the rheological properties of LS, allowing us to detect smaller changes in surface tension.
- Allows automation and ease of analysis
- Makes analysis less restrictive.

2. Results from exposure of lung surfactant to Benzalkonium chloride (BAC,surfactant), Acudyne™DHR (film-forming polymer).

a) Determination of LS inhibition

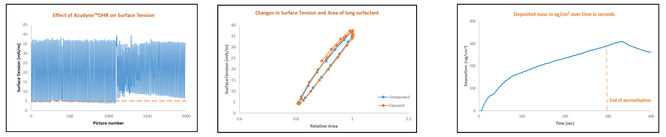
Effect on surfactant | **Deposition**

BAC LS function inhibited



Concentration of BAC: 0.1% , Exposure rate: 0.25ml/min

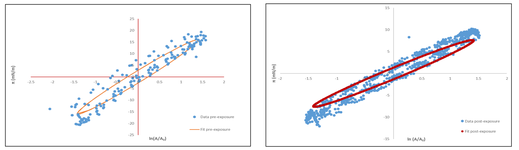
Acudyne™DHR LS function not inhibited



Concentration of Acudyne™DHR : 10% , Exposure rate: 0.25ml/min

b) Analysing data by Fourier Mode Dynamic Tensiometry

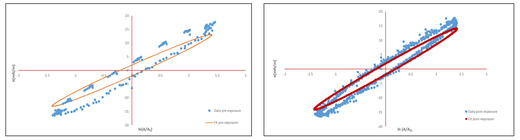
Fit data



E′ = 119.33 mN/m E″ = 20.8 mN/m
|E*| = 121.1 mN/m

E′ = 68.4 mN/m E″ = 12.19 mN/m
E* = 69.9 mN/m

$\widetilde{\Delta E} = 0.268$



E′ = 148.7 mN/m E″ = 22.7 mN/m
E* = 148.8 mN/m

E′ = 141.4 mN/m E″ = 19.5 mN/m E* = 142.8 mN/m

$\widetilde{\Delta E} = 0.02$

BAC – Exposure to 0.1% BAC for minutes at 0.25ml/min inhibits LS function resulting in a higher minimum surface tension at compression during exposure with a considerable increase in compressibility as seen in surface tension as a function of relative area graph (second breathing cycle , blue and third breathing cycle after inhibition, orange). Analysis by the Fourier Tensiometry method shows depicts that LS becomes more visco-elastic. Changes in the complex modulus determines surfactant inhibition. The effect of BAC on LS increases with increase in concentration and exposure rate due to an increase in the amount of chemical deposited on the QCM as shown in the deposition as a function of time. LS was exposed to 0.025%, 0.05%, 0.1% of BAC at a range of exposure rates – 0.1ml/min, 0.25ml/min, 0.5ml/min. LS inhibition was observed at 0.1%, and 0.05% at all exposure rates, and at 0.025% at the highest exposure rate (data not shown).

Acudyne™ DHR – Inhibition of LS function was not observed when exposed for 5 minutes to a range of concentrations of Acudyne polymer; 10%, 15%, 20% at different infusion rates; 0.1ml/min, 0.25ml/min and 0.5ml/min. The surface tension-relative area graph (second breathing cycle, blue and third breathing cycle after exposure, orange) shows no changes in compressibility pre and post exposure, although there was increase in the amount of chemical deposition on the QCM with increasing concentration and exposure rate.
E′ = storage modulus E″ = loss modulus E*= complex modulus

3: Conclusion

1. Analysing changes in rheological properties of lung surfactant when exposed to a surfactant or a film-forming polymer is a novel method to quantise their effect.
2. BAC, a quaternary ammonium compound, inhibits LS functionality, we are currently investigating the effects of other quatenary compounds and surfactants e.g. , DDAB, and PHMB, SDS. Acudyne, a film forming polymer does not change LS function, however, further analysis of the structure and chemical properties of various surfactants and polymers, will allow us to categorise the chemicals and predict toxicity.
3. Ongoing work: Collection of historical inhalation toxicity data on the use of above mentioned and various other surfactants and polymers and comparing it to the experimental data obtained from the *in vitro* LS bioassay

References:
(1.) (Da Silva et al., 2021) (2.) Sørli et al., 2016 (3.) Sørli et al., 2018 (4.) Da Silva et al., 2021