frontiers

Mucus microrheology measured on human bronchial epithelium culture

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Supplementary Material

1 Methodology

1.1 Optical tweezers for biological samples

The use of optical tweezers for microrheology involves various experimental subtleties and even more specifically when used with biological samples. We discuss here some technical aspects that required to be checked to validate our experimental approach.

Probe size and chemistry. The size of the probe (bead) a has as impact on the laser stiffness k and on the viscoelastic moduli determination (see formula in the main text). For accurate measurements a determination of the bead size a and of the corresponding optical trap stiffness k are required before each measurement. In our experiment, we use beads of carboxylated melamine resin of diameter 3 µm (Fluka Analytical, Sigma-Aldrich) with a standard deviation of 0.2 µm. Beads are checked optically under microscope and the ones outside the deviation are rejected. This incertitude on the probe size creates a relative error of 6.7%, on the viscoelastic moduli is much lower than the dispersion coming from the spatial heterogeneity of the mucus. The mean value of 3 µm given by the bead provider was then used. The bead chemistry must to have an optical index (1.68 for the melamine resin) clearly different to the sample one to be well trapped. A high optical refractive index was preferred in order to reduce the laser intensity necessary to achieve a given trap stiffness. The mechanical strength (typical elastic modulus of melamine being between 4 and 9 GPa) and the thermal stability (up to 300 °C for the melamine) of the probe are also important parameters. Moreover, the probe chemistry or its coating have to minimise the adhesive effects to the sample to don't affect the viscoelastic measurements.

Characterization and quality of the optical trap. Absorption and scattering are expected to degrade the performance of optical trapping when entering in a non-homogeneous medium. The effects are however reduced in thin samples and if near infra-red lasers (here, 1064nm) are chosen so it is still possible to trap efficiently particles well within living tissues [1]. For a quantitative analysis, an optical trap is however modified when entering deeper into the medium and, one should carefully take into account two main effects leading to a change of the trap stiffness: the light intensity loss and the shape of the trap. We have systematically characterized in situ the trap stiffness from the local optical potential analysis for the less elastic samples (obtained from the Brownian motion of the bead inside the trap). The measurement of the trap stiffness by this method does not depend of the local rheological

properties of the medium. In our article we showed microrheology results on several kind of samples that have different concentrations in impurities. Inside the mucus sample from ALI cultures the impurities are mainly dead cells and cell debris (the mucus by itself is transparent) and for the "ex vivo" mucus there is also a limited amount of red and white blood cells. In the Figure S3 we show typical examples of impurities concentrations corresponding to each kind of sample. In FigS1A and FigS1B is shown the concentration inside mucus collected from ALI cultures with BEGM medium, in FigS1C the typical concentration of the mucus layer close to the tissue of ALI cultures with BEGM medium, and in FigS1D the typical concentration of the mucus from ALI cultures with Pneumacult medium and the "ex vivo" mucus. In order to evaluate more systematically the influence of the height and the presence of the cell and debris on the optical trap, we prepared water samples, pure and with cells and debris concentrations corresponding to the mucus from ALI cultures with BEGM medium (FigS1B and FigS1C). Thanks to the Brownian motion recorded and analysed on 10 min at low laser power (8 mW) we have finely characterized the evolution of the optical trap with the height. Results are shown in Figure S2 and Figure S3. Analysis of the trap at several heights through the water with increasing concentrations (FigS1B and FigS1C) of dead cells and debris show that the trap homogeneity is conserved and there is no major deformation of the optical trap, as shown in Figure S3. The laser trap remains axisymmetric as illustrated on the FigS3C. After crossing the whole sample (100µm thick), the image of the reflection of the laser beam on the glass slide (FigS3D) has still a circular shape even if tiny speckles are present. The change of trap stiffness with height over 100 µm is very similar for the 3 concentrations: pure water (FigS2A), less than 1% in volume like the mucus collected from ALI cultures with BEGM medium (FigS2B), and less than 5% like the mucus layer close to the tissue of ALI cultures with BEGM medium (FigS2C). A typical decrease of 35% is observed through the sample. Even if we pass through a cell with the cell directly vertical under the laser trap, the loss of the trap stiffness k is also around 35% (FigS2D). One can conclude that the effect of the debris can be neglected in a first approximation.

Effect of the temperature. In our experiments a thermocouple, directly stuck on the coverslip, gives the global temperature of the sample. The local temperature of the bead is however unknown accurately and can be higher. Observations performed with the same beads on other dilute aqueous samples such as lyotropic liquid crystals or giant micelles give indirect indications of the temperature shift due to the laser intensity. At the most powerful intensities used here (~1W), both the phase transitions of the liquid crystals and the very sensitive rheology measurements of giant micelles [2], indicate a limited heating below 10°C. This in typical accordance with the rule of thumb that 1064nm laser in vivo give a typical increase of about 1 °C/100 mW [3]. We can assume that inside the mucus, a poorly absorber in the near infra-red, the local heating doesn't exceed this value. Moreover, as shown in Figure S4, the temperature doesn't change the mucus viscoelastic behaviour as a function of the frequency. The decrease of the viscoelastic values doesn't exceed 50% for a 7°C temperature increase. Macrorheology measurements of BEGM collected mucus sample, not shown here, also confirm a limited change of viscoelastic moduli (40 %) when the temperature is increased by 17°C. These variations remain within the variations in rheological response due to heterogeneities and in all cases are negligible compared to the high increase in elasticity observed when decreasing the distance probe/epithelium.

1.2 Effect of the bead dragging on the local mucus viscoelasticity.

Macrorheology experiments performed on collected BEGM mucus samples show that a stationary regime is reached in less than 30 seconds and that sample recovering (perfect overlapping of the curves) occurs within 5 min. In collected mucus samples, recovery is also observed in our microrheology

measurements. Successive measurements (after three minutes waiting) on the same bead at the same location give similar results, as illustrated in Fig S5A. We also tested the possible influence of a large perturbation before measurement. When a bead is tested locally, then moved at a large distance (typically 20µm) and replaced at its initial position (see a typical example in Figure S5B), some difference can be observed with a maximum change of 35% of the local viscoelasticity. This change is similar to the one due to the spatial heterogeneity. We thus neglected the effect of residual stresses or plasticity that could be induced by dragging on the microrheology response.

2 Distinction of several bead environments within the mucus (cases I and II)

For the "ex vivo" mucus, with a high concentration in impurities and aggregates inside, we chose to differentiate two cases, illustrated on the Figure S6: when the bead is close to aggregates and trapped by the strong network around them (corresponding to the case I of the Figure 4), illustrated on the FigS6B; and when the bead is away from aggregates and free of their influence (corresponding to the case II of the Figure 4), illustrated on the FigS6C. The criterion is not the distance to the aggregate, although it is informative, but the existence of a large restoring force (around 1nN with the maximum power of the laser) when the bead is moved away from the aggregate beyond a certain distance (typically 10-20µm). The FigS6A illustrates a particular case corresponding to a bead inside a cell aggregate. In this particular case the viscoelastic response is an elastic plateau (power law exponent equal to 0) around several Pa. Within the mucus from BEGM cultures (poorly concentrated in dead cells and debris), either collected or directly on the epithelium, we always did the measurements in a configuration corresponding to the case II. For the mucus from Pneumacult cultures (very concentrated in dead cells and debris), however, we did the measurements in both configurations and obtained similar results.

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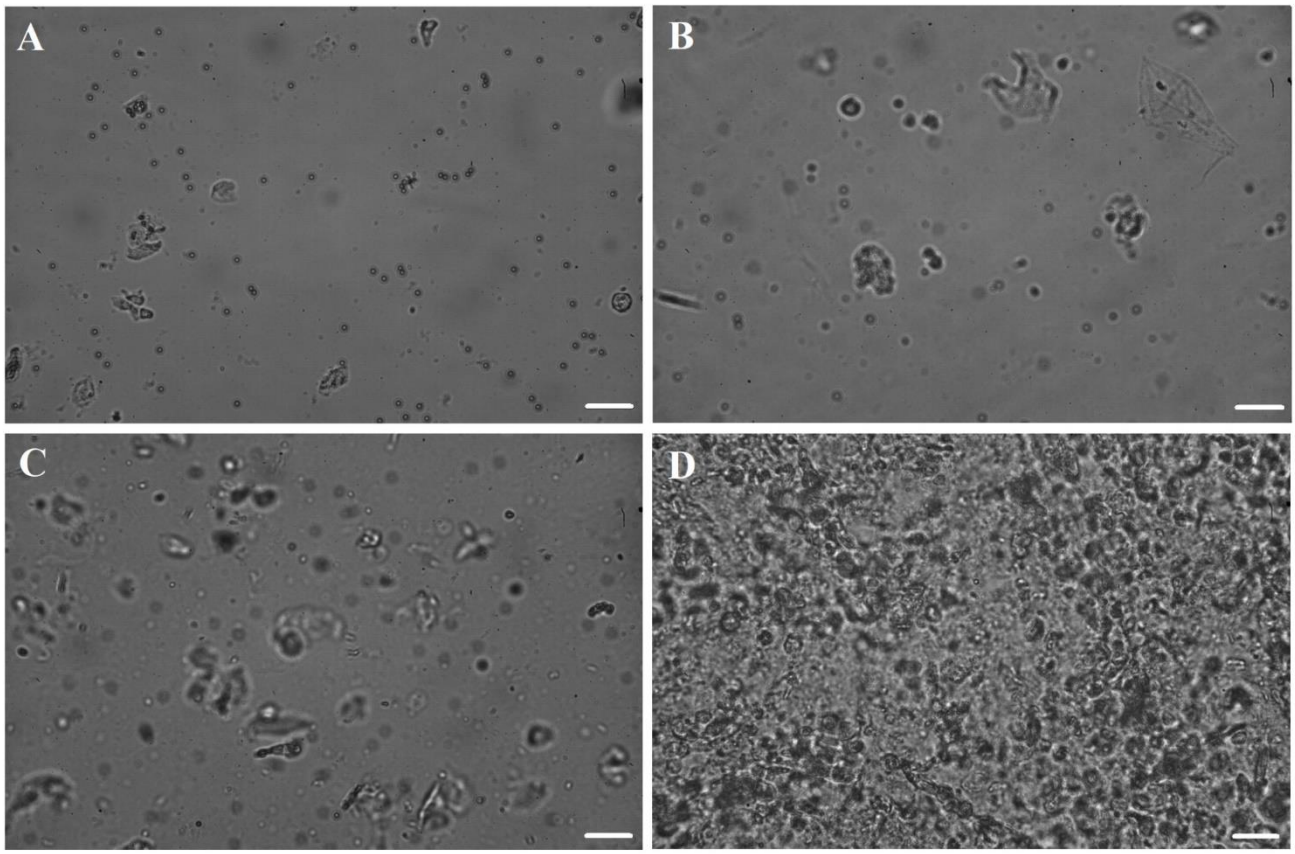

Figure S1. Image of the impurities concentration inside the samples. A) Mucus collected from BGEM cultures. We observe typically less than 1% in volume of cells and debris inside. B) Water with approximatively the same concentration of cells and debris inside than the samples of mucus collected from BEGM cultures. C) Water with a concentration of cells and debris inside (less than 5% in volume) similar to the mucus layer close to the epithelium of BEGM cultures and also like the "ex vivo" mucus corresponding to the patients 5 and 7. D) Mucus collected from Pneumacult cultures. We observe a critical concentration of cells and debris inside (> 10%). This example is representative of all the Pneumacult mucus samples, collected and on the epithelium, and also the "ex vivo" mucus at the exception of the patients 5 and 7. Scale bars: 30 µm.

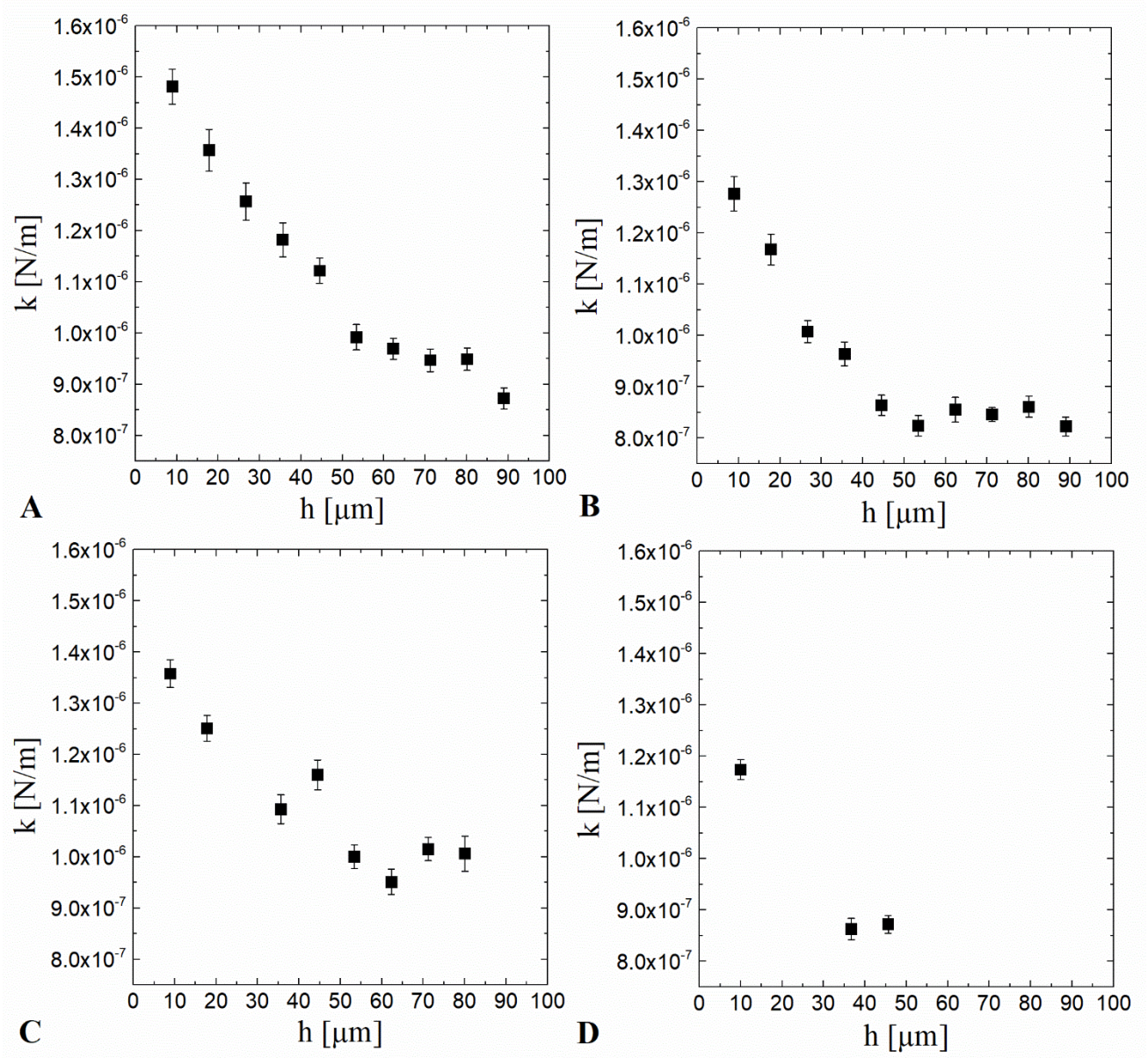

Figure S2. Optical trap stiffness as a function of the height in water with several concentrations of dead cells and debris inside. Measurements were done at a low laser power of 8 mW to determine the trap stiffness k from the analysis of the Brownian motion of a bead inside the trap. The laser power and the bead are the same for all the measurement in height. In A) measurements in pure water, without cells or debris, in B) with a concentration in dead cells and debris similar to the one found inside collected BEGM mucus (see illustration FigS1B) and in C) with a concentration similar to the one found inside the mucus layer close to the epithelium of BEGM cultures (see illustration FigS1C). The panel D) was also obtained with the water sample with dead cells and debris (<5%) similar to the one found inside collected BEGM mucus (FigS1B), but in the most unfavorable condition with a large debris occulting the bead. The first point, is taken near the cell (around 5 µm), at the same height h=10µm. The last two points are taken above the cell. In these four cases we observe the same global loss of trap stiffness k of 35%.

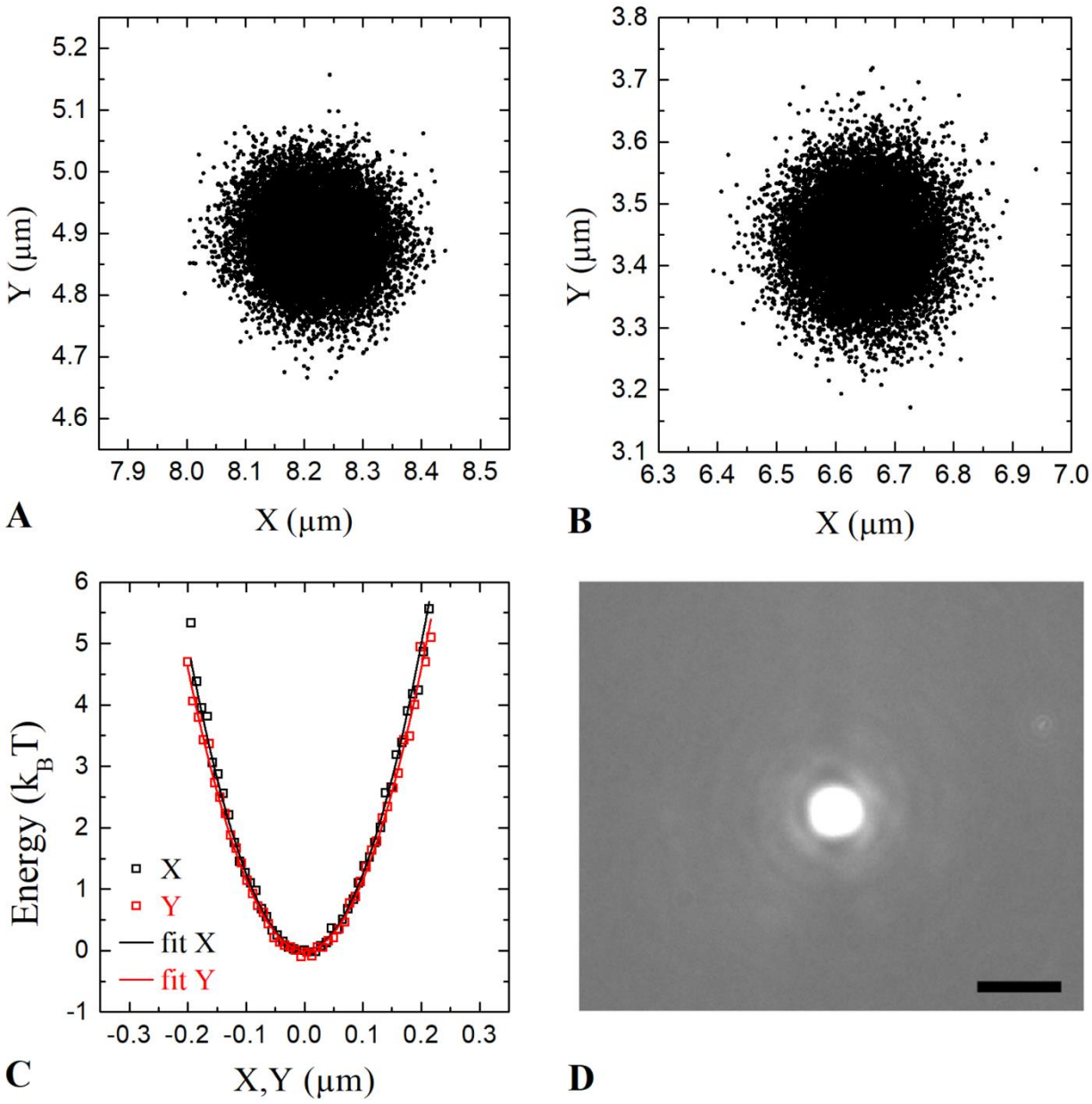

Figure S3. Characterization of the optical trap quality inside water with a concentration of dead cells and debris less than 5% similar to the one found close to the epithelium of BEGM cultures (see illustration FigS1C). The Brownian motion is recorded during 10 min at 30fps: in panel A) at a distance of 17.8 µm from the coverslip, and in panel B) at a distance of 71.2 µm from the coverslip. Panel C) shows the energy potential of the laser trap for the height 71.2 µm on the X (black squares) and Y (red squares) axis. The solid lines are the corresponding quadratic fits with k_x=1.03pN/µm and k_y=0.95pN/µm. Panel D) shows the reflection of the laser beam on the slide after crossing the 100 µm of sample. Scale bar, 2.5 µm. The homogeneity of the Brownian motion is well conserved, there is not deformation of the laser trap focus nor additional ghost traps.

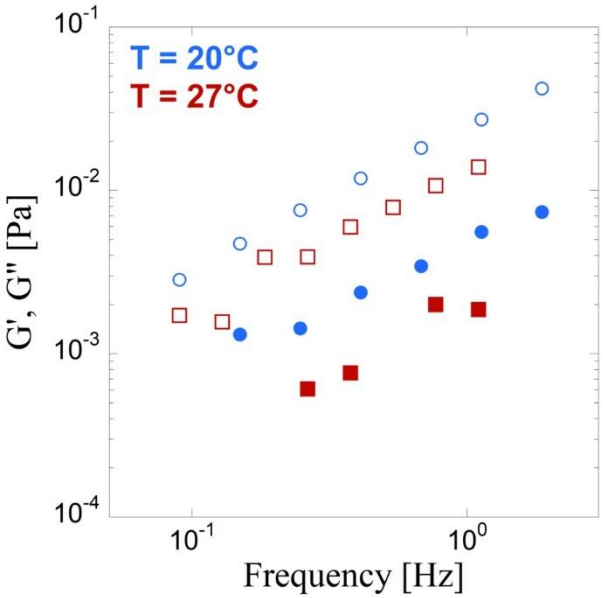

Figure S4. Elastic and viscous moduli G' and G'' (full and empty symbols) as a function of the frequency on a collected BEGM mucus for two temperature values T: T=20°C (blue circles) and T=27°C (red squares). We can see that an increase of the global temperature around 7°C decreases the viscoelastic values by 50% (relatively to the initial temperature of 20°C). This is in the range of the mucus heterogeneity observed for a same height on the BEGM cultures and lower than the difference measured in function of the height to the epithelium.

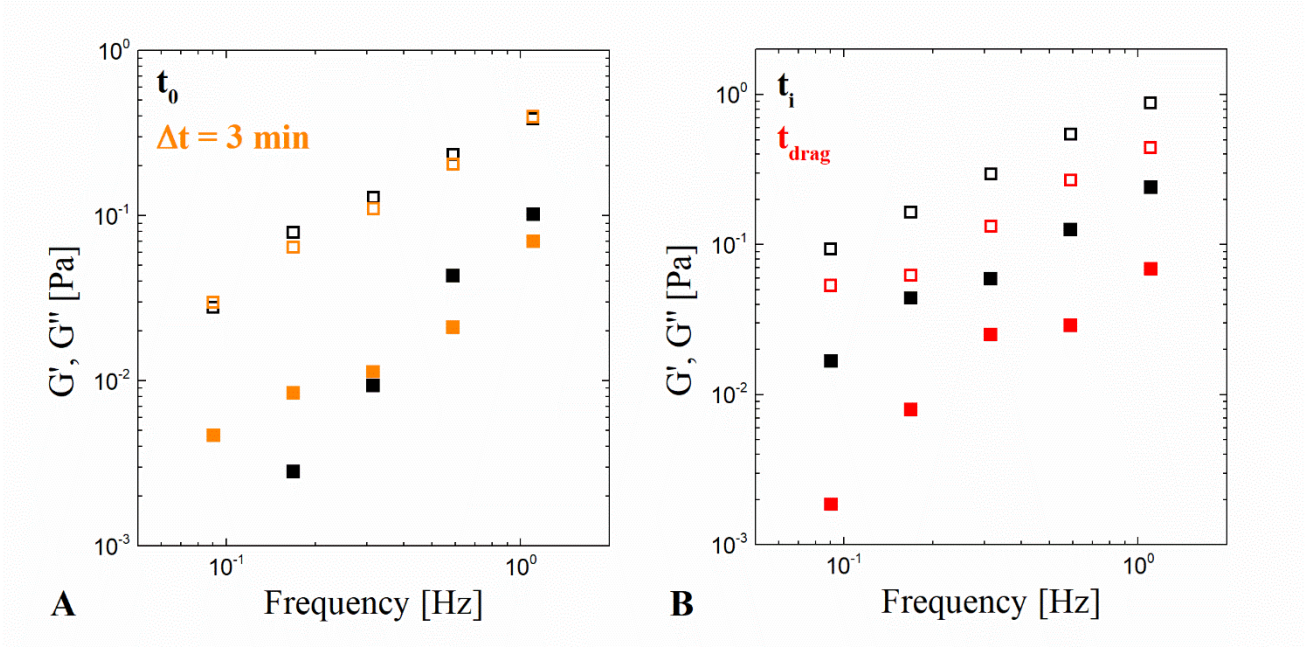

Figure S5. Microrheology of mucus collected from a "Pneumacult" bronchial epithelium culture. Viscoelastic moduli G' (full symbols) and G'' (empty symbols) are plotted as a function of the frequency. The panel A shows a microrheology measurement (black squares) followed by a second one (orange squares) done at the same location with the same bead and same laser power. We waited 3 min between the two measurements. We obtain an overlap of the 2 curves. The panel B show a measurement (black curve) taken with a bead already present at its location. After this first measurement we dragged the bead 20 µm to the top, then 20 µm to the bottom to its former position. We waited 3 min before taking the second measurement (red curve). The two curves do not overlap, the viscoelasticity after dragging is here 35% lower than initially.

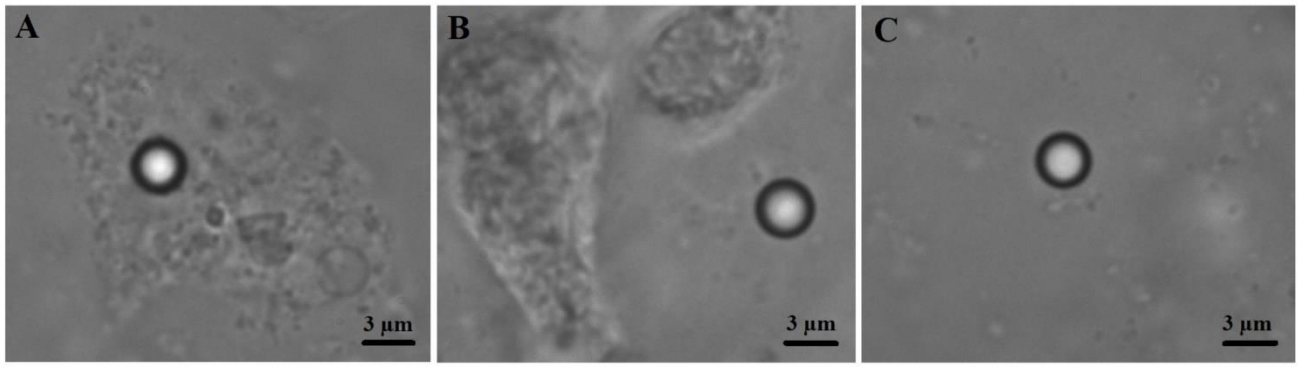

Figure S6. Images of the 3 types of bead locations considered for the mucus "ex vivo". A) Case of a bead embedded in cells and debris. B) Case I of a bead close to a cells and debris aggregate. C) Case II of a bead far from aggregates and free of their influence.