

# The Role of Cholesterol in Lung Surfactant

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## Lung Surfactant

Lung surfactant is a complex mixture of lipids and proteins secreted by alveolar type II epithelial cells and is essential to the mechanism of breathing.

### Function:

Lung surfactant forms a film at the air-water interface inside the alveoli where it functions in reducing surface tension, easing breathing and preventing alveolar collapse.



Courtesy of Zasadzinski Lab



A deficiency or dysfunction of lung surfactant results in disorders:

- Neonatal Respiratory Distress Syndrome (NRDS)
  - Acute Respiratory Distress Syndrome (ARDS)
  - Animal derived surfactants, such as Survant, are only successful in treating NRDS.
- Other drawbacks include possible viral contamination and batch to batch variation.

### Goal:

To understand the roles of the various components of lung surfactant in order to determine an optimum composition which will result in the best performance. This will hopefully lead to the development of a synthetic replacement surfactant.



## Cholesterol

Cholesterol is a neutral lipid component of lung surfactant.

- It accounts for 3-10 wt % of healthy native surfactant.
- Role of these physiological amounts of cholesterol is not well understood.
- ARDS is associated with elevated levels of cholesterol – 20 wt % or greater – which inhibit surfactant function.

### Replacement Surfactants:

Due to a debate over whether lower levels of cholesterol also inhibit surfactant function, cholesterol is removed from most FDA approved animal derived replacement surfactants.



|                                | Native Surfactant | Curosurf | Infasurf | Survanta |
|--------------------------------|-------------------|----------|----------|----------|
| Cholesterol (Avg. % by weight) | 5                 | 0        | 5        | 0        |

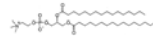
### Questions:

- Do pharmaceutical companies need to remove small amounts of cholesterol from replacement surfactants?
- What amount of cholesterol should be included in a synthetic surfactant?

Cholesterol Chart: Braun et al., Biophys. J. 93 (2007)

## Major Components of Lung Surfactant

### Phospholipids: 80-90 wt %



The main phospholipid, dipalmitoylphosphatidylcholine (DPPC), is responsible for the ability of lung surfactant to reach very low surface tensions.

### Proteins: 5-10 wt %



Survanta has two of the four proteins present in native surfactant: SP-B and SP-C. These aid in re-absorption by holding lipids close to the interface.

### Cholesterol: 3-10 wt %



Cholesterol is present in native surfactant but is removed from Survanta.

## Research Methods

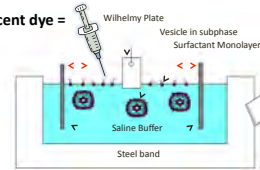
The functioning and morphology of a surfactant mixture is measured and observed.

Survanta lipids + cholesterol + SP-B + fluorescent dye =

Langmuir trough: an *in vitro* model of the breathing cycle.

Steel band: compresses and expands the surfactant film

Wilhelmy plate: measures surface pressure



Langmuir trough is mounted on a fluorescence microscope

## Results

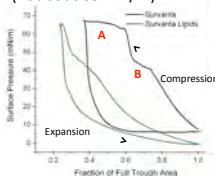
### Pressure vs. Area Isotherms:

These isotherms display the change in surface pressure as the area inside the Langmuir trough is changed. An ideal surfactant will reach surface pressures as close to 72.2 mN/m as possible with as little compression as possible.

### Survanta and Survanta Lipids

Survanta is used as a model of a healthy, good performing lung surfactant. Its isotherm has:

- A characteristic shoulder
- A collapse plateau (At about 65 mN/m)

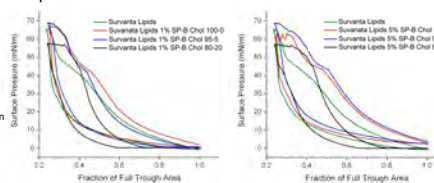


Survanta from which the proteins have been removed is called Survanta lipids. Its isotherm has:

- No shoulder
- No collapse plateau

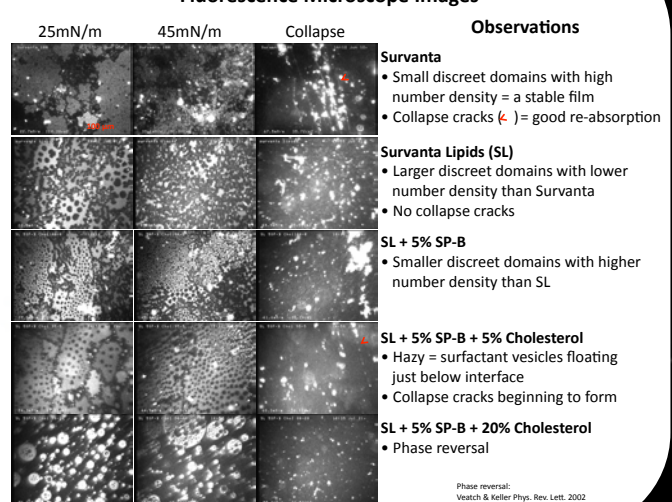
### SP-B and Cholesterol

The addition of SP-B to Survanta lipids improves absorption. This is indicated by the shift of the compression line to the right. The shift is more pronounced with 5% SP-B than with 1%.



- 5% cholesterol improves surfactant function:
  - improved absorption
  - higher surface pressures (about 69 mN/m) reached sooner
- 20% cholesterol inhibits surfactant function
  - absorption inhibited
  - high surface pressures not reached

### Fluorescence Microscope Images



Phase reversal: Veitch & Keller Phys. Rev. Lett. 2002; Radhakrishnan & McConnell Biochemistry 2002; Keller et al. J. Phys. Chem. 2000

## Acknowledgements

- Zasadzinski Group members
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- Cottage Hospital



## Summary

- The addition of SP-B and small amounts of cholesterol to a surfactant which lacks proteins and cholesterol improves function and alters surface film morphology.
- The addition of large amounts of cholesterol inhibits surfactant function and causes phase reversal.

### Future Work

- Study interaction of cholesterol with SP-C or a combination of SP-C and SP-B
- Use additional concentrations of cholesterol at smaller intervals to narrow down what is the exact optimum concentration
- Run experiments at physiological temperature