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Microrheology of complex systems and living cells using AFM

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1. Introduction

Biological cells display viscoelastic mechanical properties that are a key factor in the regulation of cell processes, such as migration, adhesion and deformability. One interesting tool to probe the mechanical properties of living cells and other complex systems is the Atomic Force Microscopy (AFM). Rheological properties of the sample are obtained usually from the force-indentation (F - δ) measurements and by fitting with the Sneddon's modification of the Hertz model [1]. This model describes the relationship between the force applied by a stiff cone, a purely elastic indentation in a flat and soft sample and the Young's modulus E . AFM uses a flexible cantilever with a pyramidal tip (or sphere) at the end that will locally indent the viscoelastic material. Another approach is to superpose low-amplitude sinusoidal oscillations to an initial indentation δ_0 (polyacrylamide gel or cells). Then one can determine the complex shear modulus G^* [2-3] using the following formula:

$$G^*(\omega) = \frac{1 - \nu}{3 \delta_0 \tan \theta} \left\{ \frac{F^*(\omega)}{\delta^*(\omega)} - i\omega b(0) \right\} \quad (1)$$

$\omega=2\pi f$ is the angular frequency (f is frequency in Hz), θ describes the half angle to the face of the pyramid, δ_0 is the initial indentation and ν is the Poisson's ration (usually $\nu=0.5$ for gels and cells). $F^*(\omega)$ and $\delta^*(\omega)$ are the Fourier transforms of the measured force F and the sample indentation depth δ , and are functions of ω . The last term $-i\omega b(0)$ represents the hydrodynamic drag. $G^*(\omega)=G'(\omega)+iG''(\omega)$ is the complex modulus and contains the elastic part $G'(\omega)$, and the loss modulus $G''(\omega)$.

2. Validation of the protocol using polyacrylamide gels

Measurements were done in a liquid, so the measured force is the sum of the force due to the sample's response and the hydrodynamic drag force F_d due to the liquid around the cantilever. Similarly to the Stokes drag, F_d can be shown experimentally to be linear in

terms of frequency $F_d = i\omega b(0)$, where $b(0)$ is the drag factor measured by extrapolation at a distance close to the surface. In our experiments the frequency f can vary from 1 to 400 Hz.

We probed a sample of acrylamide (7.5%) mixed with bis-acrylamid (0.03%) in water using the oscillation mode of the AFM (JPK Instruments, Berlin, Germany, oscillation force mode). Samples for the AFM were prepared in Petri dishes using our protocol [4] and were square samples 15mmx15mm, and 70 μ m in height. Triangular cantilevers with a pyramidal tip (Veeco MLCT, $\theta=18.75^\circ$) and a small spring stiffness around 0.015N/m were chosen for the study.

Measurements were also carried out with a rheometer (Bohlin Gemini 150), using a small oscillatory strain while recording the stress (dynamic mode) and parallel plate geometry (diameter = 20mm, gap = 1mm), in a lower frequency range [0.001Hz-5Hz]. Samples were prepared by polymerizing a drop located between two glass slides separated by a calibration blade (1mm in height).

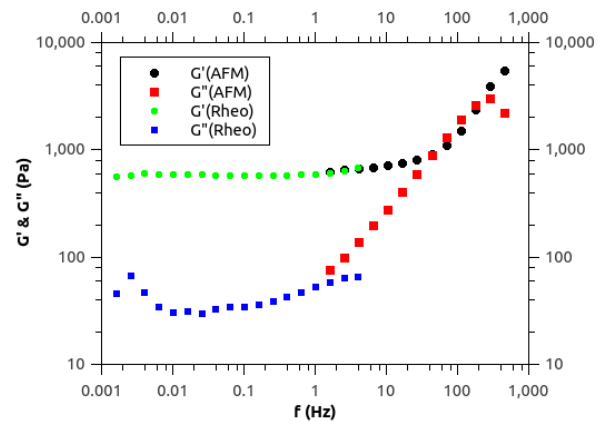


Figure 1: Plot of the modulus $G'(\omega)$ and $G''(\omega)$ of a polyacrylamide gel (with acrylamide concentration 7.5%) as a function of frequency, measured by rheometry or using force modulation with AFM. The variances, not represented, are in the range of 10%.

By using these two methods, we can compare the overlapping data and conclude on the accuracy of the

technique. Both $G'(\omega)$ and $G''(\omega)$ can be seen in figure 1 below. The elastic modulus shows a plateau at low frequencies (rheometry) whereas the loss modulus is one decade below.

The AFM measurements at higher frequencies match very well the rheometry data and prolongates the curves into the glassy regime where G' and G'' have a typical gel behavior $G' \sim G'' \sim \omega^{0.75}$. This is typical of previous experiments done on such gels [5].

3. Microrheology on cells

The viscoelastic properties of cancer cells can be probed using the same force modulation technique. T24 cells, a cell line established from a human bladder carcinoma, were cultured at 37° and 5% CO₂ atmosphere, and plated on a glass cover slip, at the bottom of a Petri dish, specially designed to be used with the AFM. Measurements are carried out on isolated cells (n=2). The pyramidal cantilever tip used with these cells had a spring constant of $k=0.011$ N/m. The initial indentation and oscillations were done on the nucleus with a loading force of 600 pN, ensuring a good contact, but not too much pressure in order to avoid any mechanotransduction due to force application. Another thing is that the indentation needs to be large enough (800nm) so that the superposed oscillations (around 20nm) can be considered small, in the linear regime. Figure 2 represents the dynamic moduli as a function of frequency. Such cells behave similarly to gels, in fact the gel was chosen with mechanical properties close to the cell's moduli.

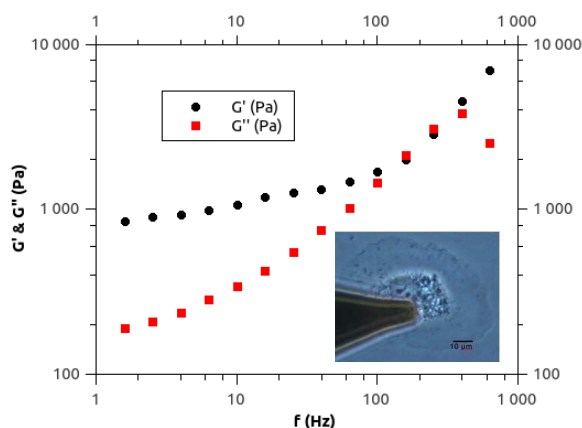


Figure 2: Plot of the moduli $G'(\omega)$ and $G''(\omega)$ on top of the nucleus of a cancer cell T24. These measurements are carried out using $\delta_0 = 811$ nm, with a spring constant of $k=0.011$ N/m.

At low frequencies, the elastic modulus seems to reach a plateau around 800 Pa. This is similar to what we obtain when performing experiments in the classical contact mode and using Hertz model [6]. This result makes sense, as the cytoplasm can be considered to be similar to a gel. Our result is therefore in agreement

with static experiments and goes further as compared to initial results [2].

At higher frequencies, gels moduli G' and G'' have a slope of roughly 0.8, again revealing a glassy transition. It is interesting to note that such properties may correspond to a viscoplastic material, with the existence of a yield stress at low frequencies. Indeed, since this biological material does not flow at low frequencies (slope of G' very small close to zero), it will eventually reach a plateau (close to 800 Pa).

4. Conclusion

Microrheology experiments seem to be an adequate tool for characterizing the cell microrheology. They reveal intriguing gel-like viscoelastic properties, with the existence of a plateau modulus at low frequencies, corresponding to the possible existence of a yield stress. Further studies are still needed to explore the different cell regions (lamellipodium, cytoplasm, nucleus, etc.) in order to understand cell microrheology better. Finally, this tool may be an important one to study the viscoelastic properties of living cells, in particular it may help differentiate different cancer cells, and it could also be used as cells undergo particular functions (spreading, interactions, transmigration).

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