

In vivo effects of rhDNase and saline solution on sputum rheology in cystic fibrosis patients

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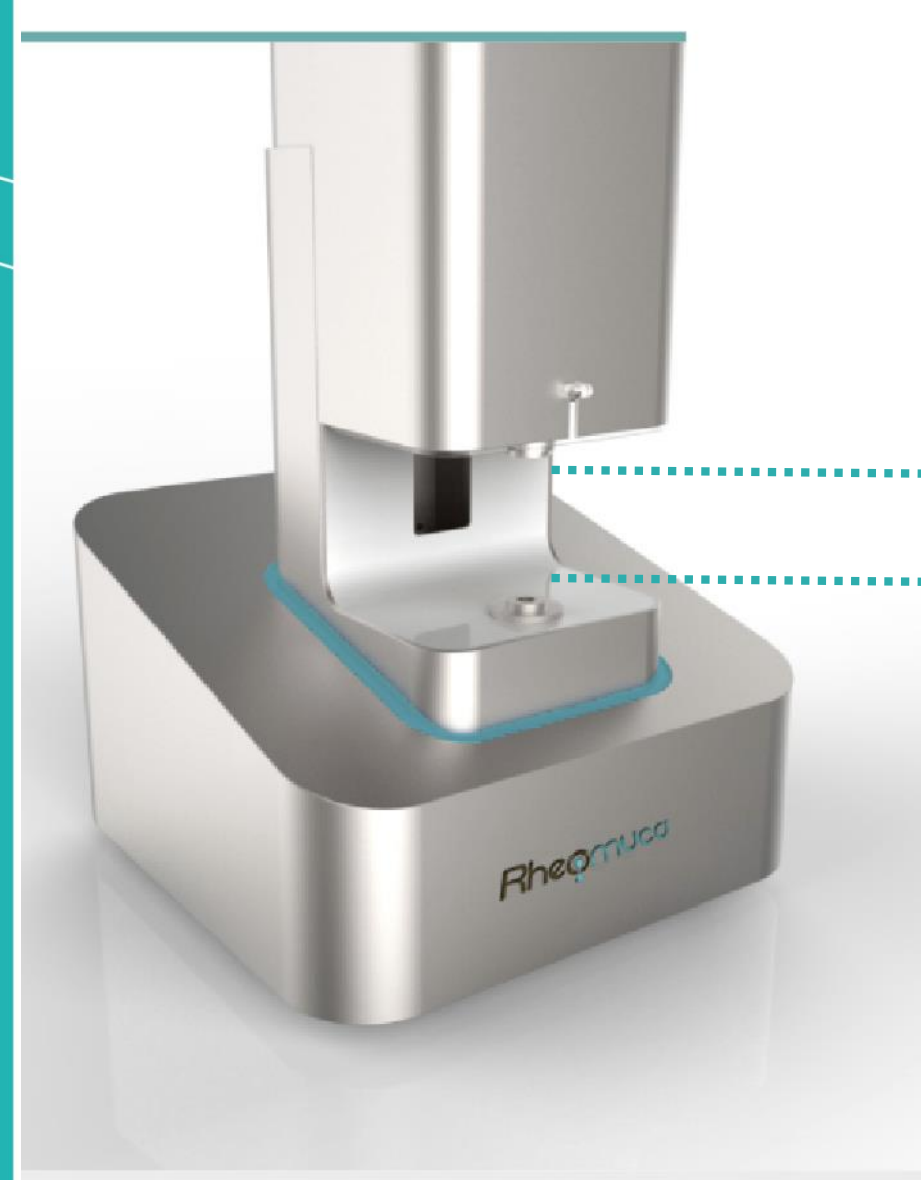
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Context

Sputum rheology provides **biophysical markers** based on elasticity and viscosity measurements. Those markers can be used to follow the status of patients with cystic fibrosis (CF), and to evaluate the **personalized efficiency of current treatments**. The purpose of this study is to benchmark, with *in vivo* samples, the effect of rhDNase and hypertonic saline solution (HSS) on the rheological properties of mucus.

Protocol

Rheology is the science of flowing matter: human sputum are fluids that exhibit a combination of elastic, viscous and plastic behaviors. The Rheomuco rheometer was used to measure viscoelastic properties in a clinical study (NCT02682290). Three properties are mainly extracted: the modulus of elasticity G' , the viscous modulus G'' and the damping ratio $\tan \delta$. The ViscaleTM score is a combination of these rheological properties.

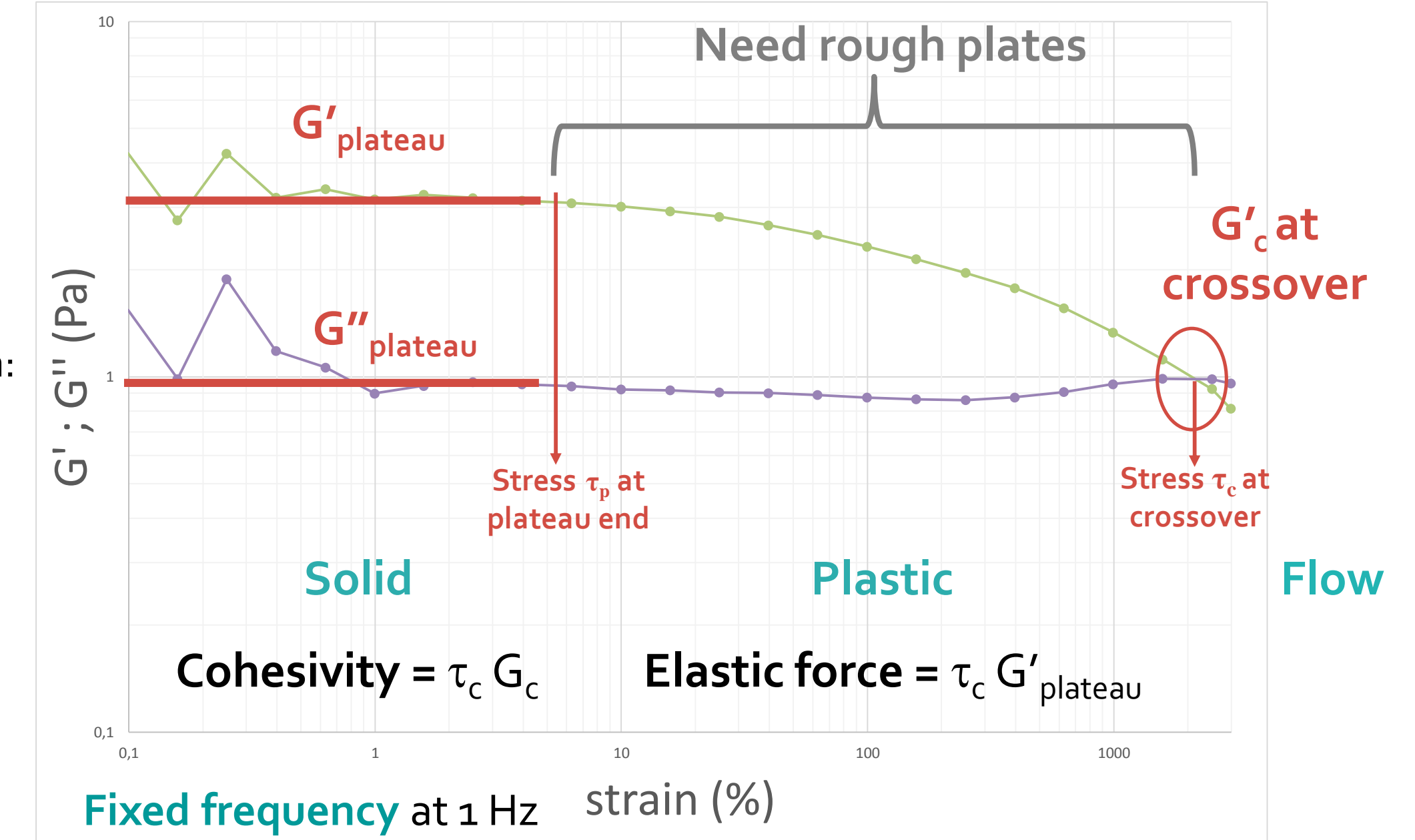


Output: phase δ
torque τ

In phase contribution:
 $G' = \frac{\tau}{\gamma} \cos(\delta)$
 G' elastic modulus

Out of phase contribution:
 $G'' = \frac{\tau}{\gamma} \sin(\delta)$
 G'' viscous modulus

$\tan(\delta)$ Phase tangent
 $\tan(\delta) = \frac{G''}{G'}$



Clinical trial

Assessment of Rheological Parameters of Human Sputum [Rheomuco, NCT02682290]

10 CF patients

Eligibility criteria:

- Adult CF patients with bronchial disorder, confirmed by Grenoble Reference Center

Exclusion criteria:

- $FEV_1 \leq 40\%$, contraindications for rhDNase, $PaO_2 < 60$ mmHg at rest
- Acute exacerbation during the last month
- Contraindications for spirometry

Two visits 48 hours apart

First visit:

Patients with CF have a spontaneous expectoration. Then all participants will have an induced expectoration with hypertonic saline solution (4.5%) during 10 minutes maximum. First incoming sputum in the following statistics.

Second visit:

Patients with CF have a spontaneous expectoration followed by 20 minutes rhDNase nebulization and again a spontaneous expectoration 1 hour after.

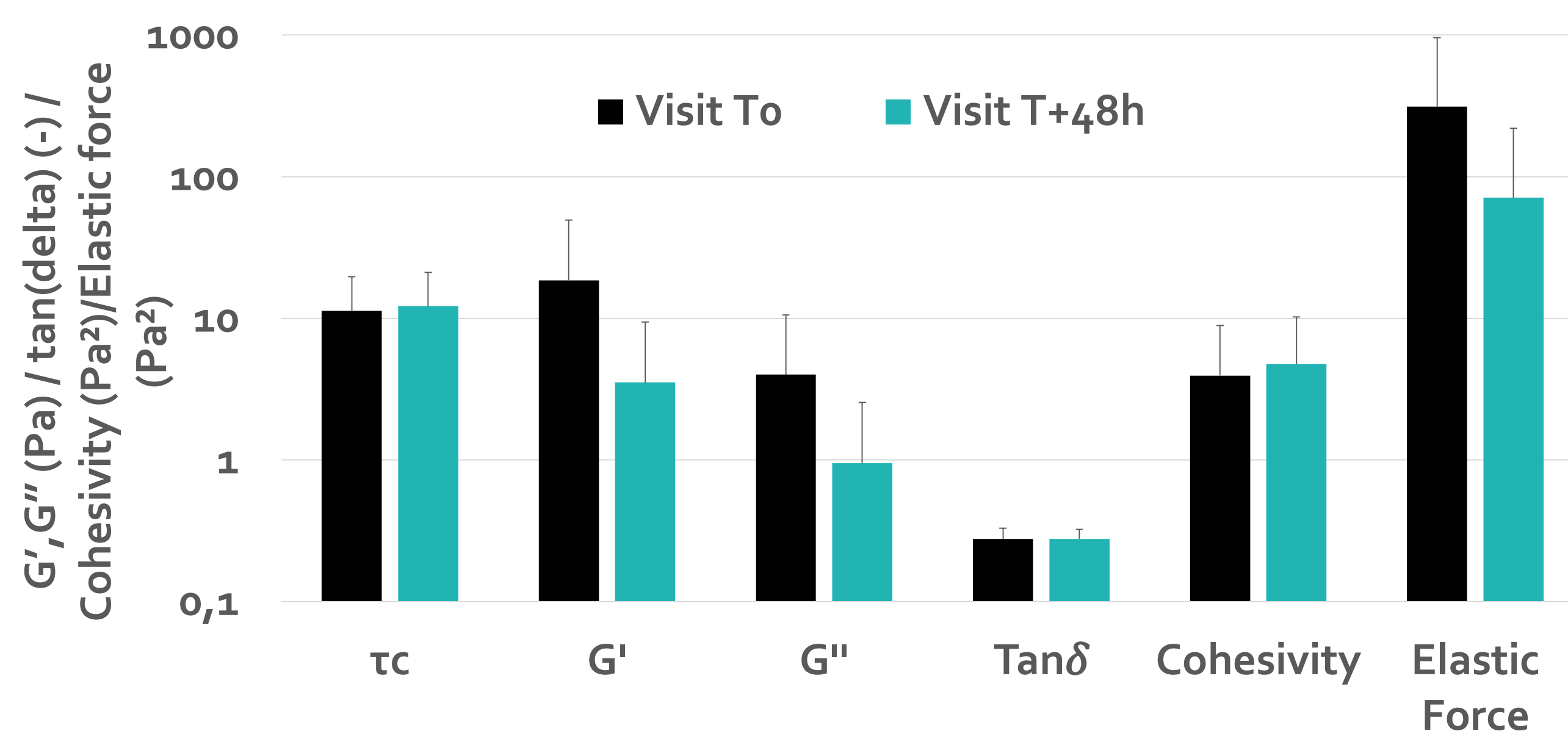
Expectoration were homogenized and **Rheology measurements** at 37°C are made on each of them on the Rheomuco device (Rheonova, France) dedicated to the measurement of rheological properties of sputum.

Results

Measurements for modulus G' and G'' are showed at a frequency $F=1$ Hz.

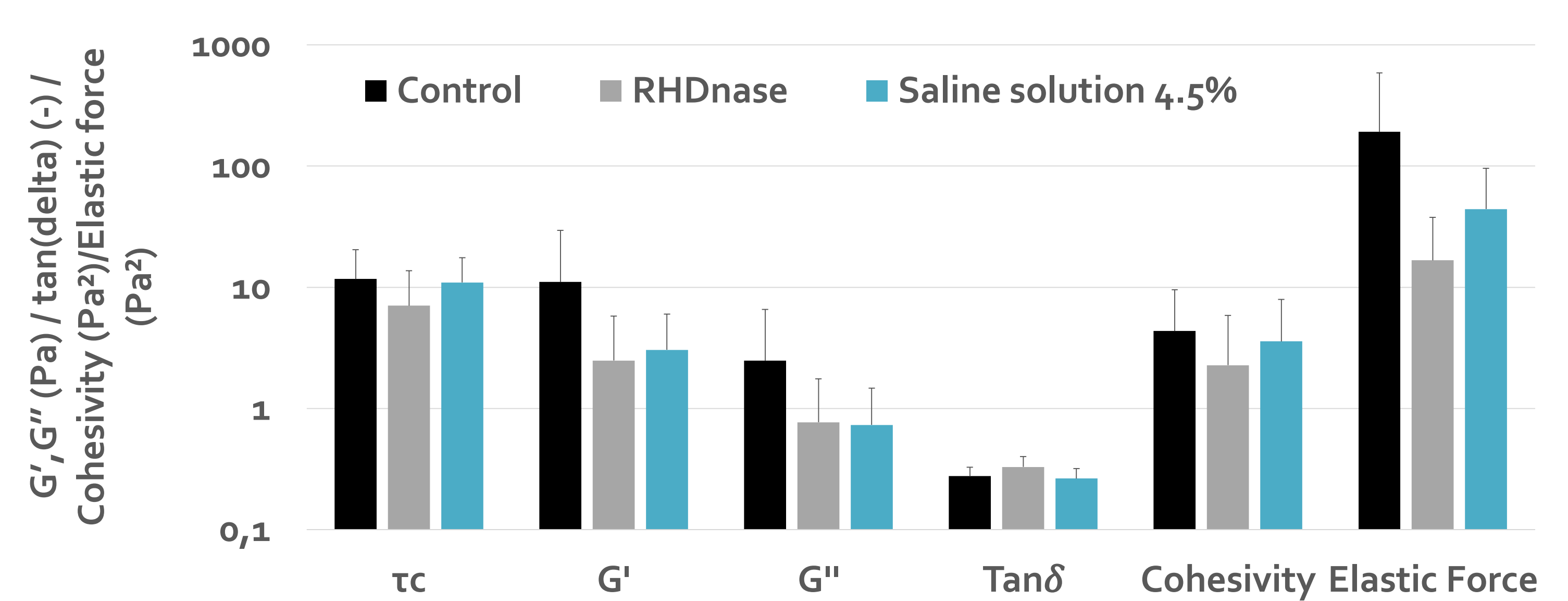
Statistical treatment of the data will be finalized for the end of the study which is scheduled for June 2018.

CF patients: 48-h evolution (n=10)



Rheological data from CF spontaneous sputum. Elastic and viscous moduli G' , G'' , and elastic force decrease with time ($p < 0.05$). $\tan \delta$, τ_c and cohesivity are stable between the two visits in CF patients.

Effect of HSS 4.5% and rhDNase on CF mucus (n=10)



RhDNase significantly reduces G' by 77%, G'' by 69% and elastic force by 91%. **HSS 4.5%** reduces G' by 72%, G'' by 70% and elastic force by 77%. But the relative decreases of those properties for both condition is not significantly different. Only rhDNase significantly affects τ_c (reduced below 10 Pa) and $\tan \delta$ (increased).

Conclusions

- Within 2 consecutive visits, CF patients sputa exhibit 3 varying (G' , G'' , and elastic force) and 3 very stable rheological properties (τ_c , $\tan \delta$, and cohesivity). These results consistently compare with those obtained with conventional rheometers.
- The varying rheological parameters drastically decrease following either HSS or rhDNase nebulization, more pronounced in the rhDNase case.
- RhDNase reduces the yield stress below 10 Pa, which suggests a possible restoration of the mucocilliary function. The increase of $\tan \delta$ is likely a rheological signature of the molecular effect of rhDNase (DNA cutting), while the fact that $\tan \delta$ remains constant in the HSS case suggests its effect is only due to dilution.