Surface Properties of Pulmonary Surfactant Sampled by Bronchoalveolar Lavage and by Electrostatic Exhaled Aerosol Trapping

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Abstract. The development of efficient methods for non-invasive collection of alveolar lining fluid (ALF) samples containing pulmonary surfactant (PS) components and the study of the surface activity of the obtained native material is relevant for the diagnosis of inflammatory pneumopathies of the lungs. The paper presents an electrostatic aerosol trapping (ESAT) mobile complex for capturing droplets of ALF contained in an exhaled air. Passing the exhaled air through the corona discharge area results in the aerosol droplets charging and their further transferring by electrostatic force into a water surface, where they accumulate forming an adsorbed layer. Additionally, ALF samples were collected using a bronchoalveolar lavage (BAL). The surface properties of the PS obtained by both methods have been examined using the capillary wave method, which was previously modified by the authors specifically for biomedical applications. Significant difference was found in the results obtained with ESAT and BAL in the group of healthy subjects, which can be explained by different origin of the samples obtained by these techniques. Furthermore, significant difference in surface properties was established in the samples collected from healthy volunteers and patient with disseminated tuberculosis, while we did not find significant differences in the limited inflammatory process. The results presented in the paper demonstrate high potential of the proposed non-invasive technique for clinical usage.

1. Introduction

Pulmonary surfactant (PS) complex consisting of a mixture of different lipids and proteins is the main part of alveolar lining fluid (ALF) covering the surface of alveoli and distal airways. PS plays a vital role in pulmonary physiology. The most important biophysical function of PS is to reduce the surface tension on the interface of the alveoli with the air, which decreases the energy consumption at breathing and prevents atelectasis (collapse of lung alveoli) at the end of expiration. Non-biophysical functions include protecting the lungs from injuries and infections caused by inhaled particles and microorganisms.

A variety of pulmonary diseases (such as asthma, pneumonia, adult respiratory distress syndrome, tuberculosis, etc.) are able to cause surfactant deficiency or to change its composition, which reduces the PS surface activity, provoking the alveolar instability and development of inflammatory processes in the lung [1-4]. Since each PS component plays an important role in the surface properties, any noticeable deviations from the optimal PS composition or changes of PS concentration can lead to dysfunction of the entire PS system. The examination of the surface-active properties of PS is an effective way for early diagnosis of pulmonary diseases and for treatment monitoring. A native material comprising the PS can be obtained from a lung by invasive or non-invasive way. Today the most well-known and well-elaborated invasive method is bronchoalveolar lavage (BAL) [5, 6]. It provides a high PS concentration in the samples which makes it possible to study the surface-active properties of PS.
with tensiometric methods [7]. Because of the risk of affecting the airways during BAL this method of PS collection cannot be used frequently and is contraindicative to some groups of patients. A certain amount of PS can be detected in the exhaled breath condensate (EBC) [8]. This method was actively developed from the early 1980’s. However, since this noninvasive method has been specifically designed for collecting volatile substances, the content of non-volatile PS’s components turns out to be rather low and the results of surface-active properties examination demonstrate low repeatability.

Human breathing is accompanied by the formation of small droplets of ALF, which are emitted from the lungs with exhaled air in aerosol form. The droplet formation mechanism is associated with closure and reopening of the airways during breathing [9, 10, 11]. The exhaled air of a healthy human during normal breathing contains, on average, a few submicron particles per cubic centimeter [12-14]. Although the particle droplets size distribution and related particle concentrations demonstrate high reproducibility within one subject, there also exist high inter-subject variability [15] and strong dependence on the type of breathing and the breathing maneuvers before sampling [16, 17]. Analysis of the droplet composition has revealed that the aerosol particles originate from ALF and contain all its components in undiluted concentration [18-20]. Significant differences were established between the aerosol characteristics and the droplet composition collected from the groups of subjects with chronic obstructive pulmonary disease [21], asthma [21], cystic fibrosis [11] or pulmonary tuberculosis [22] and from healthy subjects. Thus, the emitted droplets are ALF microsamples, and therefore their trapping provides a non-invasive way for obtaining native material directly from the respiratory tract.

In recent works [23, 24] an electrostatic precipitation on solid substrate was proposed as an effective way to trap the exhaled particles. The method demonstrated high efficiency, allowing to trap up to 80% of the exhaled particles. The collected material was applied for structural analysis with atomic force microscopy [23] or for biochemical examination with microfluidic lab-on-chips [24]. Electrostatic precipitation on a water surface was recently proposed by the authors of this paper [25]. The PS containing in the trapped droplets formed an adsorbed layer, which surface-active properties can be examined with a tensiometric method. For that purpose we applied the modified capillary wave method [26] which is sensitive to even small changes in surface properties. The electrostatic aerosol trapping (ESAT) system, measuring module and data processing were carefully tested both with model aerosol and with exhaled air of healthy subjects.

In this paper, first, we present a compact ESAT system (hereafter ESAT-mobile) for the clinical usage. Second, we demonstrate that capillary wave method can be effectively applied to the study of surface-active properties of the PS samples obtained by BAL. This requires much less amount of the sample and allows to measure dynamic characteristics, which are inaccessible for other tensiometric methods. Finally, we present the results of comparative analysis of the surface-active properties of the samples achieved with ESAT and BAL collected within the groups of both healthy volunteers and subjects with lung pathology.

2. Experimental setup

The ESAT-mobile complex designed for clinical usage is presented in figure 1. The air exhaled by a subject 1 moves through a silicone tube 3 of 1.2 cm inner diameter and goes to the reservoir 3. After the end of the exhalation, the air is squeezed out into the electrostatic collection system with less flow rate using motorized moving guide 5 installed on the platform 4. The presence of such motorized guide is the main distinction of the ESAT-mobile setup from that described in [25]. Its usage allows to provide the low flow rate through the ESAT system increasing thus the trapping efficiency, keeping at the same time the subject’s breathing free.

The air from the reservoir 3 goes to the trapping system described in [25]. The exhaled air comes through a corona discharge created near a thin tip of a stainless steel needle connected to a negative electrode of the high voltage power supply. The aerosol droplets, which become electrically charged, are transported by the electrostatic force toward the grounded collecting electrode placed at the bottom of the cylindrical glass cuvette 6 of 1.6 cm in diameter and 0.05 cm in depth. The droplets moving towards the collecting electrode coalesce with the water surface and adsorb on the water surface forming the surface-active layer which properties are investigated by the modified capillary wave method [26]. For that purpose the cuvette was moved in the upper position (see figure 1(b)).
A cylindrical capillary wave was excited by the acoustic impact (1-10 kHz) by the speaker 7. The profile of the surface was registered using digital interferometry by the optic system 8. The implementation of this method for the study of lung surfactant layers is described in [25]. Further post-processing of the surface profiles images were carried out with the original algorithm introduced in [27], which allows to measure capillary wave wavelength and attenuation coefficient in automatic regime with high accuracy. The instantaneous surface profile is complex, it is a superposition of the static meniscus and small-scale capillary waves. As the spurious signal and the capillary wave have different spatial scales, the post-processing filtration procedure allows us to split these signals, which makes vibroinsulation of the whole setup unnecessary. The key advantage of the modified capillary wave method is very small surface area (1 cm$^2$) and, as a consequence, small amount of the native material is required. All modules of the setup were fixed at the support frame (0.5 × 1 × 0.5 m$^3$) having three-point base for the setup alignment.

To quantify the surface-active properties, we have plotted the surface pressure $\Pi$ as a function of the relative surfactant surface concentration $\Gamma/\Gamma^*$. For normalization we have used surfactant surface concentration $\Gamma^*$ which corresponds to the phase transition in the adsorbed layer. The surface pressure $\Pi$ is introduced as a difference between surface tension on a clean water surface $\sigma_0$ and that in water surface containing the adsorbed layer $\sigma$. By the definition $\Pi$ is equal to zero at clean interface. Additionally, we have plotted the attenuation coefficient $\beta$ as a function of the relative surfactant surface concentration $\Gamma/\Gamma^*$, which characterizes viscous and viscoelastic properties of the adsorbed layer.

Typical dependencies of the surface pressure $\Pi$ and attenuation coefficient $\beta$ on the dimensionless surfactant concentration $\Gamma/\Gamma^*$ for the samples obtained with the ESAT-mobile system in a healthy subject are presented in figure 2. The different curves correspond to different capillary wave excitation frequencies. It is seen that the $\Pi$ is frequency-independent and all curves form a unified dependence, which is called the surface pressure isotherm. Contrary, the attenuation coefficient essentially increases with the excitation frequency. The dependence is non-monotonic with a pronounced maximum, which position along the abscissa axis is slowly shifted towards lower surface concentrations as the excitation frequency rises. The excitation of a capillary wave on the water surface containing surfactant is accompanied by the appearance of a dilatation wave associated with stretching and compression of the surfactant film. This additional dissipative mechanism amplifies wave attenuation. It is known that the local maximum of the attenuation coefficient of the capillary wave is observed when excitation its frequency coincides with the natural frequency of the dilatation wave [28].

Two groups of subjects participated in the study. The first group consists of healthy volunteers: 10 subjects aged from 25 to 70, 6 males and 4 females. The second group included 5 subjects with tuberculosis: 4 subjects with infiltrative pulmonary tuberculosis with a prevalence of up to 1 lobe of the lung on one side (BAL was taken from the lower parts of the affected lung), and 1 subject with...
disseminated pulmonary tuberculosis with a widespread bilateral lesion, progressive course. The study was approved by the local ethical committee of Perm State Medical University, all subjects were informed about purposes of the study and signed informed consent. Two kinds of native material, sampled with ESAT and with BAL methods, were used in the study. In the latter case a small amount (about 30 μl) of BAL was put on the clean water surface with a micropipette.

![Figure 2](image1.png)

**Figure 2.** The typical dependence of the surface pressure Π (a) and attenuation coefficient β (b) on dimensionless surfactant concentration Γ for different capillary wave excitation frequencies.

### 3. Results

Figure 3 compares the Π(Γ/Γ*) (a) and β(Γ/Γ*) (b) dependencies obtained in healthy subjects with ESAT and BAL techniques. It is seen that the surface pressure isotherms essentially differ from each other, whereas the dependencies for the attenuation coefficient coincide. The observed discrepancies between the results obtained for the native materials sampled from the lung in a different way can be explained as follows.

Due to the relatively large diameter of the probe (several millimeters), a sample collected with BAL is originated from both the bronchial part and the alveolar part of the lung. Moreover, most of the lavage occurs from the bronchial part. At the same time, due to the mechanism of ALF droplets formation the aerosol particles can form only in alveoli and the farthest, closest to the alveoli, airways, which diameter is no more several hundred micrometers. Thus, the difference in composition of alveolar and bronchial lining fluid causes the distinctions in the surface-active properties of the native materials sampled with these two methods.

![Figure 3](image2.png)

**Figure 3.** The dependence of the surface pressure Π (a) and β (b) on dimensionless surfactant concentration Γ of the samples obtained in the group of healthy volunteers by BAL and EAST techniques.

The surface pressure isotherms and attenuation coefficient as a function of relative surface concentration measured for the samples obtained with BAL are presented in Figure 4. For comparison,
the results obtained within the groups of both healthy volunteers and subjects with lung pathology are presented. A significant difference between the results of healthy volunteers and a patient with a disseminated form of the disease is clearly seen. The low surface activity of the sample in the latter case might be caused both by PS deficiency and by essential deviations in its composition owing to disease. At the same time, the dependencies obtained in patients with a limited specific process differ slightly from the isotherms of the healthy group. The revealed difference can serve as a marker for assessing PS system dysfunction.

Figure 4. The typical dependencies of the surface pressure \( \Pi \) (a) and \( \beta \) (b) on dimensionless surfactant concentration \( \Gamma / \Gamma^* \) of alveolar fluid samples obtained in the group of healthy volunteers and subjects with lung pathology by BAL method.

4. Conclusion
In this paper, we present the results of a studying the surface-active properties of samples of an ALF collected non-invasively due to the electrostatic trapping of aerosol droplets from the exhaled air. The study was carried out with ESAT-mobile system specially designed for the clinical usage. The system is compact and mobile and requires no special conditions for application. Moreover, automated data processing requires minimal skills from the operator. The surface-active properties of the samples collected with the ESAT system were examined with modified capillary wave method [29]. The method allows us to measure the surface pressure in the adsorbed layer and the attenuation coefficient of the capillary wave as a function of the relative surface concentration of the PS. The results obtained in a group of healthy subjects show good agreement between each other. Taking into account that concentration and size distribution of the aerosol particles demonstrate wide data spread among subjects, the results obtained in the paper allows us to consider them as a promising criterion for assessing the state of PS system.

The surface properties measured for the samples collected with ESAT-mobile system were compared with those measured for the samples obtained with BAL which is the most common invasive method for ALF collection. Until now, studies of the surface properties of lavages have been carried out by static tensiometry methods, for example with Langmuir trough, which requires large volumes of material. The modified capillary wave method utilized in this work is dynamic which gives us possibility to get additional information. Moreover, the amount of BAL required for the study is less than 1 ml which makes the procedure of lavage sampling easier and safer for subjects. The comparative analysis of the results obtained for the samples collected with these two methods demonstrated essential difference in pressure isotherms. The possible explanation might be that the BAL is the mixture of alveolar and bronchial fluids, whereas aerosol particles collected by ESAT system originate from alveoli and distal airways and, therefore, consist of solely alveolar fluid. These results indicate the native material trapped from exhaled air to be more preferable at the assessment of the state of PS system.

We have used the capacity of the modified capillary wave method to study surface-active properties for comparative examination of the BAL collected in healthy subjects and subjects with pulmonary tuberculosis. We have found a significant difference between the results of healthy volunteers and a patient with a disseminated form of the disease, whereas the dependencies obtained in patients with a
limited specific process differ slightly from the isotherms of the healthy group. These results indicate the high potential of the method as regards to a primary detection of the pulmonary surfactant system dysfunction and to a pulmonary diseases treatment monitoring.

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