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Cell Motility Probed by Noise Analysis of Thickness Shear Mode Resonators

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The quartz crystal microbalance (QCM) technique is an emerging bioanalytical tool to study the behavior of animal cells in vitro. Due to the high interfacial sensitivity of thickness shear mode (TSM) resonators it is possible to monitor the formation and breakage of cell−matrix interactions and changes in viscoelasticity of the cell bodies, as well as minute cell volume alterations by the time course of their resonance frequency even with millisecond time resolution. We found that mammalian MDCK-II cells grown on TSM resonators impose characteristic fluctuations on the resonance frequency, which are a quantitative indicator for dynamic activities of the cells on the surface and report on their vitality and motility. Applying noise analysis to the fluctuating resonance frequency allows one to quantify the response of the cells to environmental changes such as osmotic stress, addition of fixation reagents, or the influence of drugs such as cytochalasin D. The corresponding power density spectra of the noise imposed on the resonance frequency by the dynamic activities of the cells show a characteristic resonance at 1−2 Hz, which can be substantially altered by osmotic stress, fixation agents, or cytochalasin D. Comparison of QCM-based fluctuation readings with electric cell−substrate impedance sensing (ECIS)—a well-established technique to monitor cell dynamics—provides substantially different results, indicating that both techniques may complement each other with respect to their biological information. Whereas ECIS readings report solely on cell shape changes, QCM-based fluctuation analysis is also influenced by fluctuations in the viscoelasticity of the cell bodies.

Invasive and metastatic behavior of malignant cells is the major cause of mortality in all cancer patients. Migration of cancer cells, as opposed to normal ones, is critically regulated (i) by physical adhesion of cells to each other and to their noncellular surroundings, i.e., the extracellular matrix and (ii) by transmission and interpretation of signals from the extracellular environment into various intracellular signaling cascades. The ability of cells to move—often considered as a direct measure for malignancy—can be inferred from their vertical motility, i.e., the dynamics of forming and breaking focal contacts without net lateral movement. This "stepping on the spot" is associated with the continuous formation and breakage of noncovalent bonds between extracellular matrix proteins and corresponding receptors embedded in the cellular plasma membrane.[1] Integrins are the major family of cell surface adhesion receptors than bind to ligands in the extracellular matrix. They are responsible for the formation of so-called focal contacts and serve as mediators between the intra- and extracellular compartments. Moreover, vitality of cells in general is also manifested in their ability to perform shape fluctuations most often induced by dynamics of the cytoskeleton as is impressively demonstrated by the collective contraction of cardiomyocytes in vitro.[2−4] Among other contributors to cell motility, the dynamics of actin polymerization and filament turnover play important roles.[5] Many aspects of animal cell motility are directly correlated with dynamic changes in cell volume, viscoelasticity, cell−cell and cell−matrix contacts. Thus, it was our objective to establish a new and powerful experimental technique to sense these cellular shape fluctuations in real time and to derive a direct measure of cell motility from fluctuation measurements.

Fluctuations in biological systems have recently been investigated with regard to the enzymatic function of proteins,[6] molecular machines,[7] gene expression,[8] individual cells,[9] and ion channels.[10] The stochastic nature of ion transport through transmembrane channels has been used as a sensor to detect individual molecules (stochastic sensor). Recently, we characterized the dynamics of protein−membrane interactions by recording and analyzing the noise created by the adsorption and desorption of proteins on a biomimetic surface.[11]

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(1) Lambrechts, A.; Van Troys, M.; Ampe, C. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 1890−1909.
(2) Szabó, B.; Selmeczi, D.; Környei, Z.; Madarász, E.; Rozlosnik, N. *Phys. Rev. E* **2002**, *65*, 1−6.
(3) Luong, J. H. T. *Anal. Lett.* **2003**, *36*, 3147−3164.
(4) Pax, M.; Rieger, J.; Eibl, R. H.; Thielemann, C.; Johannsmann, D. *Analyst* **2005**, *130*, 1474−1477.
(5) Carlier, M.-F.; Pantaloni, D. *J. Mol. Biol.* **1997**, 459−467.
(6) Tang, K. E. S.; Dill, K. A. *J. Biomol. Struct. Dyn.* **1998**, *16*, 397−411.
(7) Ishijima, A.; Kojima, H.; Funatsu, T.; Tokunaga, M.; Hijuchi, H.; Tanaka, H.; Yanagida, T. *Cell* **1998**, *92*, 161−171.
(8) Simpson, M. L.; Cox, C. D.; Sayler, G. S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4551−4556.
(9) Szabó, B.; Selmeczi, D.; Környei, Z.; Madarasz, E.; Rozlosnik, N. *Phys. Rev. E* **2002**, *65*, 0419101−0419106.
(10) Bayley, H.; Cremer, P. S. *Nature* **2001**, *413*, 226−230.
(11) Lüthgens, E.; Janshoff, A. *ChemPhysChem* **2005**, *6*, 444−448.

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A cell, however, is a nonequilibrium soft material whose fluctuations are actively driven. Highly regulated biochemical signaling pathways control the energy-dependent dynamics of living cells, which are a sensitive indicator for their metabolic activity. In fact, fluctuations in living cells are a measure of the cells' adaptability to changes of the environment.[12] Typically, mechanical noise originating from living cells is orders of magnitude higher than fluctuations produced by thermal motion and it shows a different frequency dependence. A soft or plastic system is characterized by a large variability of its viscoelastic properties; thus, it can be tuned in meet the cells' requirements. Hence, measuring the stochastic micromechanical properties of living cells may serve as an extremely sensitive indicator to monitor the response of cells to external challenges and may form the basis for a new type of whole-cell biosensor.

So far, only electric cell−substrate impedance sensing (ECIS) has demonstrated sufficient sensitivity to detect fluctuations in cell shape—so-called micromotion—when the cells are grown on electrically conductive substrates.[13−15] In ECIS, the electrical impedance of a cell-covered, substrate-integrated microelectrode is recorded with a time resolution of 1 s. Since the electrode impedance is modulated by the shape of the cells that are attached to it, the ECIS readout can be applied to monitor fluctuations in cell shape. A single study based on TIRAF microcopy has picked up micromotion-like fluctuations in the area of cell-to-substrate adhesion.[16]

Recently, we showed that the quartz crystal microbalance (QCM) is an excellent tool to monitor the de novo formation and modulation of cell−matrix interactions with high time resolution. It additionally provides quantitative information about the micromechanical properties of the cell−matrix contact sites.[17−19] This sensitivity for cell−matrix adhesion together with its superior time resolution renders the QCM approach a promising transducer to monitor shape fluctuations of living cells. Thus, we report on a new approach to assess the dynamics of adherent cells by measuring the fluctuations that are imposed on the resonance frequency of a thickness shear mode (TSM) resonator that is covered by a monolayer of cells and serves as a cell culture substratum. Compared to ECIS or TIRAF, the QCM approach is limited to neither transparent nor conductive surfaces and provides a fast readout in the millisecond regime.

EXPERIMENTAL SECTION

QCM-Based Fluctuation Experiments. AT-cut quartz crystals (5 MHz fundamental resonance; KVG, Neckarbischofsheim, Germany) coated on both sides with circular gold electrodes (i.d. = 5 mm) were mounted as the bottom plate of a cell culture dish into a homemade crystal holder (Teflon). To study frequency fluctuations of the quartz resonator, we used a phase-lock oscillator (QCM100; SRS, Inc., Sunnyvale, CA). The frequency was recorded by a frequency counter (Agilent 53181A; Agilent Technologies, Palo Alto, CA) and then transferred via GPIB to a computer (time resolution 70 ms). QCM- and ECIS-based (next paragraph) fluctuation measurements were carried out in incubators with 5% (v/v) CO_2 atmosphere at 37 °C.

To characterize the mechanical load of the quartz resonator under the various experimental conditions we also measured the Q-factor of the shear oscillation by impedance analysis using a continuous-wave impedance analyzer (SI-1260; Solartron Instruments, Farnborough, UK) in the frequency regime close to the quartz fundamental resonance (5 MHz). Impedance data were analyzed by adjusting the parameters of the Butterworth−Van Dyke (BVD) equivalent circuit as described elsewhere,[17] providing energy storage (inductance) and energy dissipation (resistance) as fitting parameters, thus, the Q-factor.

ECIS-Based Fluctuation Experiments. ECIS measurements were performed using eight-well cell culture dishes with circular gold film electrodes ($d = 250\ \mu$m) deposited upon the bottom of each well (Applied Biophysics, Inc., Troy, NY; www.biophysics.com), a lock-in amplifier (SR830, SRS, Inc., Sunnyvale, CA) with an internal oscillator, and a personal computer that controls the measurement and stores data.[15,20,21]

Noise Analysis. To analyze resistance (ECIS) and frequency (QCM) fluctuations, we applied fast Fourier transformation (FFT) and variance analysis (i.e., computing the variance of the normalized and detrended raw data along the entire data set as a direct measure for the fluctuation amplitude). Whereas computation of the variance along the data set provides an easy to interpret measure for the overall amplitude of the observed fluctuations, FFT is particularly useful either to look for periodicities or to demonstrate aperiodicity. Applying FFT to a fluctuating time series—or noise—provides the corresponding power spectral density function or short power spectrum of the measured time series. The power spectrum plots the relative contribution of an individual harmonic function of a specified frequency (sinus or cosinus) to the overall time series as a function of frequency. Thus, any periodicity within the experimental data can be easily spotted as a peak in the power spectrum at its characteristic frequency. The absence of peaks is indicative for a time series without any periodicity. But even without well-defined peaks, the power spectrum of a fluctuating or noisy time series provides a useful tool to characterize the frequency-dependent fluctuations in the data. In fact, different types of noise are typically classified by the character of their power spectrum. So-called white noise is characterized by a power spectrum independent of frequency (horizontal line), while pink noise is represented by a $1/f$ behavior, and Brownian noise displays a $1/f^2$ power law. Taken together, Fourier transformation of the recorded data provides a frequency-resolved view on the composition of the measured time series.

Mathematically speaking, the power spectrum of a given time series is the square of the magnitude of the continuous Fourier transform of the signal. Particular attention has to be paid to

(12) Sato, K.; Ito, Y.; Yomo, T.; Kaneko, K. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14086−14090.
(13) Lo, C.-M.; Keese, C. R.; Giaever, I. *Exp. Cell Res.* **1993**, *204*.
(14) Pei, Z.; Keese, C. R.; Giaever, I.; Kurzawa, H.; Wilson, D. E. *Exp. Cell Res.* **1994**, *212*, 225−229.
(15) Giaever, I.; Keese, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7896−7900.
(16) Geggier, P.; Fuhr, G. *Appl. Phys. A* **1999**, *68*, 505.
(17) Wegener, J.; Seebach, J.; Janshoff, A.; Galla, H.-J. *Biophys. J.* **2000**, *78*, 2821−2833.
(18) Wegener, J.; Janshoff, A.; Galla, H.-J. *Eur. Biophys. J.* **1998**, *28*, 26−37.
(19) Reiss, B.; Janshoff, A.; Steinem, C.; Seebach, J.; Wegener, J. *Langmuir* **2003**, *19*, 1816−1823.
(20) Wegener, J.; Keese, C. R.; Giaever, I. *Exp. Cell Res.* **2000**, *259*, 158−166.
(21) Giaever, I.; Keese, C. R. *Nature* **1993**, *366*, 591−592.

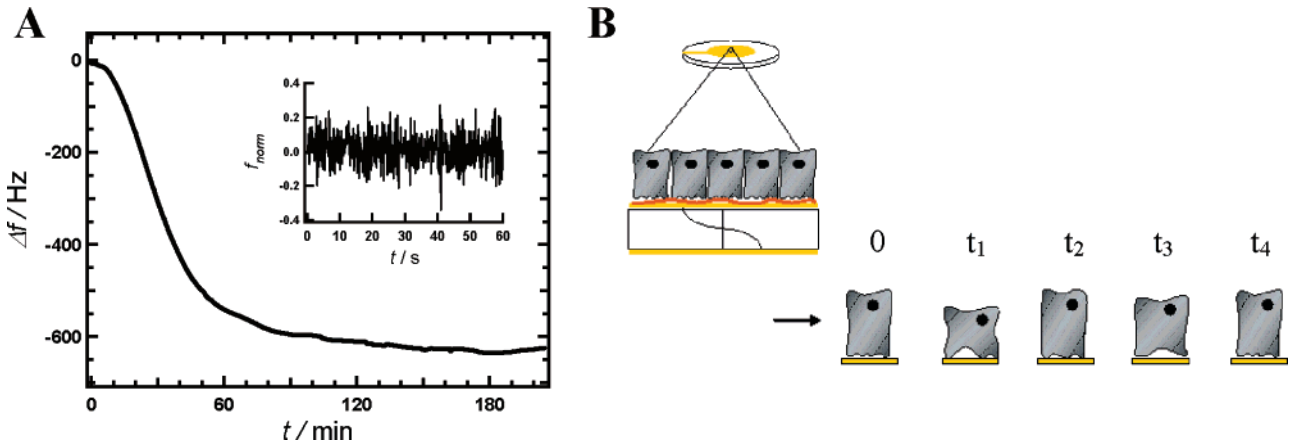

Figure 1. (A) Shift of the resonance frequency Δf when a suspension of MDCK-II cells is allowed to settle on a 5-MHz shear wave resonator. $T = 37$ °C. The inset shows fluctuations of the resonance frequency that originate from the dynamic activities of the cells on the surface. (B) Schematic diagram of a confluent cell monolayer, cultured on the gold electrode of a thickness shear mode resonator, and a sketch illustrating shape fluctuations.

normalization. Assuming that we record an N-point sample of our signal $c(t)$ at equal intervals and use the FFT compute its discrete Fourier transform (DFT)

$$C_k = \sum_{j=0}^{N-1} c_j e \exp\left[\frac{2\pi ijk}{N}\right] = DFT(c(t)) \quad k = 0,...,N-1 \quad (1)$$

The power spectrum $S(f)$ is then calculated according to the following equations:

$$\Phi(0) = \Phi(f_0) = \frac{1}{N^2}|C_0|^2$$

$$\Phi(f_k) = \frac{1}{N^2}(|C_k|^2 + |C_{N-k}|^2) \quad k = 1,2,...,\left(\frac{N}{2}-1\right)$$

$$\Phi(f_c) = \Phi(f_{N/2}) = \frac{1}{N^2}|C_{N/2}|^2 \quad (2)$$

where $f_k = k/N\Delta t = 2f_c k/N$ ($k = 0,1,..., N/2$) and N is an even number. f_k is defined for zero and positive frequencies, Δt is the sampling interval, and $f_c = 1/2\Delta t$ is the maximum frequency within the Nyquist interval. Finally, $\Phi(f)$ is divided by the sampling frequency Δf and multiplied by N to obtain the power spectral density function $S(f)$ in accordance with Parseval's theorem. Data with equidistant time scale is recorded, subdivided into sets containing multiples of 2^n data points. Each subset of the original data is subjected to a fast Fourier transformation, and the mean of the power spectrum from all data subsets is calculated.

The data of this study have been checked for stationarity and ergodicity as described elsewhere.[22] We found that neither the mean resonance frequency value nor its variance changed with time within one measurement, hence fulfilling the requirements for "weak stationarity". Power spectra recorded at different times of one experiment did not show any noticeable differences as required for ergodic systems.

(22) Bezegh, A.; Janata, J. *Anal. Chem.* **1987**, *59*, 494A−506A.

Cell Culture. MDCK-II cells were cultured in Earle's minimum essential medium supplemented with 4 mM glutamine, 100 μg/mL of both penicillin and streptomycin (all purchased by Biochrom, Berlin, Germany), and 10% (v/v) fetal calf serum (PAA Laboratories GmbH, Cölbe, Germany). Stocks of these cells were grown in incubators with a 5% CO_2 atmosphere. Since the fluctuation amplitude might depend on the passage number (age of the cell line), we performed each series of experiments with cells originating from the same passage number to provide absolute comparability of the power spectra.

RESULTS AND DISCUSSION

Figure 1A traces the shift of the frequency response of a 5-MHz TSM resonator upon seeding 1.6×10^5 MDCK-II cells on the gold electrode of the quartz resonator. A typical frequency decrease of ~600 Hz due to the attachment and spreading of the cells on the surface can be observed.[18] It is important to note that the mass sensitivity of the quartz resonator is a function of position on its surface. Cells attached to the center of the electrode contribute more than cells in the periphery. The radial sensitivity behaves like a Gaussian function. However, with a homogeneous load situation—i.e., a homogeneous distribution of cells on the gold electrode—all further considerations can be reliably based on an average integral sensitivity.

Fluctuation analysis is performed after attachment and spreading of the cells is completed and a confluent cell monolayer is established on the surface. Accordingly, adhesion of cells does not interfere with motility recordings. The schematic in Figure 1B illustrates shape fluctuations as envisioned for confluent cell monolayers grown on a quartz resonator. The formation and breakup of focal contacts produce a fluctuating contact area and as a consequence a transient change in free volume between the cell monolayer and the substrate. Moreover, the cells themselves might exhibit transient changes in volume and their micromechanical properties. Therefore, the signal of the TSM resonator shows a fluctuating resonance frequency (compare inset in Figure 1A) arising from the fluctuating load situation on the crystal surface.

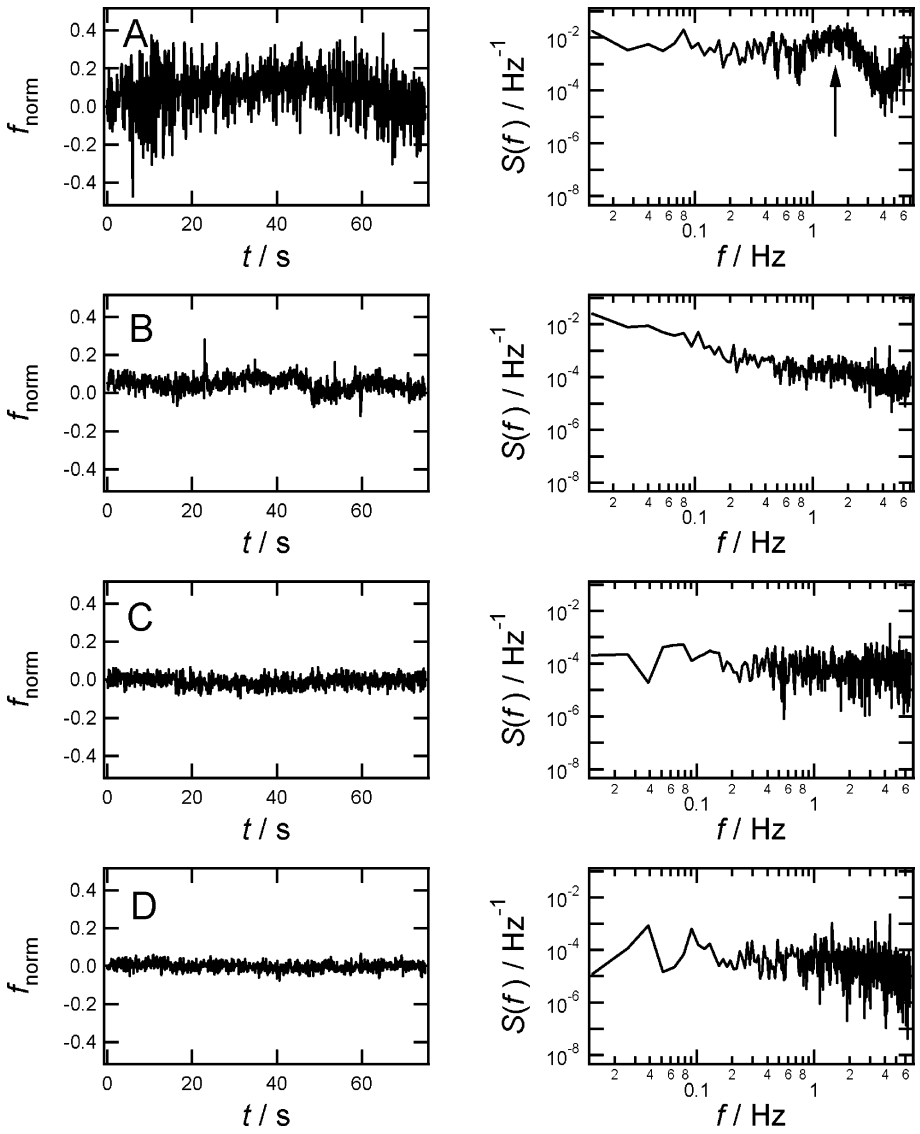

Figure 2. Frequency fluctuations of TSM resonators under various load situations. Power density spectra were obtained after subtraction of a linear trend from the raw data and applying an FFT algorithm to subsets of the data set comprising 1024 points. The resulting power density spectra of the individual subsets were averaged to yield the spectrum shown in the figure on the right side. The graphs on the left show the time course of the fluctuating frequency after normalization. For normalization, the average has been set to zero. (A) MDCK-II cells in isotonic culture fluid. (B) MDCK-II cells in hypertonic (200 mM sucrose added to isotonic medium) culture fluid. (C) MDCK-II cells fixed with PFA. (D) Resonator after removal of cells (immersed in buffer).

The impact of different load situations on the resonance frequency fluctuations of the quartz crystal is shown in Figure 2, in which the resonance frequencies (the average is set to zero) are plotted as a function of time in the left panel, while the corresponding power density spectra are shown on the right. The resonance frequency was recorded with a sampling rate of 13 Hz, i.e., one data point every 75 ms. Figure 2A (left) shows a typical time course of the fluctuating resonance frequency for a quartz crystal covered with a complete monolayer of living MDCK-II cells in culture medium, while Figure 2B (left) shows the frequency noise of the same cell layer exposed to hypertonic medium (200 mM sucrose added to the isotonic culture medium). The amplitude of the fluctuations is reduced significantly when the cells are forced to shrink and condense in a hyperosmotic environment. A further reduction in noise is achieved by cross-linking all cell protein via paraformaldehyde (4% (v/v) in PBS^{2+}), a common fixative to stabilize biological samples (Figure 2C). Finally, the cell layer was removed from the quartz surface mechanically (Figure 2D) but the cell debris was allowed to settle back on the surface. Even without any quantitative analysis, it is already apparent from the raw data that the amplitude of the resonance frequency fluctuations observed for living MDCK-II cells (Figure 2A) is considerably higher than the noise level found for the cell-free electrode (Figure 2D). A more detailed characterization is provided by the power density spectra of the fluctuation data. The power density spectra of the cell-free quartz resonator (Figure 2D, right) is essentially similar to white noise with almost no frequency dependence and no significant peaks. Living MDCK-II cells, however, show a typical peak around 1−3 Hz (arrow) indicative of an intrinsic resonance. Moreover, we find a higher overall noise level together with a slightly negative slope of the spectrum. The peak has to be considered as an indication for

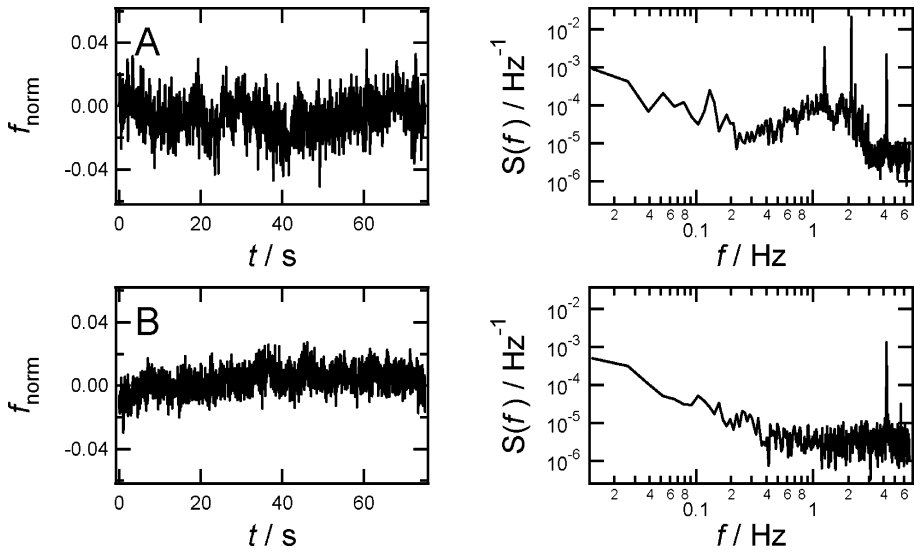

Figure 3. Impact of cytochalasin D (1 μM) on resonance frequency fluctuations of MDCK-II cells. The graphs on the left-hand side show the time course of the fluctuating frequency after normalization. For normalization, the average has been set to zero. The corresponding power density spectra are grouped on the right-hand side. (A) MDCK-II cells under vehicle control conditions. (B) MDCK-II cells exposed to cytochalasin D.

synchronized and periodic fluctuations in viscoelasticity or shape of the cell bodies. The origin of this collective and periodic behavior remains to be elucidated. However, AFM studies, in which the cantilever tip was allowed to settle on the bodies of adherent cells and follow their shape fluctuations, show a similar power spectrum with resonance features at ~5 Hz using 3T3 fibroblast cells.[2] Exposing the cells to hypertonic conditions (Figure 2B) reduces the peak substantially as well as the overall noise. The noise level decreases further when the cell's proteins were cross-linked by PFA.

The cell shape, cell−substrate anchorage, and cell movement are mainly determined by the static and dynamic properties of the actin network. Controlled polymerization of actin is a prerequisite for any movement of the cell. Therefore, we experimentally hindered f-actin formation by cytochalasin D, a fungal alkaloid, which specifically inhibits actin polymerization (Figure 3). We found that adding cytochalasin D (Sigma; 0.2 mM stock solution in DMSO) in a final concentration of 1 μM to vital cells leads to a substantial reduction of the resonance peak at 1−2 Hz in the power spectrum. The cellular noise level is then similar to the situation observed for fixed cells (Figure 2D). Vehicle control experiments revealed that adding the solvent DMSO alone in the same concentrations does not have any impact on the appearance of the peak. The impact of cytochalasin D on the dynamic properties of the cells is by far more pronounced than the associated changes in overall energy dissipation of the shear oscillation as demonstrated by impedance analysis. Here, cytochalasin D reduces the motional resistance by 25%. Since the fluctuation pattern of living cells, as reported here, is highly susceptible to drugs acting on the cytoskeleton, it is straightforward to conclude that the dynamics of actin polymerization are a likely source for the 1−2-Hz peak observed in the power spectra of unchallenged cells. In the AFM work mentioned above, the resonance peak was also assigned to the dynamics of the actin cytoskeleton.[2] These experiments also demonstrate that QCM-based fluctuation analysis is a sensitive new bioanalytical means to study subtle changes within the physiological state of adherent cells when they are mediated by the cellular cytoskeleton.

We compared the frequency fluctuations observed for cells with those obtained for chemically defined model systems. Deposition of giant liposomes (data not shown) shows essentially the same frequency fluctuation magnitude as cell-free resonators. Thus, those fluctuations observed for living cells can be attributed to their biological activity and energy-dependent motility rather than thermal membrane undulations or a decrease in the quality factor of the oscillation due to increased damping. Moreover, application of hypertonic conditions, which results in a dehydration and compression of the cell bodies, provides a significant but reversible decrease of the fluctuation amplitude and a concomitant decrease of the quality factor. Thus, the observed fluctuations are not a simple consequence of a reduced quality factor of the oscillation in the presence of cells but are indeed biological in nature.

It is instructive to compare the frequency fluctuations in a QCM experiment with resistance fluctuations obtained from ECIS analysis. ECIS is a highly sensitive electrochemical technique to investigate morphological changes of adherent cells without using an optical microscope.[23] The technique reports on changes in cell−cell and cell−matrix contacts when the cells are adherently grown on gold film electrodes. The electrical impedance of the electrodes increases when the cells attach and spread on the surface as the current has to bypass the cellular bodies instead of leaving the electrode directly into the bulk. With the cells behaving like insulating particles the impedance is a function of cell shape and can be used to monitor shape fluctuations as described above. Fluctuations in electrode resistance when MDCK-II cells completely cover the electrode surface (diameter of 250 μm) are shown in Figure 4A. The sampling rate for the impedance measurements performed at 4 kHz was 1 Hz. The power density spectra (right) of the living cells display a slope in the range of −2.1 to −2.7 s^{-2},

(23) Lo, C.-M.; Keese, C. R.; Giaever, I. *Biophys. J.* **1995**, *69*, 2800−2807.

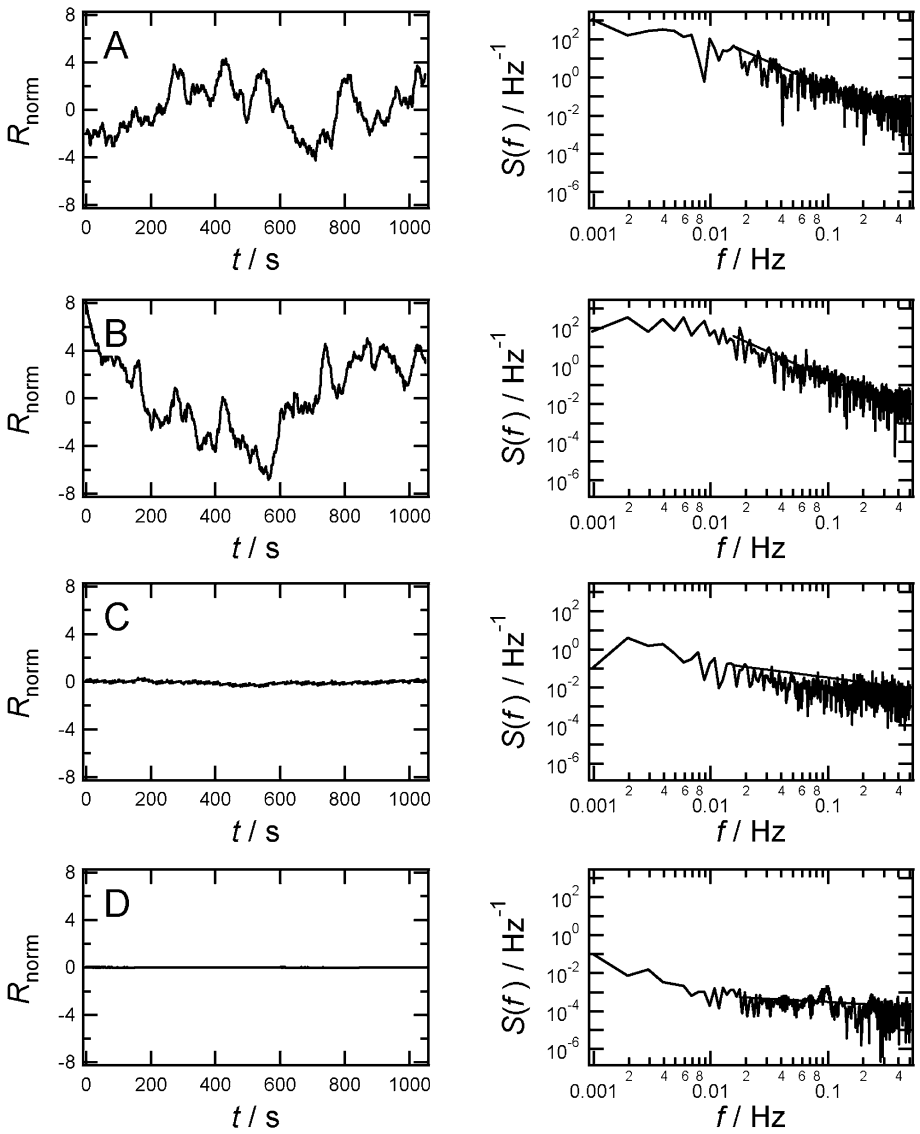

Figure 4. Analysis of ECIS-based resistance fluctuations under various experimental conditions. Prior to FFT, a linear trend was subtracted from the raw data. The power density spectra of data subsets comprising 1024 points were averaged to yield the spectra shown in the right panel. The graphs on the left show the time course of the normalized resistance. For normalization, the first data point has been set to zero. (A) MDCK-II cells in isotonic culture fluid. (B) MDCK-II cells in hypertonic (200 mM sucrose) culture fluid. (C) MDCK-II cells fixed with PFA. (D) Bare electrode just immersed in buffer.

indicative for biologically active cells.[24] Exposing the cells to hyperosmolar solution (Figure 4B) does not induce any significant change in the power density spectrum, while fixation with PFA (Figure 4C) substantially decreases the noise (slope, -0.8 s^{-2}) as consistently observed in QCM measurements as well. Removal of the cells (Figure 4D) reduces the slope of the power spectrum to its lowest value (slope, -0.3 s^{-2}). In more recent studies, we learned that thermal membrane fluctuations of giant liposomes are easily detected in an ECIS setup with the liposomes attached to the same ECIS electrodes that were used in cell experiments here. The power spectrum of liposome fluctuations is typically characterized by a slope close to -2 s^{-2} indicative of Brownian noise.[24−26]

Looking for reasons to explain the observed differences between QCM-based and ECIS-based fluctuation experiments, one has to recognize two technical details. While the sensing area in ECIS experiments is only 0.0005 cm^2, the active area of the quartz resonator amounts to 0.33 cm^2. Since the surface area of the measuring electrode determines the number of cells that contribute to the signal, and thus signal averaging, it is not surprising to find individual readouts for both techniques. Moreover, the bandwidth of the ECIS technique is different from the QCM approach. While ECIS is limited at the high-frequency end of the power spectrum to 0.5 Hz, the QCM approach provides data up to 7 Hz. Thus, it remains to be elucidated whether ECIS readings with a better time resolution would also show a resonance peak at 1−2 Hz in the power spectrum. Also the origin of both signals has to be considered: the real part of the complex impedance measured by the ECIS technique mirrors the width of the electrolyte-filled spaces between adjacent cells and between cell

(24) Giaever, I.; Keese, C. *IEEE Trans. Biomed. Eng.* **1986**, *33*, 242−247.
(25) Sapper, A.; Reiss, B.; Janshoff, A.; Wegener, J. *Langmuir* **2005**.
(26) Marinari, E.; Parisi, G.; Ruelle, D.; Windey, P. *Phys. Rev. Lett.* **1983**, *50*, 1223−1225.

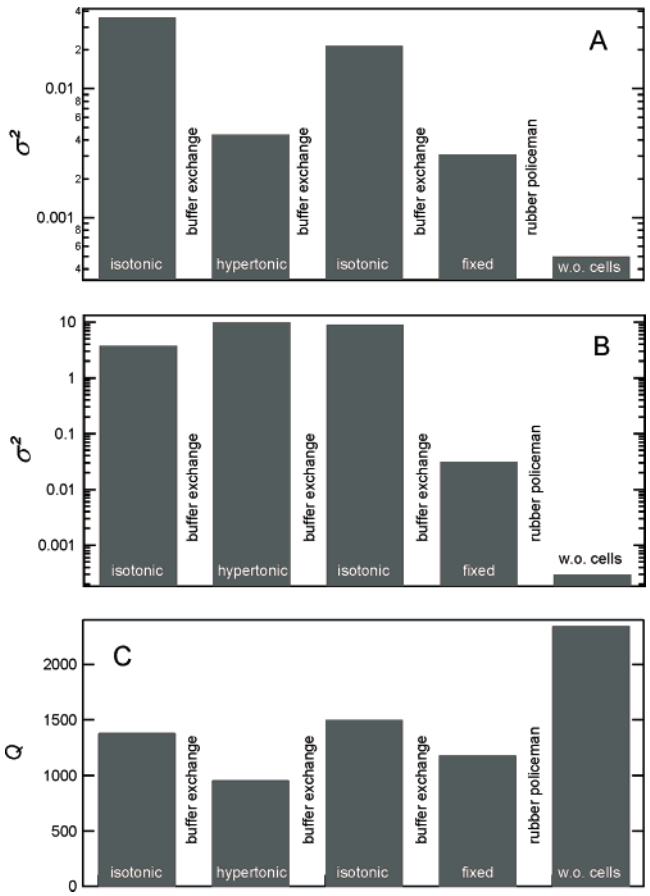

Figure 5. Variance σ^2 of the normalized resonance frequency (A) or resistance (B) fluctuations under various experimental conditions. After subtraction of a linear trend from the raw data, σ^2-values were calculated for 2048 data points (A) and 1000 data points (B). (C) *Q*-Factor of the quartz resonator under different conditions as extracted from impedance analysis and subsequent equivalent circuit modeling using the BVD network.

and electrode surface.[23] The QCM, however, reads the overall viscoelastic load on the resonator, which is governed by the space between ventral cell membrane and gold electrode but also by changes in cell volume and viscoelasticity. These individual signal sources might account for the different frequency dependences in the ECIS- or QCM-based power density spectra.

Taken together, QCM measurements provide a novel means to monitor the dynamics of living adherent cells by detecting their transient shape fluctuations. However, data hold different information than in ECIS recordings. This conclusion is supported by the different cell reactions observed in fluctuation analysis upon exposure to osmotic stress. Figure 5A and B summarize the variance σ^2 of the resonance frequency (A, QCM) and resistance (B, ECIS) fluctuations, respectively, for cells under different osmotic conditions. Here the variance along the entire data set is used as an easy to grasp and intuitive parameter that directly mirrors the fluctuation amplitude. A hypertonic environment results in a substantial decrease in frequency fluctuations (10%) that recovers to 60% of the initial value after switching the solution back to isotonic conditions. Compared to fixed cells, the fluctuations of cells in hypertonic medium are only slightly higher, while the bare quartz provides the smallest variance. Interestingly, we found that ECIS measurements under the same conditions give a different answer (Figure 5B). Cells in hypertonic fluid still impose the same fluctuation amplitude on the measured resistance. Chemical fixation of the cells, however, results in substantial decrease of resistance fluctuations, which are even further reduced by removal of the cells from the electrode surface.

In addition, QCM- and ECIS-based fluctuation analysis report differently on the cells' motility after addition of the drug cytochalasin D. Using QCM, cells exposed to cytochalasin D show a complete loss of the cell resonance, while Lo et al. reported that the ECIS fluctuations do not decrease significantly after addition of cytochalasin D.[13] These findings support our claim that fluctuations measured by ECIS and QCM contain different information about the dynamics of the cells that may complement each other.

Figure 5C summarizes the changes in *Q*-factor (*Q*, energy stored per cycle/energy dissipated per cycle) for the cell-covered quartz resonators under different experimental conditions. Energy dissipation of the cell-covered quartz resonator is significantly increased (*Q*-factor reduced) when the cells are exposed to hypertonic medium. The cells slowly recover from the osmotic stress within 2 h even under constant hypertonic conditions (data not shown). The initial *Q*-value is, however, quickly recovered after switching back to isotonic buffer. Protein cross-linking also increases the energy dissipation of the resonator, while cell removal yields a *Q*-value almost twice as high as recorded for the cell-covered resonator. Although the load on the quartz resonator is increased considerably by applying hyperosmotic stress, the fluctuations decrease, indicating its biological origin. We interpret this finding as follows: the osmotically driven water efflux from the cells results in a compression of the cells, and as a consequence, they adopt a flat shape with the membrane closely attached to the cytoskeleton. Hence, the viscoelasticity of the cells increases considerably and shape fluctuations are damped down. It is important to note that hyperosmotic medium has virtually no effect on the oscillation of the bare quartz. Moreover, in hyperosmotic media, the cells show essentially the same mechanical properties compared to cells exposed to a cross-linking reagent. QCM readings indicate that paraformaldehyde not only increases the stiffness of the cells but significantly freezes their motility as shown in Figure 5A by the dramatically decreased variance. In contrast to the impact of fixation, the response of the cells to hyperosmotic stress is more or less reversible after buffer exchange.

Comparing ECIS- and QCM-based micromotion readings, we found that both methods show a clear difference between living cells and those killed by paraformaldehyde. On the other hand, both techniques provide different answers when the cells are challenged by hypertonic conditions. Whereas ECIS does not indicate any difference in shape fluctuations, QCM-based readings exhibit reduced cell dynamics. These differences suggest that signal fluctuations originate from different parts of the cell body and that they reflect different subcellular activities. A precise assignment of the individual contributions awaits further studies in particular those that combine both techniques.

CONCLUSIONS

We show for the first time how the quartz crystal microbalance can be applied to monitor the dynamics of living adherent cells in real time, which may serve as the basis for a new type of whole-

cell biosensor. QCM-based fluctuation analysis provides a quantitative means to monitor periodic micromechanical alterations of living cells with a time resolution that can be easily improved when quartz resonators with higher fundamental resonance frequency will be used. Future applications of this new technology comprise cytotoxicity screening, drug screening, or—more ambitious—a direct method to quantify the motility of tumor cells derived from biopsy material. One may envision that it might become possible to characterize the metastatic potential of tumor cells from a detailed analysis of their dynamics.

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