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Flexible microfluidic device for mechanical property characterization of soft viscoelastic solids such as bacterial biofilms

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Abstract

We introduce a flexible microfluidic device to characterize the mechanical properties of soft viscoelastic solids such as bacterial biofilms. In the device, stress is imposed on a test specimen by application of a fixed pressure to a thin, flexible poly(dimethyl siloxane) (PDMS) membrane that is in contact with the specimen. The stress is applied by pressurizing a microfabricated air channel located above the test area. The strain resulting from the applied stress is quantified by measuring the membrane deflection with a confocal laser-scanning microscope. The deflection is governed by the viscoelastic properties of the PDMS membrane and of the test specimen. The relative contributions of the membrane and test material to the measured deformation are quantified by comparing a finite element analysis and an independent (control) measurement of the PDMS membrane mechanical properties. The flexible microfluidic rheometer was used to characterize both the steady-state elastic modulus and transient strain recoil of two soft materials: gellan gums and bacterial biofilms. The measured linear elastic moduli and viscoelastic relaxation times of gellan gum solutions were in good agreement with the results of conventional mechanical rheometry. The linear Young's moduli of biofilms of *Staphylococcus epidermidis* and *Klebsiella pneumoniae*, which could not be measured using conventional methods, were found to be 3.2 kPa and 1.1 kPa, respectively, and the relaxation time of the *S. epidermidis* biofilm was 13.8 s. Additionally, strain hardening was observed in all the biofilms studied. Finally, design parameters and detection limits of the method show that the device is capable of characterizing soft viscoelastic solids with elastic moduli in the range of $10^2 - 10^5$ Pa. The flexible microfluidic rheometer addresses a need for mechanical property characterization of soft viscoelastic solids common in fields such as biomaterials, food and consumer products. It requires only ~ 200 pL of test specimen.

1 Introduction

Characterization of the mechanical properties of soft materials is important for fields such as consumer products, food science, biomaterials and pharmaceuticals. Although existing rheological methods are well suited to the measurement of homogeneous materials, their applicability is more limited in cases where mechanical properties such as the viscoelastic relaxation time and linear elastic modulus exhibit microscale heterogeneity because of spatial variations in local structure. To quantify the properties of such heterogeneous materials, microscale characterization is required. Many such heterogeneous materials can be classed as soft viscoelastic solids. These materials do not readily flow, are highly elastic ($E < 0.1$ GPa)^{1, 2}, but are capable of some dissipation as evidenced by their transient viscoelastic response. Examples of soft viscoelastic solids include many gels, biomaterials and foods.

To address the need for such local viscoelastic characterization, new techniques based upon microrheology, microfluidic rheometry and flexure-based rheometry have been developed. For example, microrheology correlates the motion of colloidal particles dispersed in complex fluids with the fluid's viscoelastic properties^{3–7}. Microrheology measures elastic moduli in the range of 10^{-1} – 10^3 Pa^{8, 9} and relaxation times on the order of micro to milliseconds^{10–12}. Spatially resolved¹³ and two-point⁸ techniques have been introduced to extend the method to heterogeneous samples. Methods to characterize viscoelastic properties by means of microfluidics have also been developed. Droplet retraction methods quantify relaxation times as fast as microseconds¹⁴. Weak elastic effects found in contraction and expansion flows have been measured^{15, 16}. The addition of pressure sensing in these geometries yields simultaneous determination of shear and extensional rheology¹⁷. To date microfluidic methods have been principally applied to weakly elastic fluids such as dilute polymer solutions. Flexure-based rheometry has been applied to 1–10 μ L samples of test material. The stresses imposed by these methods are in the range of 10–1500 Pa^{18, 19}. Piezoelectric methods have likewise been applied to deform polymer solutions and measure their dynamic response to sinusoidal inputs²⁰. Finally, microindentation devices have been applied to characterize material properties of biofilms. These devices probe elastic moduli over the range of 1–10 kPa²¹.

Even given these recent developments, methods to measure elastic moduli in the range of 10–100 kPa are few. Yet, such methods could be fruitfully applied to characterize properties of soft viscoelastic solids at microscopic length scales. In this manuscript we report a device suitable for mechanical property characterization in this range. The device uses simple methods

and materials previously developed for microfluidic applications. We apply the method to the two soft viscoelastic solids: gellan gums and bacterial biofilms.

To develop the flexible microfluidic rheometer we adapt the design of poly(dimethyl siloxane) (PDMS) devices previously developed for pumping and valving in microfluidic systems²². These devices use a two-layer assembly in which a PDMS membrane separates an air channel from a main fluidic channel. In response to an applied pressure in the air channel, the PDMS membrane deforms and the valve closes. We find that when the main fluidic channel is loaded with a soft viscoelastic solid (e.g. Young's modulus, $E \sim 10\text{kPa}$) the open/close response of the microfluidic channel changes significantly relative to the control case of a valve filled with water. We use this change in deformation to perform rheometry. The change in valve deformation is a function of its geometry, the mechanical properties of the test specimen, and the elasticity of the membrane. To quantify the test specimen elastic modulus, we compare the membrane deflection, measured by confocal microscopy, to finite element simulations of the valve's mechanical response to the applied air pressure. This comparison transforms qualitative differences in the open/close response of the flexible valve into a quantitative characterization of the test specimen's elastic modulus.

Attributes of this flexible microfluidic rheometer are its simple fabrication and operation. By tuning the mechanical properties of the PDMS membrane (by changing its crosslink density), devices can be prepared which are suitable for characterization of materials with a wide range of moduli (from 10^2 – 10^5 Pa) and relaxation times as small as ~ 10 s. The microfluidic design allows for microscale characterization using confocal microscopy, measurements of both linear and non-linear response and performance with small (~ 200 pL) samples. The microfluidic format also allows for easy integration with multi-process lab-on-a-chip designs²³. Although here we use confocal microscopy to characterize the deformation applied to the test sample, we note that other high resolution optical methods would also be suitable for strain measurement. A particular advantage of the confocal microscopy method is that it provides additional information about the underlying microstructure of the soft material tested.

A particular aim of this work is to use the flexible microfluidic rheometer to characterize the mechanical properties of bacterial biofilms. Biofilms are communities of sessile bacteria embedded in an extracellular, polymeric matrix^{24–27}. Biofilms are a common phenotype of bacteria relevant to human health, because they are implicated in diseases such as bacteremia²⁸, and to environmental engineering, since they can form in industrial water systems²⁹. This device is ideal to study biofilms for the following reasons: First, heterogeneities in the structure make them difficult to study with conventional rheometry^{30–32}. These heterogeneities exist on scales as large as $100\mu\text{m}$ ^{33–35}. Thus, microscale characterization can potentially resolve differences in the mechanical properties of biofilms relevant to physiological scales^{27, 36}. Second, the microfluidic environment allows for *in situ* growth of the biofilms at shear rates consistent with those found in the cardiovascular system and *in situ* fluorescent labeling for imaging. Finally, bacterial biofilms of clinical interest are thought to display elastic moduli characteristic of soft viscoelastic solids that this device was designed to resolve³⁰.

The organization of this manuscript is as follows: First, we present techniques to design, manufacture and characterize the device using confocal microscopy and finite element analysis. We then determine the linear elastic (Young's) modulus and viscoelastic relaxation time of model gellan gum solutions. We compare the results with those acquired by means of conventional mechanical rheometry to assess the accuracy of the new method. Then, as an example application of the device to a soft viscoelastic solid, we characterize the elastic modulus and relaxation time of natively grown bacterial biofilms. These materials have proven difficult to characterize with conventional rheometers³². Finally we present quantitative

recommendations for device design and fabrication for characterization of the mechanical properties of soft matter with elastic moduli in the range $\sim 0.1 - 100$ kPa.

2 Methods and materials

2.1 Microfluidic device design and fabrication

The device is shown schematically in Fig. 1a. The test chamber volume is $25 \times 125 \times 75 \mu\text{m}^3$ (~ 200 pL). The top surface of the chamber is a flexible membrane upon which a stress can be imposed by applying pressurized air to an overlying channel²². In Fig. 1b we show the horizontal test chamber with two vertical pressure chambers allowing for two test areas (circled) on the same device. The air pressure channel is $75 \mu\text{m}$ wide channel while the test chamber is $\sim 25 \mu\text{m}$ high and $\sim 125 \mu\text{m}$ wide. The lateral structure of the device is shown in Fig. 1c, as imaged by reflection mode confocal microscopy. The three layers, from bottom to top, are the test chamber, the flexible PDMS substrate and the air channel. The device was fabricated from crosslinked elastomeric PDMS (Dow Sylgard 184 Silicone Elastomer Kit) whose properties were tuned by varying the ratio of elastomer to curing agent, and thus the cross-link density. In typical valve applications (e.g. ref [27]) this ratio is 30:1 and the PDMS elastic modulus is $\sim 600-750$ kPa^{22, 37}. By varying the base to linker ratio 30:1 to 60:1, membranes with moduli of 20 kPa – 500 kPa were reproducibly fabricated.

Devices were fabricated using soft lithography³⁸. Air and test channel masters were fabricated independently on 10 cm silicon wafers (University Wafer, Boston, MA). SU-8 2015 (MicroChem, Newton, MA) was spin-coated to yield a $\sim 25 \mu\text{m}$ coating. Air channels were made with a PDMS base and curing agent at a ratio of 10:1. The bottom fluidic network was made from a low curing agent ratio PDMS. Due to the tackiness of this layer, a silane treatment was required to remove the PDMS device from the mold. The wafer was heated at 140°C for 1 hour in a vapor of (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (United Chemical Technologies, Inc., Bristol, PA). Reactive ion etching (RIE 2000, South Bay Technologies, San Clemente, CA) was used to bond the air channel and test channel assemblies into a completed device.

For confocal microscopy the device was adhered to a 45×50 mm No. 1 (thickness $0.14-0.17$ mm) glass coverslip (Fisher Scientific, Pittsburgh, PA). For low-modulus devices, a drop of water was applied to the glass substrate to wet the fluidic test channels during adhesion to prevent the channels from collapsing due to interfacial forces.

2.2 Confocal microscopy detection of flexible rheometer strain

Confocal laser scanning microscopy (CLSM, Leica TCS SP-2 with a DMIRE-2 inverted microscope, $100\times$ NA 1.4 oil immersion objective) was used to image membrane deflection in a plane perpendicular to the bottom glass substrate. The pixel and image sizes were 98 nm and $50.2 \times 50.2 \mu\text{m}^2$, respectively. The image acquisition time was 0.848 s. The membrane deflection (as shown, for example in Fig. 1 c, d) was imaged in reflection mode with 488 nm incident light. For the transient response, 300 or 150 images were collected with time steps of 0.512 s or 0.848 s, respectively. Image processing used to determine the membrane reflection to a resolution of $\sim 0.5\mu\text{m}$.

2.3 Operation of the flexible microfabricated rheometer

Air pressure (as large as about 100 kPa) was applied to generate a deflection of the PDMS membrane above the test material. A series of incrementally increasing air pressure was applied to the device and the membrane deflection measured after steady-state was reached for each. For a transient experiment, the time-dependent membrane deflection was imaged after a step-change in air pressure was imposed. Because the deflection of the flexible membrane is a

function of both the test material and PDMS material properties, a control experiment to assess the response of the device without the test material was required. For this characterization, the test chamber of the device was filled with water. The response at eight different applied pressures was compared to the results of FEA to determine the linear elastic modulus of the elastomeric PDMS. To address any potential issues with PDMS aging, this control experiment was performed within 36 hours of any experiment.

2.4 Finite Element Analysis (FEA) simulations

Because the microfluidic rheometer's principle of operation relies on the flexibility of the elastomeric PDMS of which the device is comprised, we modeled the deformation of this linearly elastic material with FEA to quantify the mechanical properties of the test materials. The FEA methodology is crucial to the performance of the rheometer because it controls for inevitable device to device variation in geometry and PDMS material properties that result during fabrication. (In fact, we found that characterization of the Young's modulus of the PDMS *in situ* by the method described here was more accurate than *ex situ* characterization by mechanical rheometry, likely because the elasticity of the crosslinked PDMS is quite sensitive to variables such as the mixing procedure and aging times.)

Finite element analysis was performed with a standard simulation tool for linear elastic analysis (Structural mechanics module of Comsol Multiphysics v 3.3a). The three sub-domains of the finite element mesh, each with different material properties, were (i) the air channel; (ii) the PDMS device surrounding the test channel and (iii) the fluidic test channel comprised of the test material. Typical mesh values over these sub-domains included 26,000 elements and ~90,000 degrees of freedom. The PDMS was modeled as a linear elastic material with unknown Young's modulus. The Poisson's ratio of PDMS was taken as $\nu = 0.45$ ³⁷. The volume modeled by FEA was approximately $4 \times 4 \times 0.05$ mm. The maximum deformation of the flexible membrane was extracted from the simulation at each pressure that had been applied during the control experiment.

Typical results of this procedure are shown in Fig. 2. Fig. 2a plots the deflection of the membrane at different applied pressures ($P = 3.4$ kPa – 58.7 kPa) and $E_{\text{PDMS}} = 350$ kPa. The shape of the membrane, as resolved by reflection confocal microscopy, agrees well with the simulated membrane deflection as shown in Fig 2b. Fig 2c plots the experimentally determined maximum deflection normalized by the width of the air channel as a function of the pressure applied to the air channel. Fig 2c shows that for devices with PDMS of three very different elastic moduli, the measured maximum deflection is a simple linear function of the applied stress. The curves plotted are the simulated membrane deflection for the optimal Young's modulus of the PDMS membrane. The Young's moduli extracted from the simulations are consistent with the expected values given the amounts of cross linker used to produce the PDMS elastomer.

FEA was similarly used to determine the elastic modulus of test specimens. The measured deflection of the flexible membrane in this case differed from the control experiment due to the elasticity of the test material. The ability to resolve the difference is a function of the relative values of the elastic modulus of the loaded material and that of the PDMS. That is, if the test material is much more compliant (lower modulus) than the PDMS, then the membrane deflection will not be significantly perturbed from the control case. Alternatively, if the PDMS is much more compliant than the test material, then the membrane will not significantly deform at all for typical air channel pressures. The optimal range was found to be intermediate between these two regimes ($E_{\text{specimen}}/E_{\text{PDMS}} \sim 0.01-1$).

2.5 Rheometry

Results from the flexible microfluidic rheometer were compared to tests performed with a stress-controlled rheometer (AR G2, TA Instruments, New Castle, DE). To determine the linear elastic modulus in a manner consistent with the microfluidic experiments, the steady-state stress-strain response of 2 and 3% (w/w) gellan gum was measured in a compression experiment with a 40 mm flat plate. Steady-state behavior was confirmed by monitoring the response over 5 minutes. To measure the longest relaxation time of the gellan gum, a (shear) creep experiment was performed with a 60 mm 2° cone geometry. A shear stress (50 Pa, in the linear regime) was applied for 150 seconds and then the elastic recoil was observed after the stress was released.

2.6 Gellan gum solutions

Gellan gum (food grade Kelcogel (Lot#4E0783A, CP Kelco, Chicago, IL) was prepared in 2% and 3% (w/w) solutions. Dry gum powder was added to distilled, deionized water at the desired weight fraction. This solution was mixed on a vortexer and then heated to 70°C for one hour so as to yield a transparent liquid solution that was easily loaded into either the microfluidic or conventional rheometer.

2.7 Bacterial biofilms

To demonstrate microscale characterization of a structurally heterogeneous soft viscoelastic solid, we studied two different strains of encapsulated biofilm-forming bacteria, *Staphylococcus epidermidis* RP62A (obtained from ATCC, catalog number 35984) and *Klebsiella pneumoniae* LM21 (a gift from Christine Forestier, Universite d'Auvergne-Clermont, Clermont, France)^{39, 40}. Organisms under study were cryopreserved at −80°C until the night before use, when they were streaked for isolation on Luria-Bertani (LB) agar. On the day of study, organisms were grown at 37°C in LB broth in a shaking incubator at 200 rpm. These organisms were loaded into test chambers and allowed to adhere for 1 hour. After adhesion, the device was continuously perfused with LB broth using a syringe pump (Harvard Apparatus, Holliston, MA) at 0.1mL/hour for a minimum of 18 hours at 37°C in room air.

3 Results

3.1 Static Elastic Modulus of Gellan Gum

Fig. 3 reports the steady-state response of 2.0% (w/w) solutions of gellan gum to a series of applied pressures in the flexible microfluidic rheometer. As described in the methods, incrementally increasing pressures were applied to the air channel. This pressure stresses the flexible PDMS membrane above the sample, thereby yielding a deflection of the membrane that is a function of the static elastic modulus of the PDMS and of the gellan gum. At each pressure, the steady-state membrane deflection (data acquired for $t > 1$ min after application of the pressure) was recorded by confocal microscopy. Fig. 3a plots the steady-state strain, ϵ , measured for each pressure. (For consistency with the typical way of reporting mechanical property characterization, here and in subsequent figures we plot the strain measure $\epsilon = \Delta h / h_0$, where Δh and h_0 are the measured deflection and undeformed height of the fluidic channel respectively, as the abscissa and stress as the ordinate; however, note that in the experiments it is stress that is controlled and the strain that is measured.)

For comparison, the deflection of the PDMS membrane in the unloaded device (control) is also plotted in Fig. 3. This comparison shows that the deflection of the device when loaded with the elastic gellan gum differs significantly from the control experiment. This difference is a direct consequence of the material properties of the gellan gum. The deformation from the control experiment is compared to the results of FEA to determine the elastic modulus of the

PDMS membrane for the particular device as discussed previously. The result, plotted as the solid line in Fig. 3, yields $E_{\text{PDMS}} = 320 \text{ kPa}$. This value is input into a multilayer FEA simulation of the combined gellan/PDMS response of the loaded device. The deformation response of the 2.0% gellan gum in the PDMS device is simulated for a range of candidate static moduli of gellan gum (dashed lines). The elastic modulus that best models the data, $E = 8.0 \text{ kPa}$, is plotted along with the linear fit of the experimental data (solid line).

Figure 4a reports the analysis of Fig. 3 applied to 2.0% and 3.0% gellan solutions. The datum points are the mean of five replications. The curve plotted is the best estimate of the elastic modulus of the gellan materials determined from FEA (per the method in Fig. 3). Error bars are standard error of the mean. Note that the linearity of the stress/strain data, as well as the good agreement with the simulations method (which is itself based on linear elasticity), confirms that these measurements are performed within the linear elastic limit. From the analysis of Fig. 4, the flexible microfluidic rheometer determines the static elastic modulus of 2.0% and 3.0% gellan are 8.0 ± 0.2 and $30 \pm 1 \text{ kPa}$, respectively.

Fig. 4b indicates results from conventional rheometry, in which normal stresses are plotted for 2.0 and 3.0 wt% gellan gum. This experiment is a good approximation of the compression test performed in the microfluidic device. The error bars are the standard error of the mean from four independent measurements. The static (linear) elastic modulus of the material is the slope of the stress-strain curve. From this slope, conventional rheometry determines the 2.0 wt% and 3.0 wt% gellan gum moduli to be $7.8 \pm 0.5 \text{ kPa}$ and $38 \pm 0.9 \text{ kPa}$, respectively. The relative error between microfluidic and conventional rheometry for the 2% gellan gum is thus 2.5%. The relative error for the 3% gellan gum is 21%. These relative errors establish the capabilities of the flexible microfluidic rheometer for assessment of the static elastic modulus of soft viscoelastic solids.

3.2 Transient strain response of gellan gum

Fig. 5a reports the transient strain response of the PDMS membrane in contact with a 2.0 wt% gellan gum sample when the initial applied stress of 16 kPa is released. Not shown is the control response for the recoil of the elastomeric PDMS membrane without test sample - in this case the membrane returns to its equilibrium position in less than 2 s. Thus, the membrane response is retarded by about two orders of magnitude by the effect of the loaded viscoelastic material. Each datum point plots the average of the measured strain for five independent experiments at each time step ($\tau = 0.512 \text{ s}$) after the step decrease in pressure in the air channel. Error bars are the standard error of the mean of the five measurements. The transient response follows an exponential decay to the original thickness of the material ($\epsilon = 0$) that is well modeled as a single exponential function as shown by the solid curve in the figure. From the characteristic time of the exponential decay we estimate the longest relaxation time of the 2.0 wt% gellan gum to be $19.4 \pm 0.9 \text{ s}$. It appears that the Fig 5a. experiment was performed in the linear regime, because a linear response between stress and strain is observed in Fig. 4 was observed up to at least $\epsilon = 0.2$. The initial strain amplitude of the release in Fig 5a was chosen as $\epsilon \sim 0.2$ for that reason.

To test the accuracy of this result, we applied a shear stress of 50 Pa to 2.0 wt% gellan gum for 150 s in a conventional rheometer. (Mechanical rheometry showed that the linear regime for this material extended to 100 Pa.) Figure 5b reports the recoil of the gellan gum sample after this stress is released. Just as for the flexible microfluidic rheometer, an exponential decay of the strain is apparent in the data. Error bars are the standard error of the mean of three independent measurements. The curve plotted is the predicted response of a single exponential decay with a characteristic relaxation time of 19.4 s, as taken from the results of the flexible rheometer in Fig. 5a. The mechanical rheometry shows evidence of an additional, very long time decay of the recoil. This small decay, with time constant $\sim 10^2 - 10^3 \text{ s}$, is consistent with

the known aging behavior of soft materials such as gellan gum⁴¹. The agreement between the response of the flexible rheometer and the relaxation of conventional rheometry is very good except at the very longest times where this aging is observed. The transient response of the flexible microfluidic rheometer thus provides a rapid method to characterize the longest viscoelastic relaxation time of soft viscoelastic solids.

We note that additional variables that might contribute to the transient response of the microfluidic set-up include the compliance of the device and the overall viscosity of the fluid in the test section. Although a control experiment with water indicated at least an order of magnitude separation in time scales between the instrument compliance and the viscoelastic relaxation of the test specimen, additional work to characterize the role of these variables on the transient response is warranted.

3.3 Static elastic modulus of bacterial biofilms

Fig. 6 reports images of a *Staphylococcus epidermidis* biofilm loaded into the test chamber. The bacteria are labeled with SYTO-9 dye (Invitrogen, Carlsbad, CA) for fluorescence imaging of individual organisms. The biofilm completely fills the test area as shown in the plane parallel to the bottom plate of the device in Fig. 6a (image dimensions $150\mu\text{m} \times 150\mu\text{m}$). The biofilm also fills the entire test area in the plane perpendicular to the bottom plate as in Fig 6b ($h_0 = 26.2\mu\text{m}$). As the applied pressure is increased the biofilm compresses in response to the deformation of the elastomeric PDMS membrane as shown in Fig 6b–d.

At the final applied pressure ($P = 11.4\text{ kPa}$), the height of the film at the point of maximum membrane deflection is $15.8\mu\text{m}$. In these and following figures each locus of fluorescence intensity in the image is due to emission from a single bacterium of the biofilm. The fluorescence images of Fig. 6 show that the biofilm microstructure responds to the forcing of the flexible membrane in a way that can be used to extract mechanical properties, just as was accomplished for the gellan gum samples.

Fig. 7 plots the steady-state response ($t \sim 30\text{ s}$) for both *S. epidermidis* as well as a *Klebsiella pneumoniae* biofilms subjected to the protocol reported in Fig. 6. As for the case of gellan gum, the control response is plotted on the figure to show the different responses of the loaded and unloaded device. At low applied strains a region of linear behavior is observed. At higher strains a transition to a strain hardening behavior is found. Below we analyze these data with the methods reported for gellan gum, thereby extracting the elastic moduli of the biofilms in these two regimes.

Using the method outlined in Sec 2.4, we determined the static elastic modulus of the PDMS membrane from the control response (not shown for *K. pneumoniae* device). By applying the analysis used for gellan gum (c.f. Fig. 3), we found the best estimate of the low-strain (linear) static elastic modulus of *S. epidermidis* to be 3.2 kPa and for *K. pneumoniae* to be 1.1 kPa . A similar analysis was completed for all biofilms shown.

We analyzed the strain hardening response observed at high strains for both biofilms. For *S. epidermidis* the transition to strain hardening occurs at $\epsilon \sim 0.2$. To extract an effective high strain elastic modulus we applied the FEA procedure of Sec. 2.4 and Fig. 3 to the high strain regime as shown in Fig. 7. This response is consistent with a high strain elastic modulus of 23 kPa , an order of magnitude greater than the low strain elastic modulus. *K. pneumoniae* displays a similar transition to strain hardening at $\epsilon \sim 0.5$. Here the FEA estimates that the high strain elastic modulus increases 4-fold, from 1.1 kPa to 4.3 kPa .

3.4 Transient response of viscoelastic biofilms

The flexible microfluidic device was used to characterize the longest viscoelastic relaxation time of a *S. epidermidis* biofilm according to the methodology applied for gellan gum. Fig. 8 plots the transient response of an *S. epidermidis* film upon release of an applied pressure. We monitor the response of the elastomeric PDMS membrane for 150 s after the step response in stress ($\tau = 0.848$ s). The transient response is well modeled by a single exponential decay with a characteristic relaxation time of 13.8 s, much more rapid than 1100 s reported in literature³⁰. The inset images show the microstructure of the biofilm during three different time steps of the recoil. (The images were acquired in the plane perpendicular to the bottom plate of the device). Importantly, these images confirm that, during stress relaxation, the biofilms maintained contact with both the upper and lower surfaces of the test chamber, a requirement for estimating the viscoelastic response in this device.

4 Discussion

The results demonstrate that the Young's modulus and transient strain response of soft viscoelastic solids such as bacterial biofilms can be characterized in a flexible microfluidic device by integrating finite element analysis and confocal microscopy. The results from gellan gum experiments agree well with those from conventional rheology. The elastic response of the biofilms agrees well with ranges presented in rheological reviews of other biofilm systems, while the transient response does not match the reported values³⁰. We also observed strain hardening in bacterial biofilms.

The measurements with bacterial biofilms demonstrate the utility of the flexible rheometer. Because the device requires just ~ 200 pL of specimen, and because it can apply high strains, it opens new avenues for non-linear rheological characterization of materials available in only small quantities, such as biological specimens. Moreover, because the material deformation is applied on scales of less than 100 μm , the device truly probes non-linear rheology at the microscale. Such a local measurement introduces new possibilities for characterization of differences between the mechanical environment probed by microscale objects such as cells and macroscale methods such mechanical rheometry.

To conclude, we discuss potential future improvements to the device, compare our material property characterization of biofilms to literature and address conditions under which the material can be applied to characterize the mechanical properties of other soft viscoelastic solids.

4.1 Potential future improvements to the device

The flexible microfluidic rheometer uses confocal microscopy to measure the membrane deflection and finite element analysis (FEA) to quantify elastic moduli. We identify two areas for potential future improvement.

First, FEA of the multiple regions of the device used only linear elasticity. This methodology worked well for the cases considered in this paper; however, in order to extend the usage of the flexible microfluidic rheometer to other classes of materials, modeling the device response for the more general case of non-linear viscoelasticity would be of interest. In addition to models of elastoplastic and hyperplastic materials available for Comsol Multiphysics and other FEA software⁴² that could be implemented, other simulation methods and tools could be applied^{43, 44}.

Second, the flexible microfluidic device could be operated so as to address frequency dependent viscoelasticity of soft viscoelastic solids by applying a sinusoidal variation in pressure. The amplitude and phase lag of the membrane response could then be monitored by CLSM. Data

analysis would then proceed as for current methods used in mechanical rheometry². A general limitation to acquisition of time dependent data such as described is the time resolution of the CLSM. For example, for our instrument the fastest image acquisition rate is ~ 0.5 Hz. To address this limitation, faster confocal microscopes are available⁴⁵. The measurement of membrane deflection could also be accomplished by means other than confocal microscopy, such as laser reflection⁴⁶. An advantage of confocal microscopy, however, is that it allows in-situ imaging of local structural changes in the test material, as in Fig. 6.

4.2 New material property characterization of biofilms

The low-strain Young's modulus of *S. epidermidis* and *K. pneumoniae* determined from our device is similar in magnitude to the shear moduli previously reported for parallel-plate rheometry experiments with *S. mutans*, *S. aureus*, and *P. aeruginosa* biofilms³⁰. In that study, biofilms of any single bacterial species were found to have effective shear moduli ranging from 1 to 1,000 Pa. A key difference between that study and the current one is the length scale at which these structures were evaluated. The biofilms grown in our test chambers were much more homogenous than films grown in geometrically unconfined conditions, and the length scale of the material in general is less than that of the water channels and other heterogeneities that characterize larger biofilms structures. As a result, our estimates for Young's modulus are at the high end of those noted by ref³⁰. Because of its ability to characterize such small bacterial structures, our method is an effective tool for evaluating the polymeric phenotype of bacterial biofilms in the absence of the macrostructural voids that historically have confounded such efforts.

Additionally, we demonstrate clear evidence of strain hardening elastic behavior, with biofilms becoming progressively stiffer upon increasing strain. A similar effect was reported from a microindentation device, but was thought to be caused by a change in the biofilm from one experimental series to the next²¹. The strain-hardening response could be linked to the composite nature of the biofilms: indeed, atomic force microscopy studies of single bacteria have shown these particles to have Young's moduli on the order of 10⁵ Pa [51]. With progressive strain, our device might increasingly interrogate first the mechanical properties of bacteria packing into smaller volumes and ultimately the mechanical properties of the individual suspended bacteria. The advanced imaging of individual bacteria possible in our system confirms that rather than being a result of just inter-experimental variability, particle ordering and strain hardening are a characteristic feature of biofilms under progressive strain.

The transient viscoelastic response of the biofilm is much more rapid than that reported by Shaw et al. They determined a common elastic relaxation time of 1100 s for several different biofilms³⁰. However in our case the transient response of the elastomeric PDMS membrane adhered to a *S. epidermidis* film yields a characteristic relaxation time of 13.8 s. We believe the difference in time scales, much as with the case of our measured elastic moduli, is a result of the structural differences in the biofilms between the two studies and the length scale at which our analysis was undertaken. Differences in the conditions under which the biofilms were grown (as the mechanical features of these structures are known to vary with the shear field in which they develop) may also contribute to differences in our observations.

4.3 Recommendations for applying the flexible microfluidic rheometer to the characterization of other soft viscoelastic solids

Our device combines microfluidic assembly techniques and FEA to provide a simple method for characterizing soft viscoelastic solids with moduli in a range difficult to study with current alternative methods. To summarize the scope for mechanical property characterization, Fig. 9 reports the FEA determined strain response of a 25µm thick PDMS membrane and a 25 µm thick test material at the pressure required for full dynamic range (closure) of the empty device

at a given PDMS modulus. The simulated membrane strain was determined over a range of moduli of the test material. The upper and lower curves are based on the resolution limits of the flexible rheometer. The lower limit of measurability (left hand curve) is for a resolution limit of $\epsilon = 0.92$, corresponding to a 4 μm difference between full-scale deflection of the unloaded vs. the loaded device. (The 4 μm limit is about an order of magnitude greater than the resolution limits of the confocal microscope.) The upper resolution limit is specified by requiring that the full-scale actuation pressure of the loaded device be no greater than 10% of the test material modulus. Fig. 9 indicates that a test material elastic modulus varying by ~ 2 orders of magnitude can be measured in a device microfabricated with a PDMS membrane of one specific modulus. The limits of the range shift (toward higher moduli) as the elastic modulus of PDMS increases. Fig. 9 also reports an estimated range of elastic moduli for 30:1 and 60:1 PDMS elastomer base to cross-linking agent ratios. This range provides a guideline to determine the appropriate ratio of elastomer base to cross-linking agent required to fabricate a flexible microfluidic rheometer to determine the test material modulus within an estimated range.

Included in the figure are data from the experiments discussed in this paper. Results for 2% and 3% gellan gum were made in two different devices each. Despite differences in device PDMS characteristics, the resulting test moduli for the same materials are acceptably similar. We also report the strain response for three different *S. epidermidis* films and the *K. pneumoniae* film. As in the case of gellan gum, the differences in elastic modulus of the PDMS membrane do not affect the measured moduli of the biofilms, even for the strain-hardened regime.

5.0 Conclusions

This paper introduces a flexible microfluidic rheometer that can characterize the elastic modulus and transient response of soft viscoelastic solids. We tested the capabilities of the device using gellan gum solutions. These results agreed well with the results of mechanical rheology. The device was applied to determine the elastic moduli of bacterial biofilms. Strain hardening in these systems was observed. By applying design recommendations reported in Fig. 9, the device can be applied to study a range of soft viscoelastic solids including biomaterials, foods and consumer products.

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References

1. Holyst R. *Soft Matter* 2005;1(5):329–333.
2. Ferry, JD. *Viscoelastic Properties of Polymers*. Vol. 3rd Edition. New York: John Wiley and Sons; 1980.
3. Mason TG, Weitz DA. *Physical Review Letters* 1995;74(7):1250–1253. [PubMed: 10058972]
4. Solomon MJ, Lu Q. *Current Opinion in Colloid & Interface Science* 2001;6(5–6):430–437.
5. MacKintosh FC, Schmidt CF. *Current Opinion in Colloid & Interface Science* 1999;4(4):300–307.
6. Gisler T, Weitz DA. *Current Opinion in Colloid & Interface Science* 1998;3(6):586–592.
7. Mason TG, Ganesan K, vanZanten JH, Wirtz D, Kuo SC. *Physical Review Letters* 1997;79(17):3282–3285.

8. Crocker JC, Valentine MT, Weeks ER, Gisler T, Kaplan PD, Yodh AG, Weitz DA. *Physical Review Letters* 2000;85(4):888–891. [PubMed: 10991424]
9. Mason TG, Gisler T, Kroy K, Frey E, Weitz DA. *Journal of Rheology* 2000;44(4):917–928.
10. Savin T, Doyle PS. *Biophysical Journal* 2005;88(1):623–638. [PubMed: 15533928]
11. Shukla A, Fuchs R, Rehage H. *Langmuir* 2006;22(7):3000–3006. [PubMed: 16548549]
12. Teixeira AV, Geissler E, Licinio P. *Journal of Physical Chemistry B* 2007;111(2):340–344.
13. Popescu G, Dogariu A, Rajagopalan R. *Physical Review E* 2002;65(4):041504.Artn 041504
14. Steinhaus B, Shen AQ, Sureshkumar R. *Physics of Fluids* 2007;19(7):073103.Artn 073103
15. McKinley GH, Rodd LE, Oliverira MSN, Cooper-White J. *Journal of Central South University of Technology* 2007;14:6–9.
16. Rodd LE, Cooper-White JJ, Boger DV, McKinley GH. *Journal of Non-Newtonian Fluid Mechanics* 2007;143(2–3):170–191.
17. Pipe, C.; Kim, NJ.; McKinley, GH. *Microfluidic Rheometry on a Chip*; Italy, 2007. 4th AERC, Italy, 2007;
18. Clasen C, Gearing BP, McKinley GH. *Journal of Rheology* 2006;50(6):883–905.
19. Clasen C, McKinley GH. *Journal of Non-Newtonian Fluid Mechanics* 2004;124(1–3):1–10.
20. Sanehez AM, Prieto R, Laso M, Riesgo T. *Ieee Transactions on Industrial Electronics* 2008;55(1):427–436.
21. Cense AW, Peeters EAG, Gottenbos B, Baaijem FPT, Nuijs AM, van Dongen MEH. *Journal of Microbiological Methods* 2006;67(3):463–472. [PubMed: 16820233]
22. Unger MA, Chou HP, Thorsen T, Scherer A, Quake SR. *Science* 2000;288(5463):113–116. [PubMed: 10753110]
23. Chovan T, Guttman A. *Trends in Biotechnology* 2002;20(3):116–122. [PubMed: 11841863]
24. Costerton JW, Stewart PS, Greenberg EP. *Science* 1999;284(5418):1318–1322. [PubMed: 10334980]
25. Daniels R, Reynaert S, Hoekstra H, Verreth C, Janssens J, Braeken K, Fauvart M, Beullens S, Heusdens C, Lambrichts I, De Vos DE, Vanderleyden J, Vermant J, Michiels J. *Proceedings of the National Academy of Sciences of the United States of America* 2006;103(40):14965–14970.10.1073/pnas.0511037103
26. Eboigbodin KE, Ojeda JJ, Biggs CA. *Langmuir* 2007;23(12):6691–6697.10.1021/la063404z
27. Hall-Stoodley L, Costerton JW, Stoodley P. *Nature Reviews Microbiology* 2004;2(2):95–108.10.1038/nrmicro821|ISSN 1740-1526
28. Lockhart PB, Brennan MT, Sasser HC, Fox PC, Paster BJ, Bahrani-Mougeot FK. *Circulation* 2008;117(24):3118–3125.10.1161/circulationaha.107.758524
29. Donlan RM, Costerton JW. *Clinical Microbiology Reviews* 2002;15(2):167–193.10.1128/cmr.15.2.167-193.2002
30. Shaw T, Winston M, Rupp CJ, Klapper I, Stoodley P. *Physical Review Letters* 2004;93(9):098102. [PubMed: 15447143]Artn 098102
31. Stoodley P, Cargo R, Rupp CJ, Wilson S, Klapper I. *Journal of Industrial Microbiology & Biotechnology* 2002;29(6):361–367. [PubMed: 12483479]
32. Towler BW, Rupp CJ, Cunningham AB, Stoodley P. *Biofouling* 2003;19(5):279–285. [PubMed: 14650082]
33. deBeer D, Stoodley P. *Water Science and Technology* 1995;32(8):11–18.
34. Debeer D, Stoodley P, Lewandowski Z. *Biotechnology and Bioengineering* 1994;44(5):636–641. [PubMed: 18618799]
35. Stoodley P, Yang SN, LappinScott H, Lewandowski Z. *Biotechnology and Bioengineering* 1997;56(6):681–688. [PubMed: 18642340]
36. Enderle, JD.; Blanchard, SusanM; Bronzino, JosephD. *Introduction to Biomedical Engineering*. San Diego: Academic Press; 2000. p. 1062
37. Studer V, Hang G, Pandolfi A, Ortiz M, Anderson WF, Quake SR. *Journal of Applied Physics* 2004;95(1):393–398.
38. Duffy DC, McDonald JC, Schueller OJA, Whitesides GM. *Analytical Chemistry* 1998;70(23):4974–4984.

39. Favre-Bonte S, Joly B, Forestier C. Infection and Immunity 1999;67(2):554–561. [PubMed: 9916058]
40. Tojo M, Yamashita N, Goldmann DA, Pier GB. Journal of Infectious Diseases 1988;157(4):713–722. [PubMed: 3279138]
41. Tang JM, Tung MA, Zeng YY. Journal of Food Engineering 1998;38(3):279–295.
42. [June 23]. <http://www.femlab.de/products/sme/>
43. Krysl P, Cranford TW, Hildebrand JA. International Journal for Numerical Methods in Engineering 2008;74(5):754–775.
44. Muliana AH, Haj-Ali R. International Journal of Solids and Structures 2008;45(10):2937–2963.
45. Wang E, Babbey CM, Dunn KW. Journal of Microscopy-Oxford 2005;218:148–159.
46. Kohl MJ, Abdel-Khalik SI, Jeter SM, Sadowski DL. Sensors and Actuators a-Physical 2005;118(2) 212–221 10.1016/j.sna.2004.07.014|ISSN 0924-4247

Figure 1.

Schematic of the flexible microfluidic rheometer. (A). The dark and light colored regions are the fluidic and air channels, respectively. (B) Overhead bright-field microscopy image of the microfabricated device in the region outlined in A. The test areas are the two locations where the fluidic and air channels cross. (C) and (D) are schematics of the operating principle of the flexible microfluidic rheometer, shown in a plane perpendicular to the plane of (A) and (B). Initially, (C), a thin layer of undeformed PDMS separates the air channel from the fluidic channel containing the test specimen. When air pressure is applied (D), the resulting stress deforms the thin PDMS layer, thereby also deforming the test specimen in the fluidic chamber. (E) and (F) are images of the device before and after air pressure is applied. The difference in

the two images is due to the deformation of the PDMS membrane because of the pressurization of the air control channel.

Figure 2.

Figure 2A: Comparison between results of finite element analysis (FEA) and the membrane deformation when the test area is filled with water. (A) plots the simulated membrane deformation profile as the pressure is increased from 3.4 kPa to 58.7 kPa (EPDMS = 350 kPa). (B) shows the membrane profile detected by reflection confocal microscopy. (C) plots the relationship between applied air pressure (σ_{app}) and normalized membrane deflection ($\Delta h/w$) for three different devices. The curves are the results for FEA given the PDMS elastic moduli shown in the figure legend.

Figure 2B: Comparison between results of finite element analysis (FEA) and the membrane deformation when the test area is filled with water. (A) plots the simulated membrane deformation profile as the pressure is increased from 3.4 kPa to 58.7 kPa (EPDMS = 350 kPa). (B) shows the membrane profile detected by reflection confocal microscopy. (C) plots the relationship between applied air pressure (σ_{app}) and normalized membrane deflection ($\Delta h/w$) for three different devices. The curves are the results for FEA given the PDMS elastic moduli shown in the figure legend.

Figure 2C: Comparison between results of finite element analysis (FEA) and the membrane deformation when the test area is filled with water. (A) plots the simulated membrane deformation profile as the pressure is increased from 3.4 kPa to 58.7 kPa (EPDMS = 350 kPa). (B) shows the membrane profile detected by reflection confocal microscopy. (C) plots the relationship between applied air pressure (σ_{app}) and normalized membrane deflection ($\Delta h/w$) for three different devices. The curves are the results for FEA given the PDMS elastic moduli shown in the figure legend.

Figure 3.

Procedure by which the static elastic modulus of an unknown test specimen is determined. Datum points are measurements of the steady-state strain as function of applied pressure (stress) for the unloaded device (control, triangles) and the device loaded with 2% gellan gum (circles). The best linear fit to the 2% gellan gum data is plotted. Dashed curves are predicted stress/strain profiles for test specimen elastic moduli range from 3 – 30 kPa. From the plot, the best estimate of the 2% gellan gum elastic modulus is $E = 8$ kPa.

Figure 4.

Figure 4A: Comparison between results of the flexible microfluidic rheometer and mechanical rheometry. (A) shows the response of the flexible microfluidic rheometer for the unloaded device and the device loaded with 2% and 3% gellan gum. The curves are FEA estimate of the elastic modulus for the test specimens. (B) plots the results of mechanical rheometry. The curves are best fits to the experimental data. The elastic moduli from the fits are shown.

Figure 4B: Comparison between results of the flexible microfluidic rheometer and mechanical rheometry. (A) shows the response of the flexible microfluidic rheometer for the unloaded device and the device loaded with 2% and 3% gellan gum. The curves are FEA estimate of the elastic modulus for the test specimens. (B) plots the results of mechanical rheometry. The curves are best fits to the experimental data. The elastic moduli from the fits are shown.

Figure 5.

Figure 5A: (A) Plot of the transient response of the flexible microfluidic rheometer for 2% gellan gum after the applied stress is released. The data are modeled by an exponential decay with relaxation time $\tau = 19.4$ s. (B) plots the recoil, from mechanical rheometry, of 2% gellan gum after a shear stress (50 Pa - in the linear regime), applied for 150 s, is released. The curve is the exponential decay ($\tau = 19.4$ s) measured from (A).

Figure 5B: (A) Plot of the transient response of the flexible microfluidic rheometer for 2% gellan gum after the applied stress is released. The data are modeled by an exponential decay with relaxation time $\lambda = 19.4$ s. (B) plots the recoil, from mechanical rheometry, of 2% gellan gum after a shear stress (50 Pa - in the linear regime), applied for 150 s, is released. The curve is the exponential decay ($\tau = 19.4$ s) measured from (A).

Figure 6.

Figure 6A: CLSM images of *S. epidermidis* biofilm loaded into the test area of the flexible microfluidic rheometer. (A) is a CLSM image of a bacterial biofilm labeled with the fluorescent dye Syto-9. (B–D) show the structure of the biofilm as it responds to the deformation of the PDMS membrane. These images were acquired in the plane perpendicular to the bottom substrate at the centerline of the device.

Figure 6B: CLSM images of *S. epidermidis* biofilm loaded into the test area of the flexible microfluidic rheometer. (A) is a CLSM image of a bacterial biofilm labeled with the fluorescent dye Syto-9. (B–D) show the structure of the biofilm as it responds to the deformation of the PDMS membrane. These images were acquired in the plane perpendicular to the bottom substrate at the centerline of the device.

Figure 6C: CLSM images of *S. epidermidis* biofilm loaded into the test area of the flexible microfluidic rheometer. (A) is a CLSM image of a bacterial biofilm labeled with the fluorescent dye Syto-9. (B–D) show the structure of the biofilm as it responds to the deformation of the PDMS membrane. These images were acquired in the plane perpendicular to the bottom substrate at the centerline of the device.

Figure 6D: CLSM images of *S. epidermidis* biofilm loaded into the test area of the flexible microfluidic rheometer. (A) is a CLSM image of a bacterial biofilm labeled with the fluorescent dye Syto-9. (B–D) show the structure of the biofilm as it responds to the deformation of the PDMS membrane. These images were acquired in the plane perpendicular to the bottom substrate at the centerline of the device.

Figure 7.

Results of flexible microfluidic rhometry for biofilms of two different species: *S. epidermidis* and *K. pneumoniae*. The datum points are measurements strain vs applied pressure. The curves are results of FEA for elastic moduli listed on the figure.

Figure 8.

Plot of the transient response of an *S. epidermidis* biofilm after the release of air pressure on the membrane. The curve is an exponential decay with a characteristic time of 13.8 s.

Figure 9.

Design space for application of flexible microfluidic rheometry to characterize the elastic moduli of soft viscoelastic solids. The background color scale reports the expected strain as a function of device and test specimen mechanical properties. The two curves are operating limits based on resolution, as discussed in the text. The region between the two curves denotes combinations of PDMS and test specimen moduli for which mechanical property characterization is possible. Typical ranges of PDMS elastic moduli for different crosslinker ratios are shown. Experimental data discussed in this paper are plotted: 2% and 3% gellan are filled circles and squares, respectively. *S. epidermidis* and *K pneumoniae* biofilms are green and black open diamonds, respectively. Filled triangles are the high strain moduli measured for the two biofilm species.