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Quantitative measurement of rheological cell properties and the impact of drugs

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ABSTRACT

We study rheological cell properties with a narrow-gap rotational rheometer. At gaps of a few micrometers, the average viscoelastic behavior of a cell monolayer can be studied. It is particularly advantageous for quantifying typical storage and loss moduli in single experimental runs since cell-to-cell variations are very large, making it tedious and time consuming to repeat single-cell studies. It permits quantifying the impact of drugs on the cell mechanics without the need of treating the samples in the rheometer and envisions the use of this method as a tool e.g. for drug development.

1. INTRODUCTION

Mechanical properties have numerous vital implications for cells. In eukaryotic cells, they are mainly governed by the cytoskeleton. They are affected by diseases such as cancer. On the other hand, biochemicals altering the mechanical properties may be used as drugs against these diseases. During the last decades, different techniques have been applied to measure the mechanical properties of single cells. Kollmannsberger and Fabry (2011), for instance, provide an overview. Yet, cells show large cell-to-cell variations (Weissman-Shomer and Fry 1975) and the mechanical properties may vary by orders of magnitude, see e.g. Cai et al. (2013). Hence, it is important to quantify average values for the mechanical cell properties. This can be accomplished by studying the mechanical properties of a large number of cells in single experimental runs. Here, we report one a study of the effect of different biochemicals on hundreds of thousands of cells in a monolayer with a narrow-gap rheometer. The study uses the method previously described by Dakhil et al. (2016). It also offers the possibility for quantitatively studying the impact of pre-stress. To employ this method to

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cells, the absolute gap width between the rheometer plates has to be improved considerable compared to standard commercial rheometers. Here, we apply the approach of Dakhil and Wierschem (2014). This narrow-gap rheometer also enables to study adhesion (Dakhil et al. 2018) and load limits (Kokkinos et al. 2016) of cells in low viscous environments without harking back to thickeners, which may have an impact on the cell metabolism. Furthermore, it allows to access shear rates up to about 10^5 s^{-1} for measuring viscosity functions and normal-stress differences of biopolymer solutions (Dakhil et al. 2019).

2. MATERIALS AND METHODS

We modify a commercial rotational rheometer to improve the precision of the parallel-disk configuration by orders of magnitude to better than ±1 µm. To this end, we replace the standard disks with flat glass plates, which are usually employed for interferometric studies. The gap width between the plates is detected with a customized polychromatic interferometric sensor and set up with actuators or micrometer screws. The diameter of the gap is about 50 mm. Further details on the setup of the narrow-gap rheometer and its alignment are described by Dakhil and Wierschem (2014).

For our study, we use murine Swiss 3T6 fibroblasts cells. The cells, suspended in cell culture medium, are glued with fibronectin to both plates of the rheometer in a monolayer. The amount of cells in the monolayer is quantified by visualizing the cells with a microscope; see Fig. 1 for a transillumination image. To measure the viscoelastic cell properties, we carry out amplitude and frequency sweeps at a temperature of about 23.4°C. For further details on the cell preparation and treatment, we refer to Dakhil et al. (2016).

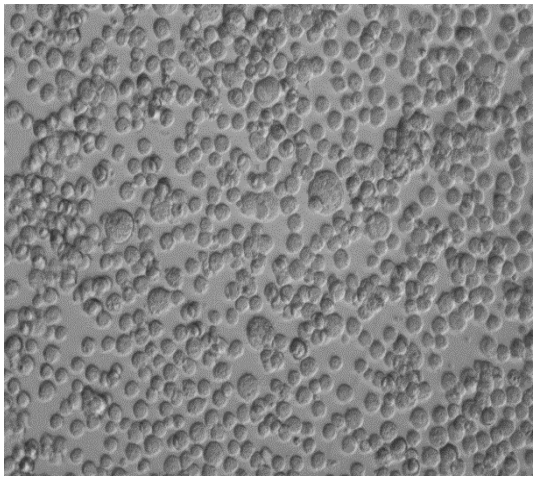

Fig. 1 Standard transillumination microscopy image of a small part of the monolayer between the rheometer plates. Cell coverage: 50%

In order to assess the impact of drugs that are known to affect the cytoskeleton structure of the cells, we recorded the rheological properties of cells in the presence of ethanol, glutaraldehyde and blebbistatin. Glutaraldehyde is a fixative agent that

denatures proteins and arrests dynamic responses of the cells (Small et al. 1999). Blebbistatin inhibits myosin motors that can generate contraction in the actin network (Kovács et al. 2004). At high concentrations, ethanol may kill fibroblasts but only minimal changes have been reported for lower concentrations (Chang et al. 2006). It also impairs cell growth in a concentration-dependent manner (Yeo et al. 2000). We treated the cells with either 0.5 vol.% ethanol with a purity of 99.8%, 0.1 vol.% glutaraldehyde or 150 micromolar of blebbistatin. Before applying blebbistatin to the medium, it was dissolved in 22.6 ml DMSO. To allow good interaction of these drugs with the cells, they were mixed into the culture medium just before leaving the cells between the glass plates.

3. RESULTS AND DISCUSSION

An example of an amplitude sweep is shown in Fig. 2(a). The storage modulus is much larger than the loss modulus, which shows that the cells behave as a viscoelastic solid. The linear viscoelastic regime extends to amplitudes of about 1%. At larger amplitudes, the storage modulus decreases. Many rheological studies focus on frequency-dependent cell properties. Fig. 2(b) depicts an example of a frequency sweep at a gap width of 5 µm. It shows that both moduli increase with frequency according to a power law with same exponent, which is typical for cells (Kollmannsberger and Fabry 2011) and, generally, for soft glassy materials (Sollich et al. 1997). The absolute values of the moduli depend sensitively on the gap width and on the cell concentration in the gap (Dakhil et al. 2016).

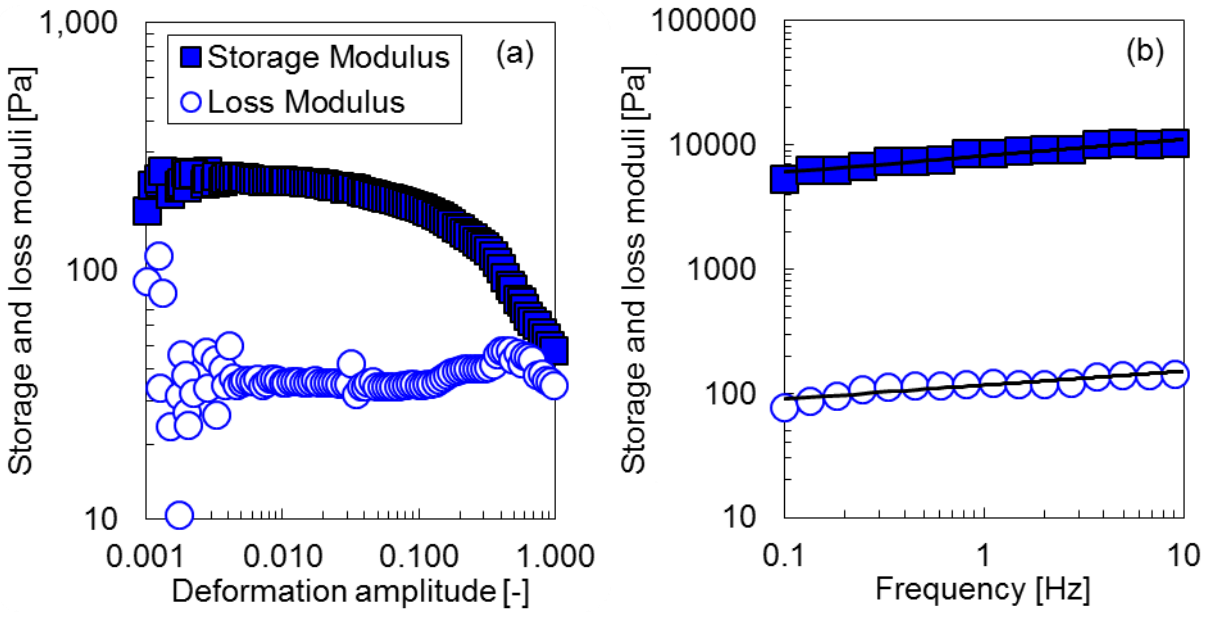

Fig. 2 Amplitude sweep at a gap width of 10 µm at a frequency of 1 Hz (a) and frequency sweep at a gap width of 5 µm and a strain amplitude: 0.2% (b). Storage modulus and loss modulus are indicated by closed and open symbols, respectively.

As had been shown by Dakhil et al. (2016), the average viscoelastic cell

properties in the monolayer can be quantified by taking into account the cell coverage in the gap, c, and the moduli of the medium. For example, the average storage modulus of the cells, $\langle G'_{cell} \rangle$, is obtained by the following equation:

$$\langle G'_{cell} \rangle = \frac{G' - (1-c)G'_{medium}}{c}, \quad (1)$$

where G'_{medium} is the storage modulus of the medium. The average loss modulus of the cells is determined accordingly. With this approach, i.e. obtaining representative data from about a million cells at once, it is possible to determine mechanical cell properties within a standard deviation of less than 20%, which is considerably smaller than typical cell-to-cell variations (Cai et al 2013), and to quantitatively compare different samples, i.e. cells from different batches, ill to healthy cells etc.

The dynamic moduli and frequency dependence of the cells can be altered with biochemical treatment. Fig. 3(a) shows the average moduli per cell in the linear viscoelastic regime. Compared to untreated cells, the application of blebbistatin lowers the storage modulus, while glutaraldehyde and ethanol tend to enhance the storage modulus. While blebbistatin slightly alters the loss modulus within range of uncertainty, the other drugs yield an increase of the loss modulus. We note that for the strain amplitude the upper limit of the linear viscoelastic regime does not change by using the drugs except for blebbistatin, which results in a higher limit. Fig. 3(b) shows the power-law exponents obtained from the frequency sweeps. While treatments with glutaraldehyde and ethanol hardly alter the exponents within the range of uncertainty, the blebbistatin treatment yields much smaller exponents.

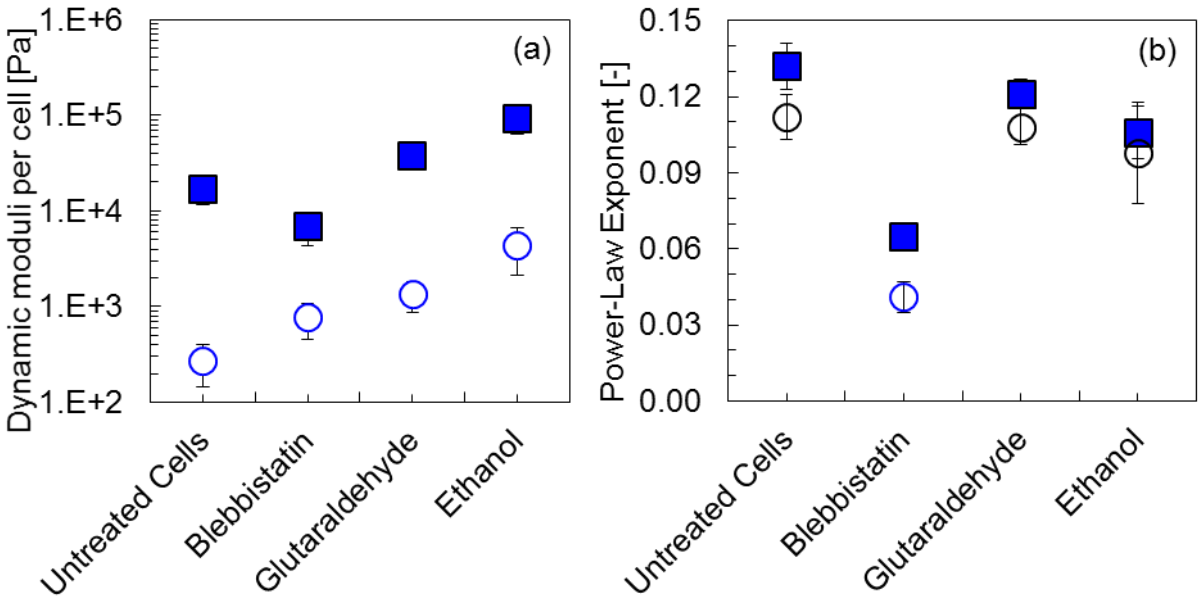

Fig. 3 Dynamic moduli per cell (a) and power-law exponent (b) for untreated fibroblasts and fibroblasts treated with different drugs. The storage modulus, the loss modulus and their respective exponents are indicated by closed and open symbols, respectively. Frequency in (a): 1 Hz; amplitude in (b): 0.2%; gap width: 5 µm.

3. CONCLUSIONS

We showed how to determine with a narrow-gap rotational rheometer dynamic cell moduli and compare them between different batches. The method allows to quantify the effect of different biochemicals that affect the cytoskeleton on the storage and loss moduli and on the frequency response.

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