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Impedance spectroscopy with field-effect transistor arrays for the analysis of anti-cancer drug action on individual cells ☆

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ABSTRACT

In this study, impedance spectroscopy measurements of silicon-based open-gate field-effect transistor (FET) devices were utilized to study the adhesion status of cancer cells at a single cell level. We developed a trans-impedance amplifier circuit for the FETs with a higher bandwidth compared to a previously described system. The new system was characterized with a fast lock-in amplifier, which enabled measuring of impedance spectra up to 50 MHz. We studied cellular activities, including cell adhesion and anti-cancer drug induced apoptosis of human embryonic kidney (HEK293) and human lung adenocarcinoma epithelial (H441) cells. A well-known chemotherapeutic drug, topotecan hydrochloride, was used to investigate the effect of this drug to tumor cells cultured on the FET devices. The presence of the drug resulted in a 20% change in the amplitude of the impedance spectra at 200 kHz as a result of the induced apoptosis process. Real-time impedance measurements were performed inside an incubator at a constant frequency. The experimental results can be interpreted with an equivalent electronic circuit to resolve the influence of the system parameters. The developed method could be applied for the analysis of the specificity and efficacy of novel anti-cancer drugs in cancer therapy research on a single cell level in parallelized measurements.

1. Introduction

Cancer is one of the leading causes of death worldwide. About 12.7 million cancer cases were diagnosed and 7.6 million people died from cancer worldwide in 2008 (Jemal et al., 2011). In many cases, cancer diseases occur in organs like mammary gland (women), prostate (men), lung, and colon. However, not every cancer progress is fatal, if timely therapy is initiated. Cancer can be treated by surgery, radiation therapy and chemotherapy. The choice of therapy depends upon the location and grade of the tumor and the stage of the disease. In many cases chemotherapy is necessary, though it has a range of side effects such as alopecia, blood count changes or mucositis. Therefore, several pharmaceutical companies are concerned with the development of anti-cancer drugs. Special attention is given to develop methods to test the efficiency and specificity of new anti-cancer drugs.

Novel anti-cancer drugs are targeting defect structures of tumor cells while leaving the healthy cells unaffected. In order to examine the efficacy and the specificity of anti-cancer drugs, it is required to study the reaction of individual cells on the medicament as well. One parameter of interest is the adhesion status of the cells to a protein-coated substrate. This is usually rated by microscopic observations. Cell adhesion can also be recorded electronically by Electrical Cell Impedance Sensing (ECIS) on gold electrodes (Lo et al., 1995; Giaever and Keese, 1991) and by impedance spectroscopy with field-effect transistors (Schäfer et al., 2009). The ECIS system is well-known, already applied in research for decades, and systems are commercially available (http://www.biophysics.com, http://www.ibidi.de, http://www.roche-applied-science.com). Typical sizes of the metal electrodes range from 25 μm to 250 μm in diameter (Giaever and Keese, 1991). Therefore, the impedance measurements are usually confined to a few cells. In contrast to ECIS, the FETs are not limited in the size reduction and can be applied to study the effect of a medicament at a single cell level. FET devices have been already used for the detection of proteins (Bergveld, 1991), neuronal (Fromherz et al., 1991; Fromherz, 2005) and cardiac cell signals (Ingebrandt et al., 2001; Sprössler et al., 1999), enzymes (Katz and Willner, 2003), and for the detection of DNA (Uslu et al., 2004; Souteyrand et al., 1997). In these earlier works published by other groups a frequency range 1 Hz–100 kHz was chosen to study enzymes (Katz and Willner, 2003; Kharitonov et al., 2001), the influence of cationic sites, receptor molecules, and the nature of a membrane matrix (Antonisse et al., 2000) attached to the gate of an FET. In our former system we used a

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frequency up to 1 MHz (Schäfer et al., 2009) and we observed the most interesting effects above 100 kHz. Therefore we decided to enlarge the bandwidth of our system in this study.

In the present work, silicon-based field-effect transistors (FETs) are used to study individual adherent cells by impedance spectroscopy. The method is based on recording the transfer function of the system (Schasfoort et al., 1989; Antonisse et al., 2000; Kharitonov et al., 2001). The transfer function of a FET, in our experimental configuration, is the bandwidth limiting effect of a combination of reference electrode, electrolyte solution, cell, transistor, and first amplifier stage. This bandwidth is affected by any biological sample, which is attached to the gate oxide of the open-gate FETs like DNA (Ingebrandt et al., 2007a), proteins (GhoshMoulick et al., 2009), or tumor cells (Schäfer et al., 2009). The response of this biological sample to the AC voltage is frequency-dependent as well. From this frequency dependence biological properties can be deduced. The transistor size of our FET structures is in the range of the size of individual tumor cells, which allows a miniaturization of the impedance approach down to a single cell level.

2. Materials and methods

2.1. FET devices

In this study, we used p-channel open-gate FET devices. The fabrication and encapsulation process of the FET devices were previously described (Offenhäusser et al., 1997; Wrobel et al., 2005). The devices were initially fabricated at the Institut für Mikrotechnik Mainz, Germany, during a former project at the Max-Planck Institute for Polymer Research Mainz, Germany. In this study the chips were wire-bonded to a 28 DIL carrier and encapsulated for cell culture at the University of Applied Sciences Kaiserslautern in Zweibrücken, Germany. The FETs were arranged in a 4×4 array with a pitch of 200 µm in the center of a 5×5 mm^2 silicon chip. The gate dimensions were 16 µm in width and 5 µm in length and the thickness of the gate oxide was 8 nm. Due to a process intrinsic under diffusion of the FET structures the electrically effective channel length is 1.5 µm.

2.2. FET amplifier system from previous works

The portable, 16 channel amplifier system was previously described (Ingebrandt et al., 2005a, 2007a; Schäfer et al., 2009). The transistor-transfer function (TTF) amplifier box was used in this study as well to characterize FET devices and to measure frequency-dependent transfer functions. Time-dependent data can be recorded in both potentiometric and impedimetric mode. All recordings can be controlled by means of read-out software implemented in Delphi 5.0 (Borland Software Corporation). The amplifier unit is operated by a microprocessor and the acquired data are transferred via an USB connection to the computer. All 16 channels can be operated simultaneously. Transfer functions are measured by applying a sinodal signal with 10 mV amplitude and varying frequency from 1 Hz to 1 MHz to the reference electrode. As pseudo-reference electrode a silver/silver chloride (Ag/AgCl) wire was used, which was immersed into the electrolyte solution. For the time-dependent measurements presented in this study, the portable amplifier box was operated inside an incubator.

2.3. Experimental setup

A second experimental setup was newly developed in this study (Fig. 1a). With this setup, cell measurements at higher frequencies compared to the previous work (Schäfer et al., 2009)

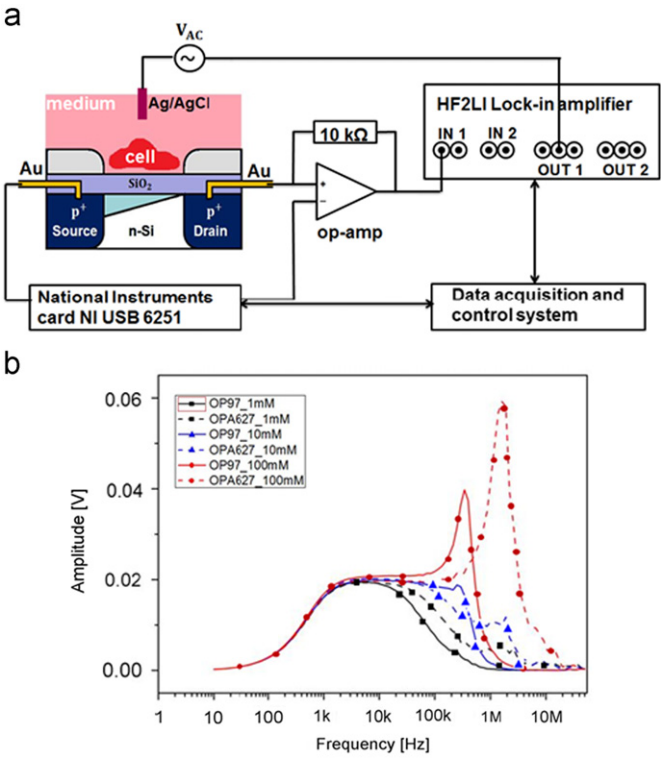

Fig. 1. (a) Experimental setup for cell measurements at higher frequencies (up to 50 MHz). (b) Comparison of measured impedance spectra using two different op-amps, namely the OP97 and OPA 627, in an electrolyte solution with different conductivity in the culture chamber of the FET chip.

can be performed. Prior to the measurement of a transfer function, a chip was characterized and the transistors were set to a working point. To control this process with a personal computer (PC), customized programs were implemented in LabView. With these programs, it was possible to measure the output and the transfer characteristics of the FET devices. From the available data, the transconductance g_m was calculated in order to set the transistors to a working point with maximum transconductance. For the respective drain-source and gate-source voltages, the analogue outputs of the data acquisition card (USB 6251, National Instruments Inc.) were used. A new preamplifier for the FETs, which uses a trans-impedance circuit, was connected to one of the two high-frequency analog inputs of a fast lock-in amplifier (HF2LI, Zurich Instruments, Switzerland). Transfer function measurements were done by applying a sinusoidal signal with 10 mV amplitude and varying frequency from 1 Hz to 50 MHz to the reference electrode. Also in this setup an Ag/AgCl wire was used as a pseudo-reference electrode. It was connected via a coaxial cable to the signal generator of the lock-in amplifier. The recorded data were transferred to a PC. The transfer functions of all 16 transistors on a chip needed to be measured consecutively with this setup. Furthermore, in order to examine the cellular responses over a longer period inside an incubator, a water-resistant preamplifier head was developed.

As mentioned above, our method is based on the recording of the transfer function of the system. A transfer function is generally defined by the ratio of the input and the output voltage of the amplifier.

$$H(j\omega) = \frac{V_{out}(\omega)}{V_{in}(\omega)} = |H(j\omega)| \cdot e^{j\phi(\omega)} \quad (1)$$

It can be separated into the amplitude $|H(j\omega)|$ and its phase $e^{j\phi(\omega)}$ component.