Rheomuco by Rheonova

Technical white paper

February 2022

A POWERFUL NEW TOOL: RHEOLOGY OF THE AIRWAYS MUCUS AND SPUTUM

A POWERFUL NEW TOOL: RHEOLOGY OF THE AIRWAYS MUCUS AND SPUTUM

INTRODUCTION

Muco-obstructive diseases, such as cystic fibrosis (CF), COPD, bronchiectasis, or severe asthma, are characterised by an impaired clearance leading to the obstruction of the lower airways through mucus oversecretion and/or accumulation. The weaker efficiency of mucus transport, together with the observed evidence that mucus structural and biochemical properties are modified in muco-obstructive diseases, strongly suggest mucus rheology as a biomarker of their severity. We review some features of mucus rheology and put forward its potential in the characterisation of muco-obstructive diseases.

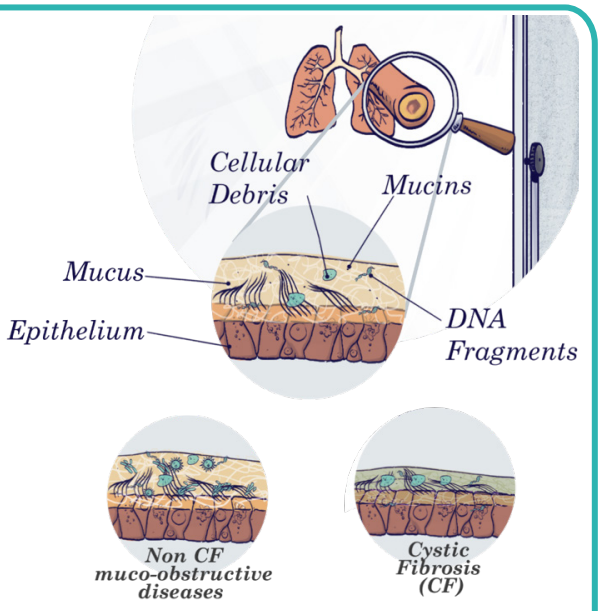

Fig. 1 – The airway surface layer and the micro-structural elements present in the bronchial mucus in healthy or diseased states.

THE LOWER AIRWAYS MUCUS

The lower airways consist of a hierarchical network of bronchi connecting the lungs to the trachea. The inner surface of the airways, the epithelium, is mostly composed of goblet and ciliated cells. The epithelium is covered by a thin layer of mucus (Fig. 1), continuously secreted by the goblet cells, and cleared by the ciliated cells through a creep mechanism called mucociliary clearance [1]. This mechanism is complemented by cough, a violent air flow that drags mucus plugs away.

Mucus is a hydrated (≈ 98% water) cross-linked network of high molecular weight glycoproteins called mucins. Due to its porous structure, mucus retains inhaled contaminants (particles, bacteria) and thereby protects the airways from inflammations or infections. Pathologic mucus is also contaminated by biological material (leucocytes, bacterial colonies, proteins, DNA) and/or is dehydrated.

In muco-obstructive diseases, patients produce sputum, a mucus solution collected with saliva. This production can be substantial (up to few mL in severe CF or bronchiectasis patients), and can alternatively be stimulated by induction with a saline solution or physiotherapy. Expectoration is the least invasive way of collecting airways mucus from patients.

MUCUS RHEOLOGY

The mechanical response of mucus, a polymeric material, under shear is primarily driven by the behaviour of its constitutive polymer strings. In shear tests, the rigidity, which corresponds to the response of the physical and chemical bonds to local extension or compression, is measured by the elastic modulus G'(Fig 2a). The dissipation, which corresponds to the friction of the polymer strings sliding along each other (Fig. 2b), is measured by the viscous modulus G". Under higher shear, the stronger solicitation of the network may break some of the bonds, which eventually translates at the macroscopic scale into the failure of the material. The strength of the material is characterised by a critical stress σ_c (Fig. 2c).

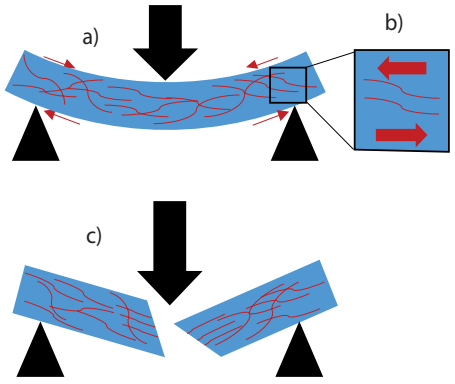

Fig. 2 – Deformation of an ideal soft polymeric material. a: small deformation while bending. b: the close-up view illustrates the sliding of polymer strings under shear. c: breakup

	Rubber	Steel	Glass	Tire: Rubber & Glass
Rigidity (G*)	Soft	Stiff	Very stiff	Soft
Dissipataion (tan δ)	High	Low	Very low	High
Strength (σ_c)	Strong	Very strong	Brittle	Very strong

Tab. 1 – Characteristic rheological behaviour of some materials.

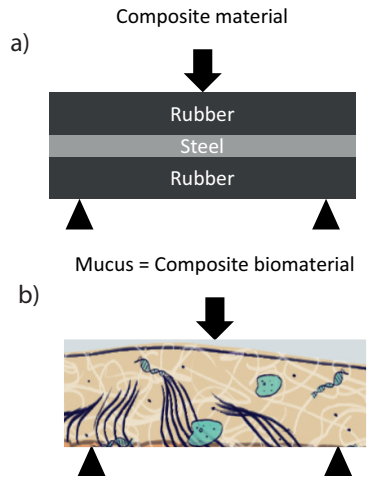

Fig. 3 – Rigidity and dissipation of composite materials are provided by their principal constituent; strength of com-posite materials is provided by their strongest constituent. (a) manufactured and (b) biological materials.

A more direct (and equivalent) description is provided by associating the viscoelastic modulus, $G^*=\sqrt{(G'^2+G''^2)}$, which is the overall response of the material to shear, together with the damping ratio, $\tan\delta=G''/G'$.

These characteristics (rigidity, dissipation, strength) are partly independent. Table 1 provides three examples of materials which distinguish on one aspect while being rather weak on the other(s): rubber is a rather soft material which dissipates large amounts of energy and resists relatively well to failure; steel is stiff, strong and dissipates poorly; glass is very stiff but fragile, and barely dissipates energy without failing. In engineering, composite materials are designed to combine the properties of several families of materials in order to combine their qualities. For example, tires are made rubber armoured with steel to benefit of the softness of rubber and the strength of steel. More precisely, the principal material, rubber, rules the rigidity, and only a small quantity of steel in rubber gives the strength (Fig. 3a). Due to its composition, mucus is by essence a composite biomaterial (Fig. 3b), and its mechanical properties are the overall signature of its components: mucins, proteins, DNA, …

A Rheomuco test consists in exerting a succession of oscillatory shear strains to a sample, and measuring the corresponding stress. For a given oscillation amplitude, the ratio of the measured stress (expressed in units of Pascals, Pa) to the exerted strain (ratio of the transverse displacement to the sample thickness, without unit) provides the viscoelastic modulus G* (in Pa). The temporal shift between the imposed and measured oscillations is caused by viscosity and provides the damping ratio tanδ (no unit). Combining G* and tanδ also gives G' and G". This procedure is iterated, increasing the oscillations amplitude, which constitutes a so-called strain sweep test, as represented in Fig. 4 for a sputum sample [2].

Under low shear, while the integrity of the structure is preserved, the rigidity and the dissipation of the polymer network dictate the material's response. The measurement therefore probes the structural properties of the mucus at rest. The viscoelastic modulus is constant, in the order of a few Pa for a sputum sample. The response in this range is predominantly elastic, i.e. $G^*\approx G'>G''$ or equivalently $\tan\delta<1$, as expected for soft gels. This low-shear regime is referred to as linear viscoelastic region. Increasing the strain, the stronger solicitation of the network may break some of the physical bonds. The viscoelastic modulus drops and the damping ratio increases. When the damping ratio overcomes 1, or equivalently when the G' and G" curves cross over, the rheological response of the sample is predominantly viscous over elastic, the sample basically starts flowing. These high shear measurements relate to the mechanical effects that drain the mucus out of the bronchi, such as cough, or physiotherapy. The strain at which this transition occurs is referred to as critical strain, γ_c, and the corresponding stress is the critical stress $\sigma_c = G_c^* \gamma_c$, which quantifies the strength of the material. Note that the critical strain of sputum, usually in the order of 10, is remarkably high among soft gelled materials. The strain sweep test thus allows to quantify the rigidity, dissipation and strength of mucus samples. This response actually encompasses those of each constituent of the composite biomaterial.

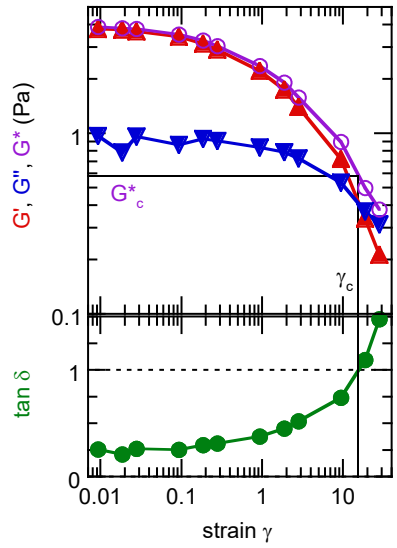

Fig. 4 – Typical rheological response (strain sweep test) of a sputum sample from a CF patient.

BIOPHYSICAL AND BIOCHEMICAL DETERMINANTS

Pathologic bronchial mucus is composed of several entangled networks (Fig. 5). The primary constituent of native mucus is the mucin network. Mucins are high molecular weight (0.4 MDa) glycosylated proteins linked by disulfide bonds. The mucin network is stabilised by weaker and reversible interactions (hydrophobic, electrostatic links), which depend on its chemical (ions, pH) environment. It constitutes the backbone of the porous structure of mucus. In patients with bronchial diseases, mucus also hosts several non-mucin networks and structures. Bacterial infections play a key role in the evolution of some bronchial diseases. For instance in CF, colonisation by *Pseudomonas aeruginosa* forms biofilms with particularly high viscoelasticity when growing in a mucous environment. Within the mucus itself, the immune response to an infection or inflammation causes the release of extracellular DNA traps (Neutrophil and Eosinophil Extracellular Traps, NETs and EETs). These traps take the form of filamentous nets which can retain intruding cells. Protein crystallisation can also lead to secondary networks in mucus.

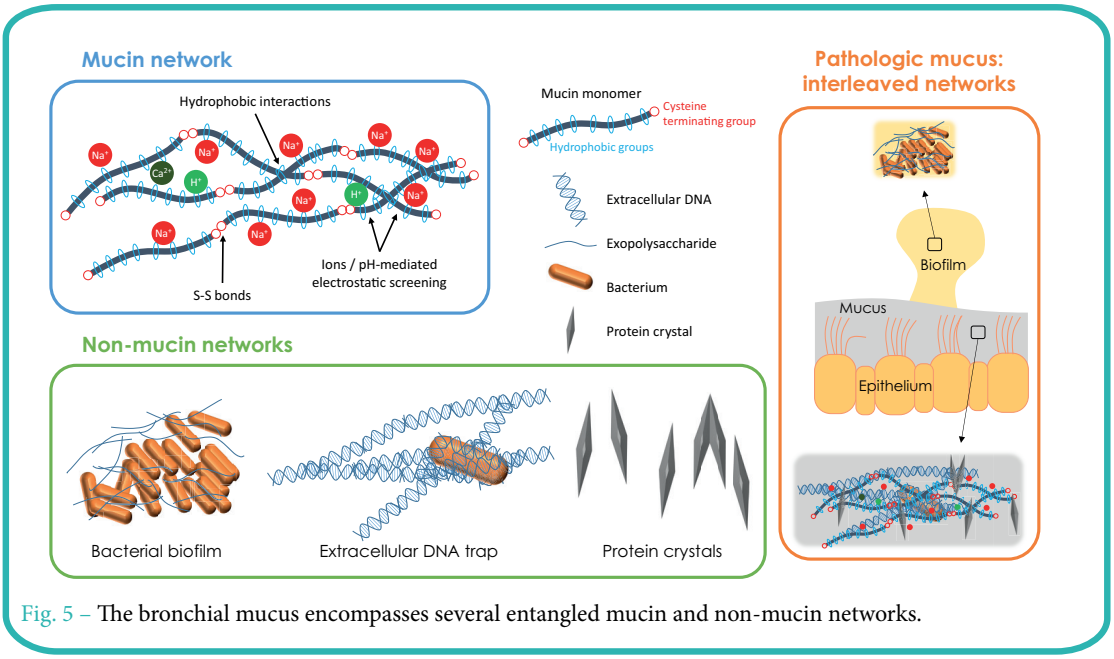

Fig. 5 – The bronchial mucus encompasses several entangled mucin and non-mucin networks.

The mobilisation of mucus within the bronchi thus involves all these entangled networks, which both contribute to the global mucus rheological properties [3,4]. Figure 6 compares the strain sweep response of three distinct samples: mucus produced by reconstituted epithelia (air-liquid interface cells, epithelix/Mucilair®) with healthy phenotype, sputum collected from a stable adult CF patient, and from a healthy volunteer. The former consists of a sole mucin network, i.e. no bacteria and no immune cells, with expectedly low hydration level as the mucus was left to accumulate in vitro before collection. The second is expected to be weakly hydrated, and contaminated with bacterial colonies, two typical features of CF mucus. Finally, the latter should feature high hydration, and normal bacterial colonisation and inflammation level.

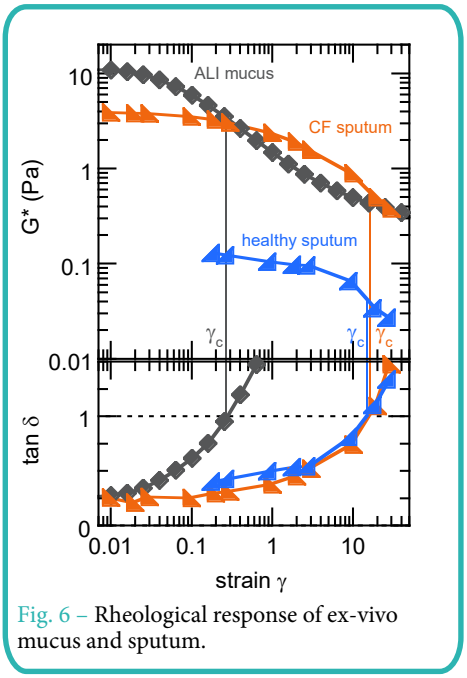

Fig. 6 – Rheological response of ex-vivo mucus and sputum.

Although both samples feature a comparable gel-like rheology, the mucus from cultured cells distinguishes by its high rigidity and its particularly low critical strain. In contrast, both healthy and pathologic sputa can undergo very large strains before flowing (60 times higher than the cultured mucus). The CF case, with higher mucin content, is stiffer by a factor of about 40 which makes it close to the cultured sample. Comparing these samples, we expect the concentration and/or dehydration level of the mucin network to primarily drive the rigidity of the sample, while the presence of DNA or proteins would rather increase its elasticity, i.e. its ability to deform before flowing, and its strength, i.e. the force needed to induce that flow.

SPUTUM RHEOLOGY IN CF PATIENTS

Cystic fibrosis evolves with periodic acute exacerbations. Recent studies strongly suggest that sputum rheology relates with these episodes [5] and their treatment [6]. Figure 7 shows the evolution of the linear elastic modulus G' from six patients in the cohort followed by Ma et al., before, during and after an exacerbation crisis. All samples feature a similar evolution pattern, with a substantial increase (by a factor of five on average) during the crisis, followed by a symmetric decrease during the recovery. Note that one patient features abnormally low rheological levels in baseline, exacerbation and recovery stages, yet the exacerbation follows a similar evolution. Sputum linear viscoelasticity is thus a credible biomarker of exacerbated states in CF.

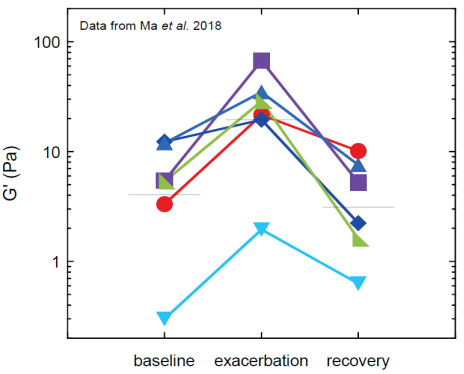

Fig. 7 – Evolution of the linear elastic modulus of 6 CF patients before, during and after an exacerbation.

TUNING MUCUS RHEOLOGY

Overall, although the mechanisms involved are diverse and yet to be identified, mucus rheology is reinforced in muco-obstructive diseases, and goes along with the severity. Thus, a tempting curative strategy consists in modifying mucus rheology in patients. Mucolytic treatments are administered in this view (Fig. 8, left). They can target either the mucin network (e.g. N-Acetylcystein which breaks disulfide bonds) or the DNA network. Among them, recombinant human DNase (rhDNase), which cleaves DNA, is probably the most widely used in CF and proves effective in reducing the viscoelasticity of mucus. Alternatively, treatments like saline solutions can swell the network by hydrating it.
The mechanism of action of these mucolytics being distinct, so should their effect on rheology. Our recent study comparing hypertonic saline solution (HSS) to rhDNase tends to suggest that although both reduce simultaneously the linear and flow properties, HSS acts stronger on the former while rhDNase has a more distinctive effect on the latter (Fig. 8, right) [2].

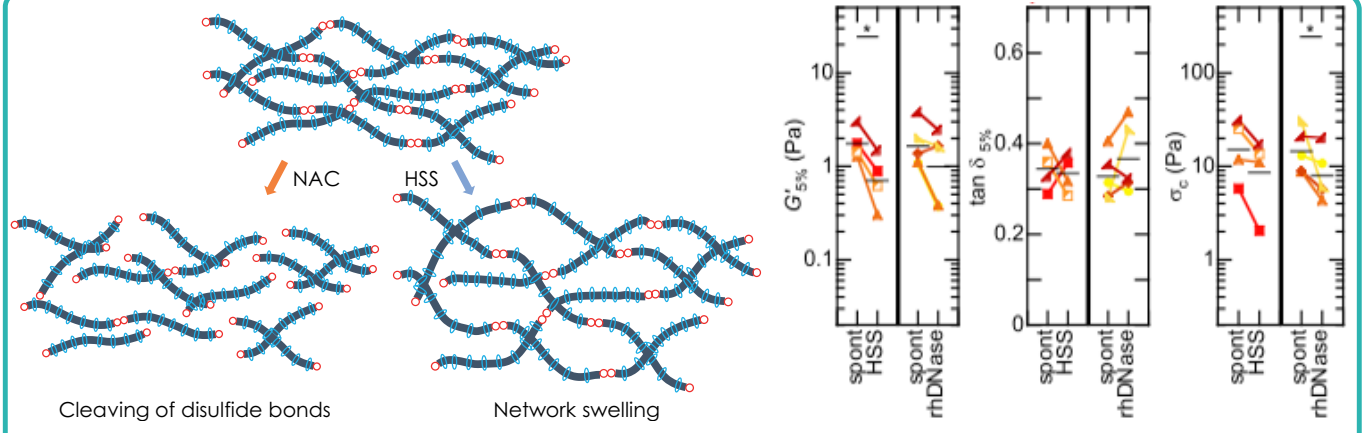

Fig. 8 – Left: Schematic effect of mucolytic agents altering mucus by cleaving or swelling the network (NAC: N-acetylcysteine; HSS: Hypertonic saline solution). Right: Individual comparison of the elastic moduli, damping ratio and critical stress of sputa obtained spontaneously and after HSS induction (N = 4), and before and after rhDNase nebulisation (N = 4) in CF patients (*p < 0.05).

CONCLUSION

In muco-obstructive diseases, bronchial mucus hosts several intruding biological networks that affect its rheology, thereby leading to bronchial obstruction. These affected rheological properties are in turn a marker of the condition. In clinical settings, analysing expectorations could therefore provide a monitoring tool to assess the severity of the disease, the outcome of a crisis, or the response of a treatment. Using cultured mucus samples, rheology can also accompany drug development by providing a simple and quantitative way to assess the efficacy of a treatment targeting mucus.

REFERENCES

[1]. B. Button *et. al* 2012
[2]. J. Patarin *et. al* 2020
[3]. R. S. Linssen *et. al* 2021
[4]. G. Tomaiuolo *et. al* 2014
[5]. J. T. Ma *et. al* 2018
[6]. D. J. Serisier *et. al* 2009

RHEOMUCO by Rheonova
contact@rheomuco.com
www.rheomuco.com