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Linear rheology as a potential monitoring tool for sputum in patients with Chronic Obstructive Pulmonary Disease (COPD).

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Abstract

Sputum samples from Chronic Obstructive Pulmonary Disease (COPD) patients were investigated using rheology, simple mathematical modelling and Scanning Electron Microscopy (SEM). The samples were all collected from patients within two days of their admission to Prince Philip Hospital due to an exacerbation of their COPD. Oscillatory and creep rheological techniques were used to measure changes in viscoelastic properties at different frequencies over time, and COPD sputum was observed to behave as a viscoelastic solid at all frequencies studied. Comparing the rheology of exacerbated COPD sputum with healthy sputum (not diagnosed with a respiratory disease) revealed significant differences in response to oscillatory shear and creep-recovery experiments, which highlights the potential clinical benefits of better understanding sputum viscoelasticity. A common power law model $G(t) = G_0(\frac{t}{\tau_0})^{-m}$ was successfully fitted to experimental rheology data over the range of frequencies studied. A comparison was made between clinical data and the power law index m obtained from rheology, which suggests that an important possible future application of this work is as a potential biomarker for COPD severity.

Keywords: Rheology, Viscoelastic Properties, Chronic Obstructive Pulmonary Disease

1. Introduction

Chronic obstructive pulmonary disease (COPD) is the term used to cover a broad range of respiratory diseases such as emphysema and bronchitis, but all having common factors that are multi-system complications, chronic inflammatory state and airflow obstruction [1]. In clinical practice the severity of airflow limitation (forced expiratory volume in the first second of expiration) is used to determine the diagnosis and severity of disease. Other measures are needed in clinical practice to evaluate patients, predict their risk, aid in treatment plans, and evaluate their response to it. Over the past few years, there has been a great deal of interest in the application of engineering to aid in the understanding of respiratory disease and to improve diagnosis and management techniques [2].

Sputum is an essential factor in normal airway clearance and lung function, which can be found in the upper airways where it forms a protective layer for the epithelium. A balance in sputum production and clearance is essential in healthy airways and changes in the viscoelastic properties can greatly affect this process.

Excess sputum production with enhanced viscoelastic properties is a common symptom of respiratory diseases such as COPD and cystic fibrosis (CF) [3]. There has been a large focus on the rheology of CF sputum with great success in modifying sputum viscoelasticity through the use of DNA severing proteins [4], for example.

The primary composition of healthy sputum is that of high molecular weight glycoproteins, however in COPD other large biopolymers can dominate. Similarly in COPD sputum there is generally a large content of biological matter such as inflammatory cells, bacteria, filamentous actin and highly polymerised DNA [5].

The glycoproteins in sputum are known to form a cross-linking polymer structure that is believed to dominate its characteristic viscoelastic behaviour [6]. At the macroscopic level, sputum can be thought of as a non-Newtonian, thixotropic gel distinguished by its response to shear stress. This network is primarily comprised of glycoproteins linked by disulphide bonds and it's rheology has been compared to that of similar mucins from other tissue in past work [7]. Changes to the glycoprotein network or the addition of abnormal constituents

can alter the rheology of sputum and these changes may be disease dependent. Relatedly, it is also known that mucus derived from the trachea is also rheologically distinct from that found in the small airways [8]. Not only is it important to take detailed quantitative measurements of the rheological properties but to also quantify the clinical data to include other factors that can influence the rheology.

The aim of the work presented here is to use linear rheology as a probe, in order to attempt to correlate the viscoelastic properties of sputum, with clinical data on the severity of disease in patients with COPD. Recently, related works on mucus viscoelastic behaviour have appeared in the literature [9] [10] [11]. In [9], for example, the link between CF mucus viscoelasticity and patient clinical status was studied, including the effects of bacterial colonisation. The effects of inflammation on airway mucus in patients with CF was investigated in [10], while the in vitro elastic properties of COPD and CF-like mucus were studied in [11] using passive microbead rheology. Reassuringly, the experimental results found in [9] [10] [11] on CF mucus are broadly consistent with the work presented here on COPD sputum.

2. Methods

Sputum samples were obtained from Prince Philip Hospital and all patients were identified during acute admissions with exacerbations of their COPD. All samples were collected during days 0-3 of admission and stored at $-80^{o}C$ within four hours of patients producing a sample. Previous work on sputum has demonstrated that freezing samples at $-80^{o}C$, then thawing, followed by rheology has no significant effect on the viscoelastic properties [12] [13] [14]. Healthy controls were obtained from consensual staff at the hospital, and upon successfully obtaining sputum, the samples were labeled and stored along with the COPD sputum. All samples obtained were spontaneously produced, and not induced by hypertonic saline. One sample was obtained per individual, with typical sample volumes of tens of millilitres. Sputum samples were initially taken from twenty patients. However several samples failed to produce meaningful rheological data, or on further inspection were found to lie outside the linear viscoelastic regime, leaving a core set of ten reliable and robust rheological samples for the purposes of this study.

Sputum samples were always thawed at room temperature for a minimum of one hour prior to performing rheology, and no homogenisation was performed in order to ensure minimal change to the underlying sputum structure. Prior investigators have predominantly applied some form of homogenisation which may detrimentally affect the sputum structure resulting in data dominated by the homogenisation technique used, rather than the properties of the intrinsic polymeric network [15].

A small amplitude oscillatory shear (SAOS) test was performed by applying a simple shear in the form of a sinusoidal strain of angular frequency ω :

$$\gamma(t) = \gamma_0 \sin(\omega t) \tag{1}$$

The use of an applied oscillatory strain allows us naturally to vary the angular frequency ω, and hence probe the viscoelastic response of a sample at different time scales $1/\omega$.

$$\sigma(t) = \mu \frac{d\gamma(t)}{dt} = \mu\gamma_0\omega \sin(\omega t + \frac{\pi}{2}) \tag{2a}$$

$$\sigma(t) = G\gamma(t) = G\gamma_0 \sin(\omega t) \tag{2b}$$

The majority of samples studied exhibited viscoelastic behaviour that was found to lie between the two extremes of an ideal Newtonian liquid and a perfectly elastic solid. As shown in Equation.2a an ideal Newtonian liquid possesses a stress response related to the shear rate and the stress oscillates out of phase with strain by $\frac{\pi}{2}$ whilst at the same angular frequency ω. Alternatively, Equation.2b for a perfectly elastic solid shows that the stress responds at the same angular frequency, and in phase with the strain.

As viscoelastic materials respond with a combination of solid and liquid type behaviour, a general form for the linear stress response can be written as:

$$\sigma(t) = \sigma_0 \sin(\omega t + \delta) \tag{3}$$

This naturally introduces the phase angle δ which is an important variable in understanding viscoelastic behaviour. The phase angle δ can vary between $0 \leq \delta \leq \frac{\pi}{2}$ and as seen in Equation.2 the ideal solid and liquid behaviour corresponds to the lower and upper limit of the phase angle respectively. Due to the sinusoidal nature of the stress, this enables us to construct the stress contribution as a combination of two functions:

$$\sigma(t) = \gamma_0[G' \sin(\omega t) + G'' \cos(\omega t)] \tag{4}$$

The storage modulus G' is a measure of the energy stored as result of the deformation by the sample oscillatory shear. After removal of the shear deformation, the stored energy is now entirely available and is used

as the restoring force. The loss modulus G" is a measure of the deformation energy that is used, or lost, by the sample during oscillatory shear [16].

In this manner, the sample is continuously excited, but not too greatly such that the strain becomes too large, which can result in non-linear response. If excess strain does occur the elastic structure will be destroyed, so care was taken to maintain a safe working strain that keeps the experiments within the linear viscoelastic region (LVR). Indeed, initial samples were destroyed through experimentally determining the LVR of COPD sputum. Furthermore, the validity of the data with respect to linearity and slip artefacts was confirmed by (a) inspection of the raw strain waveforms to ensure the absence of harmonic frequencies (which are characteristic of a non-linear response or slip artefacts [17] [18]); and (b) good agreement between the power law exponents (see below) determined using creep and dynamic oscillatory experiments.

An AR-G2 magnetic bearing rheometer was used to perform the rheological tests of the sputum. The AR-G2 is a combined motor and transducer device with a bottom component of the measuring platform fixed, and the upper component is mounted to a shaft which enables it to be rotated by a motor induced torque.

The loss modulus G" and elastic modulus G' were measured for a range of frequencies (0.01Hz - 10Hz) using a 40mm and 60mm diameter parallel plate geometry, depending on sample size. The linear viscoelastic region was determined using the standard technique of oscillatory stress sweep which resulted in the strain being set to a constant 2% which is within the LVR (see below), as excess strain can destroy the structure.

The AR-G2 was similarly used to perform the creep-recovery experiments, in which a constant stress (σ_0) is applied to the sputum sample at time t_0 and the rheometer measures the strain γ. At time t the stress is removed so the sample transitions from the creep stage to the recovery stage while the strain is measured as a function of time. Stresses between 1-10Pa were used for 1500 seconds of creep with a 150 second recovery follow up.

Preparation of sputum for Scanning Electron Microscopy (SEM) follows the process of fixation, dehydration and drying. These processes stress the delicate polymer network within its extremely hydrated state. Specific techniques have been developed to allow for the true polymer structure to be imaged with a high resolution [19] . The samples were washed 3 times by permeation with 50 mM sodium Cacodylate-HCl buffer (pH 7.4) (SPI Supplier, USA) in order to remove excess salt, followed by a fixation overnight in 2% glutaraldehyde (Sigma Aldrich, UK). Samples were then
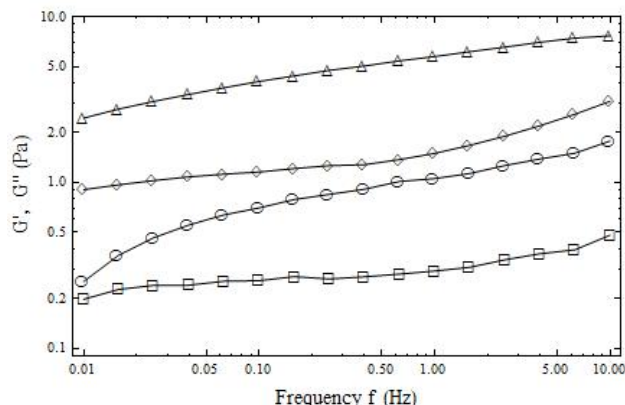

Figure 1: A representative plot for a typical COPD sputum sample of G' and G" (open triangles and open diamonds respectively), along with a typical healthy control sample (open circles and open squares respectively), against frequency.

washed an additional three times in buffer, dehydrated in an ascending series of increasing ethanol concentrations (30 to 100 %) over 3.5 hours, finished by critical point-dried with Hexamethyldisilazane 99% (440191, HDMS, Sigma Aldrich, UK).

Samples were mounted and sputtered with gold-palladium using a coating unit E5100 (Polaron Instruments, Inc., West Sussex, England) at 2.2kV, 20mA for 1-1.5 min. A Phillips 4800s scanning electron microscope (Phillips Electronics Co., Mahwah, NJ) was used to perform SEM at a operating voltage of between 5kV and 10kV, with a working distance of 7-9mm.

3. Results and Discussion

3.1. Experimental

A characteristic viscoelastic solid behaviour is observed in Figure.1 for the oscillatory shear of a typical COPD sputum sample. For all samples, G' and G" have a frequency dependence across the whole measured range where G' exceeds G" throughout, which further demonstrates the viscoelastic solid nature of sputum in this range of frequency.

Initial results of oscillatory shear performed on healthy control samples resulted in a variety of experimental data results that in some cases resemble that of COPD sputum while others appear more as a viscoelastic fluid. Two extremes were found for healthy control oscillatory shear in which sample hc1 has a similar shape to typical COPD sputum as seen in Figure.1 while sample hc2 has a drastically different shape, with G' tending towards crossing over G" at low frequency.
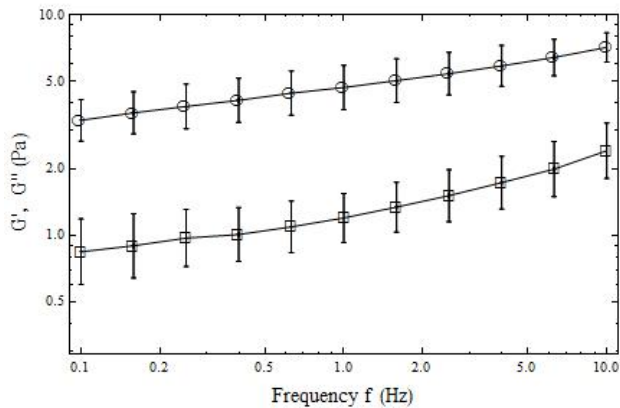
Figure 2: Plot of G' and G" (open circles and open squares respectively) versus frequency, for a representative COPD sputum sample demonstrating the typical reliability and reproducibility of our rheological data.
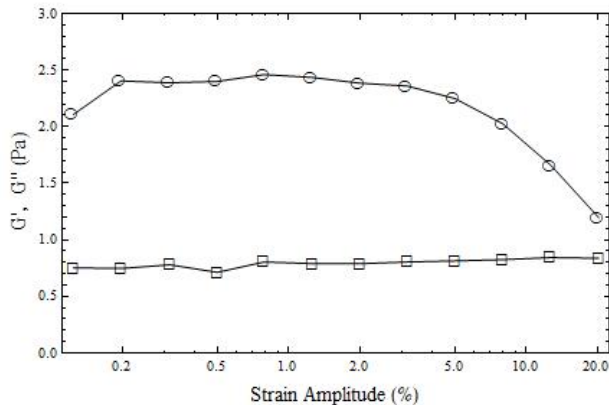

Figure 3: Plot of G' and G" (open circles and open squares respectively) for a representative COPD sputum sample, demonstrating the typical linear viscoelastic response of our rheological data at a strain amplitude of 2%

Such healthy control samples demonstrated that the viscoelastic properties of healthy sputum are influenced by factors such as water content and purulence .

The reproducibility and reliability of our rheological data is demonstrated in Figure.2, over the range of frequencies studied, where rheological measurements were carried out three times on a representative sputum sample.

Shown in Figure.3 is the linearity of our rheological measurements for a representative sputum sample, versus strain amplitude. From the figure we can clearly observe that for a strain amplitude of 2%, as considered in this work, the rheological data obtained typically lies well within the linear regime.

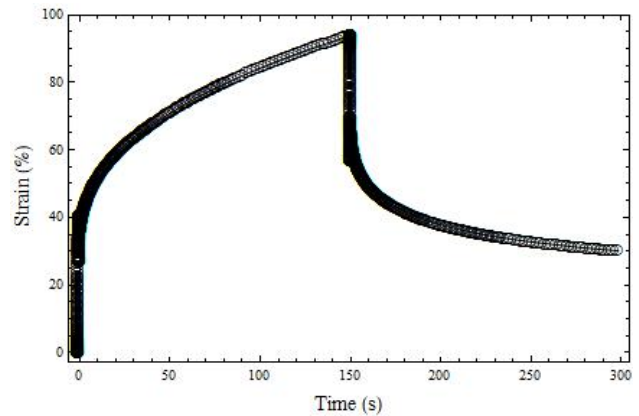The results from the SAOS shows a power law trend

Figure 4: Representative Creep-Recovery experimental data obtained (open circles), with strain plotted against time, demonstrating typical creep-recovery behaviour of a viscoelastic material.

with $G(t) \approx t^m$ where m can range from 0 to 1. Where $m = 0$, a sample would be displaying more solid-like behaviour, while $m = 1$ typifies a more liquid-like behaviour. Along with the observed trend of $G' > G''$ the complex viscosity $|\eta^*|$ was seen to increase at low frequencies which is a characteristic response for a cross-linked network [16].

The loss tangent $(\tan \delta)$ is determined as the quotient of the loss and storage moduli, as shown in Equation.7, and typically measures the ratio of the elastic and viscous portion of the deformational viscoelastic behaviour. The loss tangent varied over a small range with values between 0.26 and 0.38 across the whole physiological frequency range. Frequencies that relate to physical function of a human include ciliary beat frequency at 9Hz, while an average adult breathing rate of $18 - 22$ breaths per minute equate to a frequency of range of $0.3 - 0.36$ Hz [12].

The COPD sputum samples were also used for creep-recovery experiments and an apparent trend in the response was observed. Results of one of the trend like COPD creep-recovery experiments is shown in Figure.4; a stress of 1 Pa was used when plotting compliance $J(t)$ and strain. The time up to $150s$ in Figure.4 shows the behaviour of creep during the creep phase which shows characteristic viscoelastic response for the the sputum sample, an elastic response at the instant that stress was applied, followed by a gradual increase that can reach a steady state over time. After $150s$ Figure.4 displays the recovery phase in which the stress is removed and the resulting recovery is measured over time. In the instant the stress is removed there is a clear elastic step followed by a gradual recovery over time.

Creep testing of healthy control samples resulted often in data which showed excessively large response with no recovery. Not a single healthy control sample produced reliable data at $1Pa$, which indicates that the underlying network structure is distinctly less well formed than compared to COPD sputum.

3.2. Modelling

Initial experimental results displayed a power law behaviour over the region examined as seen in Figure.2. This type of behaviour can be understood through looking at a general network of a branched polymer that is beyond the gel point. Near and above the gel point, both the storage and loss moduli (G' & G") obey a common power law with $\omega^m \sim G' \sim G''$ [20], which suggests a fractal, or self-similar, structure for the underlying polymeric network. This characteristic behaviour results in the relaxation modulus G(t) typically decaying with time in such a way that allows for the experimental data to be fitted via a simple power law:

$$G(t) = G_0\left(\frac{t}{\tau_0}\right)^{-m} \tag{5}$$

Taking the Fourier transform of Equation.5 allows for the complex modulus G^* to be determined, and from $G^* = G' + iG''$, an expression for the storage and loss moduli can be found. Fitting of G' and G" focused on the power law index, while treating the pre-factor as a single independent variable due to the difficulty in reliably resolving G_0 and τ_0 separately.

$$G' = G_0\Gamma(1-m)(\omega\tau_0)^m \cos\left(\frac{m\pi}{2}\right) \tag{6a}$$

$$G'' = G_0\Gamma(1-m)(\omega\tau_0)^m \sin\left(\frac{m\pi}{2}\right) \tag{6b}$$

Equation.6 was used to fit the oscillatory shear data across two decades once the power index m was determined. The only variable in Equation.7 is m and allows for simple fitting.

$$\tan\delta = \frac{G''}{G'} = \tan\left(\frac{m\pi}{2}\right) \tag{7}$$

Plotting the storage and loss moduli on log-log scales, the method of least squares was used to fit the Equation.6. Figure.5 shows the result of one of the storage moduli fittings that is consistent with other sample data fittings. Of the twenty COPD samples initially used in the experiments, all but two followed the same trend and fitting as shown in Figure.5.

Table.1 displays the power law variables for all samples, and the values for the exponent m has a range
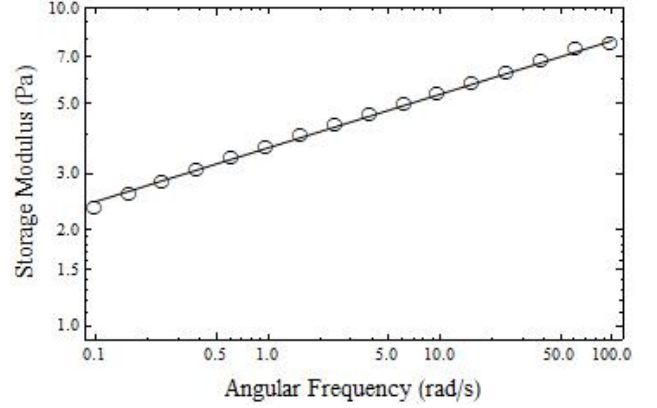

Figure 5: Result of fitting (solid line) the logarithm of G' to representative experimental data (open circles) obtained from small amplitude oscillatory shear of a typical COPD sputum sample.
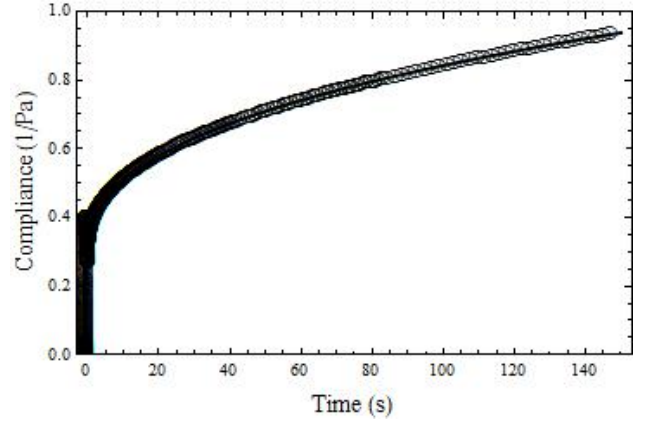

Figure 6: Result of fitting the theoretical J(t) (solid line) to experimental data (open circles) obtained from a typical sample creep experiment.

from 0.12-0.19 (excluding one anomalous sample with $m = 0.33$). This narrow range was expected for the exponent, yet the pre-factor varied significantly between samples and even between the storage and loss moduli of the same sample.

Similarly, a power law based equation was developed for fitting the creep-recovery data:

$$J(t) = J_0\left(\frac{t}{\tau}\right)^m + \frac{t}{\eta} \tag{8}$$

where t/η is a corresponding viscous term which can be linearly added to the viscoelastic equation [21]. Fitting of $J(t)$ was done over the creep region of the creep-recovery results. The results show a good fit to experimental data over the whole creep region. The fit in Figure.6 is the result of first fitting the $\tan\delta$ from os-

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cillatory data and then fitting the power law index to SAOS data which was finally used in this creep fit.

The power law index m determined from this fitting process is shown in Table.1.

Table 1: Power law index across all experiments

Sample	SAOS	CREEP
	m	m
1	0.15	0.15
2	0.15	0.15
3	0.12	0.12
4	0.14	0.14
5	0.18	0.18
6	0.17	0.17
7	0.18	0.18
8	0.15	0.15
9	0.17	n/a
10	0.14	0.14

3.3. Physiological Variables

This work was performed with sputum from patients who were admitted with an exacerbation of COPD. The general measure used to determine a diagnosis of COPD is the ratio between Forced Expiratory Volume (FEV1) and Forced Vital Capacity (FVC). Forced Expiratory Volume (FEV1), which is the volume of air expired in the first second of maximal effort, while FVC is the total volume of air expired from a total inspiration.

This data was collected and compared to the variables used in plotting the rheological data. Figure.7 shows the result of plotting FEV/FVC against the power law index m. The index m was determined through fitting to G' and the creep compliance then taking the average value of the two.

A linear fit was used to fit the data in Figure.7 and it's resulting Pearson correlation coefficient was determined to be greater than 0.8. This shows that with the limited number of samples analysed that an initial relationship between FEV/FVC and power law index m can be reliably ascertained.

3.4. Scanning Electron Microscopy

Structural analysis of COPD sputum using SEM revealed a pore size range of 50nm up to a micron, consistent with those values found in [22]. The structure of the polymer network appears to show a variety of large pores and small pores all randomly distributed in the samples imaged. Table.2 and Figure.8 demonstrate several features of the COPD sputum structure
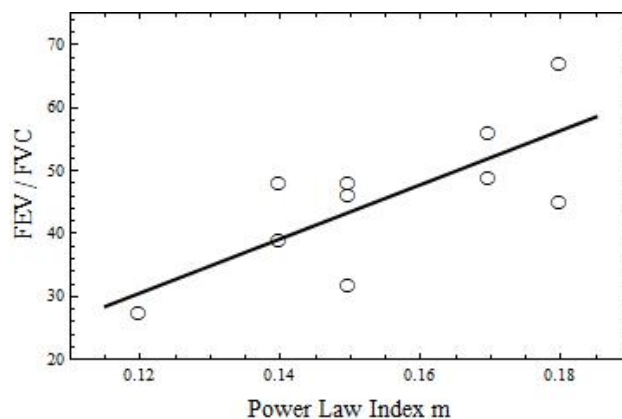

Figure 7: Forced Expiratory Volume- Forced Expiratory Volume ratio (FEV/FVC) plotted against the power index m, showing a linear trend with a Pearson correlation coefficient of R>0.8.

that may help to explain the enhanced viscoelasticity seen in some of the rheological results. Interestingly, a discussion of the possible link between the bulk modulus (G') and the average pore size of CF sputum can be found in [8], where it was suggested that as elasticity increases, so does the average pore size. Additionally, the variation in pore size will play a large role in impacting particle mobility and the potential a particle has to penetrate through the sputum polymer network. Rheology has shown that COPD sputum is a polymeric network that exhibits higher rigidity compared to healthy sputum [23]. This would imply that COPD sputum has a higher resistance to sample rupture and deformation as a result of external forces. Particles can therefore easily be caught in the small pores, or inhibited by the strong adhesive forces between the particle and sputum. This makes it difficult to identify between local sample rupture or deformations in the sputum.

Table 2: Average Pore and Fiber widths calculated from SEM images.

Sample	Pore Width (nm)	Fiber Width (nm)
A	139.06 ± 11.3	27.01 ± 1.2
B	129.37 ± 10.0	27.55 ± 1.4
C	112.11 ± 4.7	24.68 ± 0.9
D	111.61 ± 3.6	23.04 ± 0.7

Measurements of the features within the SEM image were made and a range of pore sizes was found, with average values given in Table. 2. The longest pore length measured on all samples was found to range between $60 - 450nm$, with the pore width ranging from $40 - 350nm$. This shows a considerable range between all samples and also gives an insight into the types of
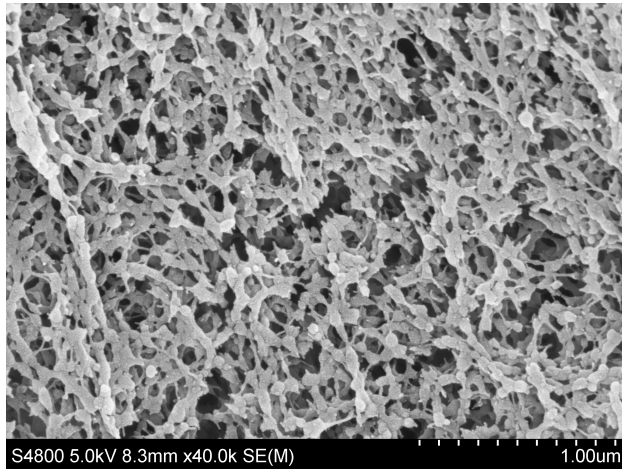
Figure 8: SEM image of sputum at a magnification of *x40k* and a working voltage of 5*kv*.

bacterium that can be hindered by this network, as discussed in [9].

Fractal analysis was also employed through the use of the box counting method to estimate a fractal dimension (d_f) for the SEM images [24]. In this method a grid of known size (spacing scale) was placed over the area for grayscale analysis. The image type required non-binary analysis in which the average intensity of each box was used to explore the scaling rule in the software package FracLac. The number of boxes was then counted followed with repeated measurements for different grid sizes and increasing spacing. The size of sampling elements ranged from $5 - 1528$ with 12 grid orientations.

Table 3: Fractal dimension

Sample	Fractal Dimension
A	1.70
B	1.61
C	1.62
D	1.63

Results of the fractal analysis of the SEM images are displayed in Table.3, where fractal dimension values ranging between 1.61 - 1.70 were found. This narrow range of fractal dimension suggests that the architecture of the polymer network is roughly similar for all the COPD sputum samples studied. Interestingly, the largest value of d_f was determined for sample A which also had the largest average pore width, a possible correlation which should be explored further and compared with rheological and clinical data in future work.

3.5. Discussion

The enhanced viscoelastic properties of COPD sputum has been proposed to significantly contribute to the clinical symptoms of COPD [25]. The rheological methods seen in this paper can directly probe physiologically relevant frequencies such as cilia beat frequency and human breathing. A person has a number of clearing mechanisms in their respiratory system, that occur at specific frequencies. These physiological frequencies have a representative average of $0.33Hz$ for breathing rate, $9Hz$ for cilia beat frequency and $16Hz$ for cough clearance [11].

Work on CF sputum conducted by Nielsen (2004) had shown that sputum has a large shear rate dependence. At high stresses sputum rupture rather than viscous flow dominates large strain deformations. This emphasised the unreliability of using viscosity as a variable that could be used as a biomarker or comparing to clinical data [8].

The rheological variables that were used to establish a correlation between clinical data included storage modulus G', loss modulus G" and loss tangent. The storage modulus G' dominates the loss modulus G" for all frequencies which shows that sputum behaves as a viscoelastic solid at small strains. Measurements made during creep-recovery tests further demonstrate the viscoelastic solid nature of sputum with a clear large elastic contribution. These result suggests that a more appropriate measure for the physical properties of sputum would be the storage modulus rather than viscosity, which has been used extensively in the past.

COPD sputum has enhanced viscoelastic properties which differ significantly from healthy controls. The results from COPD sputum showed a clear trend with each sample having a similarly consistent response under experimental conditions. This behaviour was not observed with healthy controls as experimental results observed varied from sample to sample. Comparing the creep-recovery results of COPD sputum to healthy sputum suggests that at high stresses sample rupture rather than viscous flow governs the large strain deformation. This result indicates that the polymer structure in COPD sputum dominates the viscoelastic behaviour regardless of content, unlike healthy sputum.

Scanning electron microscopy of sputum was helpfully able to picture the physical structure of the sputum network, which was further elucidated by fractal analysis [24]. The sputum displays a fractal nature in appearance, and the common power law used in modelling added to this result suggests that sputum could be treated as a open branched fractal network that could

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perhaps be better understood and explored using percolation theory [26]. Fractal dimension analysis has been performed on SEM images of COPD sputum [24]. This technique has found prior use in a variety of fields, including biological contexts, for estimating fractal dimensions from 2-d images [24]. A measure for fractal dimension was seen to range from 1.6-1.7 for several of the samples studied. Relating the fractal dimension thus obtained from image analysis, to the power law obtained via rheology, is presumably a difficult task however, not least due to the need for finding an appropriate and reliable method of extrapolating d_f from 2-d to 3-d. We leave this rather subtle issue therefore to future work, in order for further investigation.

The network structure seen in these experiments are comparable to that of other work performed on COPD sputum [23]. It is clearly distinct from other sputum SEM images such as those from cystic fibrosis sputum [22]. A fractal type of network was seen in all images for all magnifications. The COPD sputum pore size was found to have a maximum size of near a micrometer. However, the average complete pore size was found to be between 112-139 nm for all samples. This result points towards the self-similar structure that must be apparent in COPD sputum and which is responsible for the bulk response measured in rheological investigations.

With mesh sizes differing between COPD and cystic fibrosis then this may allow for different bacteria to have a higher chance of causing infections because of slower mobility in different diseased or healthy sputum, as discussed in [9]. This is also observed in COPD patients who are prescribed a treatment of inhaled steroids, as they have a higher rate of infections such as pneumonia [27]. This interesting phenomenon will be investigated further in future work.

The information generated from the modelling of the data, shown in Table.1 was used to compare to clinical measures of COPD severity such as FEV, age, gender and Forced Vital Capacity (FVC). Figure.7 displays the FEV/FVC ratio plotted against the power law index m calculated from all experiments. A correlation is apparent in Figure.7 which needs to be investigated further as a potential biomarker for COPD. We would like to emphasise that in this work, for the first time, a correlation has been shown between the rheology of COPD sputum and clinical measures, such as FEV/FVC, which could potentially predict clinical symptoms through a non-invasive process of rheology. Furthermore, this very exciting 'proof of concept' result, as provided here, should naturally prove to be of great interest in future work.

4. Conclusions

Oscillatory rheology has demonstrated that COPD sputum is a viscoelastic solid, with further results from creep-recovery experiments supporting this observation. Both oscillatory and creep techniques have shown their reliability in providing rheological data as repeated measurements return identical results, which has not always been the case for other work that has a focus on measuring viscosity alone.

The results highlight that sputum should be treated as a mature cross-linked biopolymer network and obeys a common power law in the range of frequencies measured. A power law model was successfully fitted to experimental data, and when comparing the power law index m to clinical data a significant correlation was observed. Power law rheological models for more general polymeric materials have previously been outlined in [28], for example. This shows that rheology has a great potential for use as a biomarker in patients with COPD, but more work is needed in order to increase the sample size, for example, and explore rheology as a diagnostic and monitoring tool.

Future work will include investigating the effect of mucolytic agents on the viscoelastic properties of sputum over time to see if there exists a correlation between the power law exponent from rheology and more reliable mucolytic agents. Currently diagnosis of COPD relies on spirometry which is heavily affected by the patient and practitioner and if rheology can be shown to work effectively as a monitoring tool, then it could be used to as an aid to earlier and much more accurate diagnosis.

Previous attempts to relate experimental values to clinical measures for use as a biomarker have received significant interest, and a recent review article by Paone et al (2016) shows sputum as a good source for analysis of COPD [25]. This is the first time rheology of COPD sputum has been shown to possess good potential in predicting clinical symptoms. This further highlights the current work, as presented here, as having the promising potential for an effective biomarker for COPD.

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