Supporting Text

Description of the scanning force microscope (SFM) measurements

The cells were isolated immediately before the measurement. After adding the cell suspension onto the glass slide, cells settled down and adhered slightly to the substrate within 5 min. Cells were found by phase contrast and fluorescence microscopy. The retracted cantilever (up to 15 μm distance to the substrate) was positioned above the cell area of interest by using *x* and *y* piezos. Subsequently, force-distance curves were taken by driving the *z*-piezo down until a certain specified vertical deflection of the cantilever was recorded. During this force measurement the tip of the cantilever was oscillated with a certain frequency. The principle of the measurements and the subsequent determination of the complex Young's moduli and the Poisson's ratios are described in detail in refs. 1 and 2. Since cells are viscoelastic materials, their response to an applied mechanical stress, i.e., the relationship between stress and strain, depends on time (3). Thus, the viscoelastic properties of the cells were assessed at 30, 100, and 200 Hz of cantilever oscillations. Additionally to the actual force measurement, three force-distance curves necessary for the cell height determination were taken on the substrate around the cell. The experiments were limited to 20 min to minimize unphysiological changes in cell properties. Exclusion of the supravital dye trypan blue was used to test the integrity of the dissociated cells. All experiments were carried out at room temperature.

This method abandoned both (mechanical) scanning of the cells (which was necessary, since cell adhesion was much lower than that of cultured cells) and an approach of the cantilever on the cell before the force measurement usually done in scanning force microscopy (4). Thus, a possible active cell response to a mechanical stimulus exerted by the cantilever before the actual measurement was ruled out. An additional benefit of this procedure is an immense gain in time, allowing measurements of up to 10 force-distance curves within the time frame of 20 min.

Because of the modification of the cantilever tip, which resulted in a well defined spherical geometry of the indenter necessary for the data analysis, storage and loss moduli E' and E'' could be determined very exactly. To overcome the problem of a contribution of the hard substrate to the total force, in our analysis the common Hertz-model was extended by Tu and Chen models, which correct for the substrate effects, and apply for nonadhered and well adhered regions of the cells, respectively (1). The sample height needed for these models was obtained by subtraction of the cantilever's *z*-piezo position at the moment of contact with the cell by the *z*-position of the underlying substrate. The latter could be calculated by fitting a plane through the contact points of three additional force-distance curves taken on the substrate around the sample. Unlike the cell height information obtained from an SFM scan where the cell is indented by the force the cantilever exerts on the cell (which is defined in the setpoint), this approach allows an accurate determination of the cell height. Since always only one of these models fits the data, additional information about the adherence of a cell and - in case of the Chen model - about the cell's Poisson's ratio could be obtained (1). The raw data were fed into custom built software, which yielded the valid model and the elasticity constant at the used cantilever frequency. For further data analysis Microsoft Excel and for statistical analysis Sigma Stat (SPSS Inc.) were used.

Voigt Model

A series of two viscoelastic Voigt elements can be used as equivalent combination of springs and dashpots to describe the deformation and relaxation behavior of Müller glial cells. A Voigt element is composed of a spring and a dashpot connected in parallel. The spring, which simulates the elastic response of the system, obeys the relation $\sigma = E\gamma$ for tensile stress, where σ is the stress, E the Young's modulus, and γ the strain. The dashpot, which simulates the viscous response of the system, obeys the relation $\sigma = \eta\dot{\gamma}$, where η is the viscosity. Thus, if spring and dashpot are connected in parallel, the total stress can be calculated with

$$\sigma = E\gamma + \eta\dot{\gamma}$$

Solving this differential equation for total strain γ with an applied constant stress σ_0 leads to:

$$\gamma(t) = \frac{s_0}{E}\left[1 - \exp\left(\frac{-E}{\eta}t\right)\right]$$

Two Voigt elements in series provided the most accurate fitting of the data:

$$\gamma_{extension}(t) = \frac{\sigma_0}{E_1}\left[1 - \exp\left(\frac{-E_1}{\eta_1}t\right)\right] + \frac{\sigma_0}{E_2}\left[1 - \exp\left(\frac{-E_2}{\eta_2}t\right)\right]$$

When subsequently the stress is removed, i.e., $0 = E\gamma + \eta\dot{\gamma}$, this equation can be solved at the time of stress removal with the initial condition $\gamma = \gamma_0$:

$$\gamma(t) = \gamma_0 \exp\left(\frac{-E}{\eta}t\right)$$

Applying this equation to the two Voigt elements in series leads to:

$$\gamma_{relaxation}(t) = \gamma_0\left[\exp\left(\frac{-E_1}{\eta_1}t\right) + \exp\left(\frac{-E_2}{\eta_2}t\right)\right]$$

For an applied stress of σ_0 = 100 Pa this results in the following parameters:

E_1 = 32 Pa $\quad$ η_1 = 1 Pa s $\quad$ E_2 = 40 Pa $\quad$ η_2 = 220 Pa s

The viscoelastic constants $E_{1,2}$ and $\eta_{1,2}$ do not directly describe viscoelastic properties of the cells, they describe the material constants for an equivalent in springs and dashpots. Nevertheless, the drastic difference in η_1 and η_2 indicates a relaxational behavior of the

cells on distinctively different time scales. Since the optical stretcher measures global cell properties this is not surprising. The different relaxation times might stem from different cytoskeletal or other intracellular components (5). A direct comparison with our SFM measurements should be done with caution since the SFM measures locally and the optical stretcher describes an integral whole cell behavior.

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