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Restoring the activity of serum-inhibited bovine lung extract surfactant (BLES) using cationic additives

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ABSTRACT

In this work four cationic additives were used to improve the surface activity of lung surfactants, particularly in the presence of bovine serum that was used as a model surfactant inhibitor. Two of those additives were chitosan in its soluble hydrochloride form with average molecular weights of 113 kDa and 213 kDa. The other two additives were cationic peptides, polylysine 50 kDa and polymyxin B. These additives were added to bovine lipid extract surfactant (BLES) and the optimal additive–surfactant ratio was determined based on the minimum surface tension upon dynamic compression, carried out in a constrained sessile drop (CSD) device in the presence of 50 μl/ml serum. At the optimal ratio all the BLES-additive mixtures were able to achieve desirable minimum surface tensions. The optimal additive–surfactant ratios for the chitosan chlorides are consistent with a previously proposed patch model for the binding of the anionic lipids in BLES to the positive charges in chitosan. For the peptides, the optimal binding ratios were consistent with ratios established previously for the binding of these peptides to monolayers of anionic lipids. The optimal formulation containing these peptides were able to reach low minimum surface tension in systems containing 500 μl/ml of serum, matching the effectiveness of a lung surfactant extract that had not undergone post-separation processes and therefore contained all its proteins and lipids (complete lung surfactant).

1. Introduction

There are several conditions that may lead to poor blood oxygenation associated with respiratory distress syndrome (RDS), however the lack or malfunction of lung surfactants is one of the most common conditions linked to this syndrome [1,2]. In pre-term neonatal patients diagnosed with RDS (neonatal RDS or nRDS) the syndrome is often associated with the lack of surfactants in the alveolar fluid. In patients diagnosed with Acute RDS (ARDS) the lungs may collapse due to surfactant malfunction caused by various surfactant inhibitors [1,3]. Surfactant therapy (instillation of surfactant extracts from animals) has reduced the mortality of nRDS patients from nearly 70% to less than 20%, but it has been ineffective in the treatment of ARDS [3–6].

Various inhibitors are associated with surfactant malfunction, including proteins like albumin and fibrinogen, and lipids like cholesterol, lysolecithins and unsaturated fatty acids. Bovine and human serum has been used as a broad spectrum surrogate to simulate surfactant inhibition [1,3]. Various formulations can overcome the action of low serum content (<30 μl/ml), but cannot handle higher serum content [7–9]. To assess the appropriate serum content to evaluate surfactant inhibition one should consider that bronchoalveolar lavages of ARDS patients contain up to 25 mg/ml of albumin [10]. Since bovine serum contains approximately 40 mg/ml of albumin suggests that approximately 600 μl/ml of serum is needed to evaluate surfactant inhibition.

One method used to evaluate surfactant inhibition *in vitro* involves measuring the surface tension of exogenous surfactants compressed and expanded under physiologically relevant conditions. In this work a constrained sessile droplet device (CSD) is used to measure the dynamic surface tension using the Axisymmetric Drop Shape Analysis (ADSA) [2,11–13]. Surfactant inhibition is typically characterized by either high minimum surfactant tension ($\gamma_{min} > 5$ mJ/m^2) and/or catastrophic film collapse (film collapse at high surface tensions) [1]. A typical surface tension–area–volume ADSA-CSD output for these studies is presented in Fig. 1 for a 2 mg/ml bovine lung extract surfactant (BLES) with 10 μl/ml of bovine serum, and a complete bovine lung surfactant with 750 μl/ml serum. This complete surfactant of Fig. 1 was obtained by saline lavage from calf lungs and simply lyophilized without further solvent separation steps (used to produce BLES) that remove surfactant proteins SP-A and SP-D and part of the proteins SP-B and SP-C. As indicated by Fig. 1, a common exogenous surfactant (BLES) cannot reach low surface tensions ($\gamma_{min} \sim 20$ mJ/m^2)

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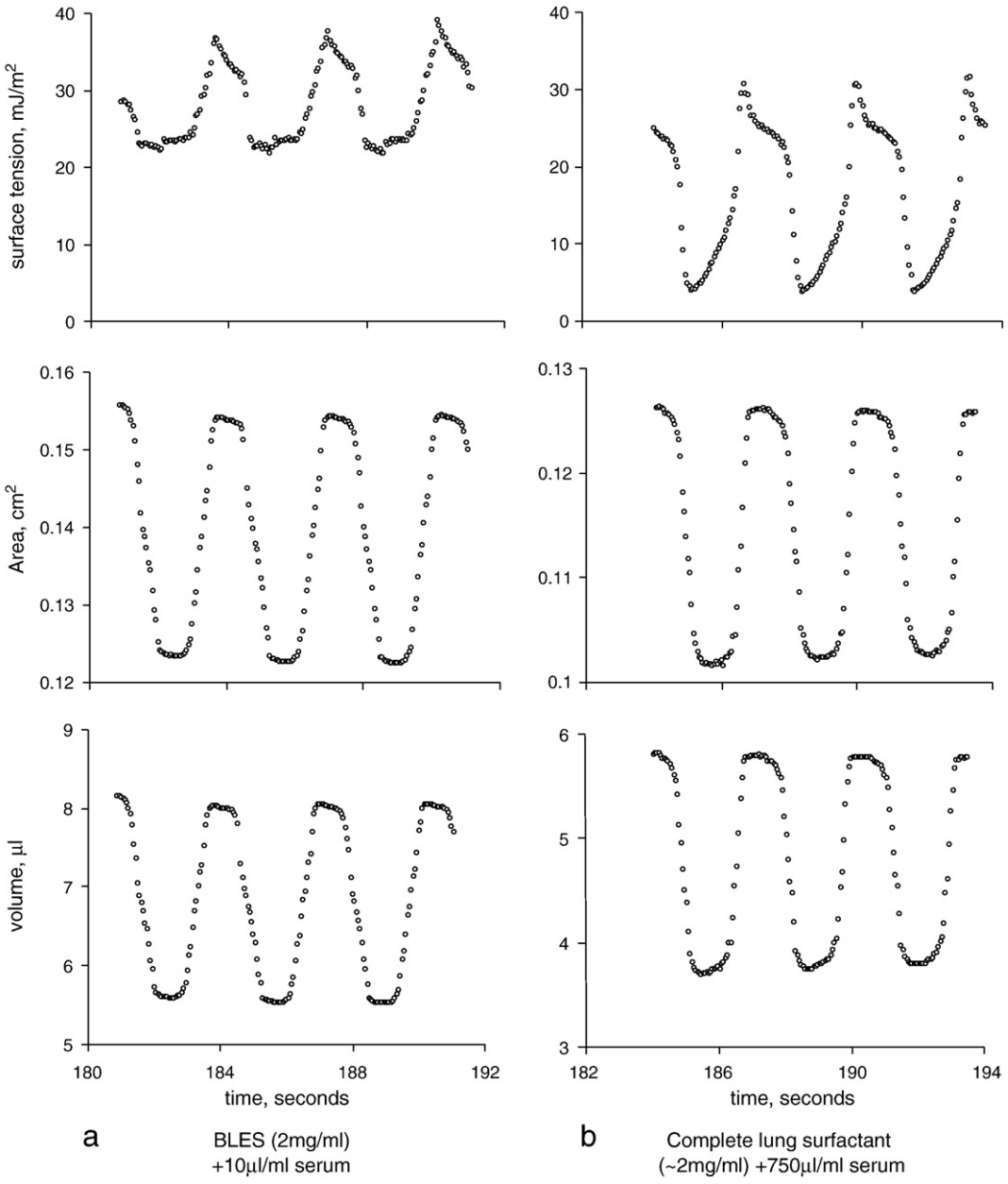

Fig. 1. Dynamic surface tension, area and volume output from ADSA for (a) 2 mg/ml BLES in the presence of 10 µl/ml serum, and (b) complete lung surfactant (~2 mg/ml) in the presence of 750 µl/ml serum. Dynamic cycling conditions: 20% compression (reduction in area), 3 s/cycle, 100% R.H., 37 °C.

when evaluated in the presence of serum and in humid air. However, a complete lung surfactant can produce minimum surface tensions of 5 mJ/m^2 or less even in the presence of 750 µl/ml of serum.

The difference between BLES and the complete lung surfactant used in Fig. 1 is that in order to produce BLES the surfactant lavage has undergone further purification with organic solvents that remove the hydrophilic surfactant proteins SP-A and SP-D, part of the cholesterol, and part of the surfactant proteins SP-B and SP-C [2,6]. Furthermore, the process of solvent extraction may also disrupt the original structure of lipid–protein complexes. Several additives have been proposed in order to make up for these changes in surfactant compositions. For example, neutral and hydrophilic polymers like dextran and polyethylene glycol have been used as surfactant additives [2,7,14–18]. Promising results have been observed *in vitro* with these additives, in particular against albumin-induced inhibition, but there are conflicting results in vivo [19]. Furthermore, mixtures of BLES and polyethylene glycol evaluated with ADSA-CSD were effective against 2.5 mg/ml albumin, but ineffective against 10 µl/ml of serum [20]. Anionic polymers such as hyaluronan and mucins have been found effective at reversing serum inhibition [20–22]. Nonionic and anionic polymers are said to simulate the role of SP-A in the preparations [2,7,18]. On the other hand, a cationic polysaccharide, chitosan, has been found to be more effective than nonionic and anionic polymers, and produce formulations that are consistently active regardless of the batch to batch variability of the extract [23,24]. A recent study has shown that chitosan chloride is effective at resisting the action of various inhibitors including albumin, fibrinogen, serum (50 µl/ml), and cholesterol [25]. Other cationic additives like polylysine, recombinant surfactant protein-C, synthetic peptide KL_4 and the biosurfactant polymyxin B have been proposed as additives that simulate the role of proteins SP-B and SP-C, which are essential for lung surfactant activity [26–30]. However, their effectiveness against high serum content has not been evaluated. These findings led to the hypothesis that using the proper concentration of cationic additives in BLES it is possible to overcome serum levels that simulate the inhibitory conditions of ARDS patients. In this work, four