Recording of cell action potentials with AlGaN/GaN field-effect transistors

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An AlGaN/GaN electrolyte gate field-effect transistor array for the detection of electrical cell signals has been realized. The low-frequency noise power spectral density of these devices exhibits a $1/f$ characteristic with a dimensionless Hooge parameter of 5×10^{-3}. The equivalent gate-input noise under operation conditions has a peak-to-peak amplitude of 15 μV, one order of magnitude smaller than for common silicon-based devices used for extracellular recordings. Extracellular action potentials from a confluent layer of rat heart muscle cells cultivated directly on the nonmetallized gate surface were recorded with a signal amplitude of 75 μV and a signal-to-noise ratio of 5:1. © *2005 American Institute of Physics*. [DOI: 10.1063/1.1853531]

Recording of electrical activity of cells with planar device arrays is a promising approach for the study of biological networks as well as for the realization of whole-cell biosensors, in which cultured cells act as the biological receptor unit.[1,2] Such hybrid sensors benefit from the high sensitivity and selectivity of the specific receptors in the cellular membrane and the signal amplification in second messenger pathways of the cells. These cell-sensor hybrids are suitable for a variety of applications, such as drug discovery and screening in pharmacology, detection of toxins, and environmental monitoring.[3,4] Most of these applications require a noninvasive multisite recording system suitable for long-term measurements under physiological conditions. This excludes the utilization of conventional invasive patch-clamp techniques[5] or voltage-sensitive dyes,[6] which are toxic on illumination.

Extracellular monitoring of electrical cell activity can be performed by microelectrode arrays[7–9] or field-effect transistor (FET) device arrays, as first reported for muscle fibers and neuronal slices.[10] Silicon-based electrolyte oxide FETs are generally used as transducers for this purpose.[11–13] The main drawbacks of these devices are their long-term drift in electrolytes due to the electrochemical instability of the SiO_2 surface[14] and a high noise level due to mobility fluctuations caused by trapping and detrapping of carriers to and from trap states at the interface to the gate oxide layer.[15] Therefore, alternative concepts such as buried-channel FETs[16] and floating-gate devices[17,18] have been developed.

Another approach is the application of alternative material systems for the realization of electrolyte-gate FETs (EGFETs) such as AlGaN/GaN heterostructure FETs. Group III-nitrides are chemically stable under physiological conditions and nontoxic to living cells[19] and AlGaN/GaN FETs have recently been demonstrated to exhibit promising properties for sensor applications in liquid and electrolyte environments.[19,20]

Here, we report the recording of electrical signals from a cardiac myocyte syncytium cultivated on the surface of AlGaN/GaN heterostructure EGFET arrays.

Transistor arrays consisting of 4×4 individual devices were processed on AlGaN/GaN heterostructures grown by metalorganic vapor phase epitaxy on sapphire substrates.[21] The heterostructures consisted of a 24–26 nm undoped AlGaN barrier and a 3 nm undoped GaN cap, grown on a 1.5 μm GaN buffer. For some devices, the GaN buffer was compensated by Fe doping. The Al content in the AlGaN barriers was between 20% and 30%, as determined by high-resolution x-ray diffraction measurements. Room-temperature sheet carrier concentrations and mobilities obtained from Hall measurements were between 1×10^{13} and 1.2×10^{13} cm^{-2} and from 1100 to 1240 cm^2/Vs, respectively. Single-transistor elements with a channel width of 35 μm and a source-drain spacing of 35 μm were patterned by Ar-ion-beam etching. The gate length was defined by photolithographically opening the epoxy-based passivation layer. The FET arrays were mounted on a standard 28 DIL ceramic chip carrier, wire bonded, and partially encapsulated to form an electronic culture dish.

The preparation for the cardiac myocyte cells of embryonic Wistar rats (Charles River GmbH, Sulzbach, Germany, E 19) was adapted from previously published protocols.[22] The AlGaN/GaN chips were cleaned with 70% (v/v) ethanol (p.a.) and coated for 30 min with 20 μl of 12.5 μg/ml fibronectin (Sigma-Aldrich) in Hanks' balanced salt solution (HBSS) at 37 °C. The surfaces were rinsed with Phosphate-buffered saline (PBS) buffer solution (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM NaH_2PO_4, *p*H 7.3). The cells were then plated onto the chips at densities of 3000–5000 cells/mm^2 exposed chip area (effective chip surface: 6.2 mm^2). The chips were kept at 37 °C and 5% CO_2

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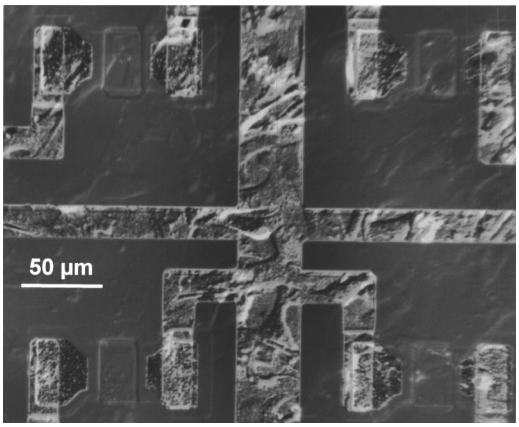

FIG. 1. Cardiac mycoyte syncytium cultivated on the device surface of a AlGaN/GaN EGFET array.

in a humidified chamber. The Ham's F10 medium (Sigma-Aldrich) containing 5% (v/v) foetal bovine serum (FBS), 1% (v/v) penicillin-streptomycin (10 000 units/ml penicillin and 10 mg/ml streptomycin, Sigma-Aldrich) and 0.5% (v/v) insulin/transferrin/selenium (ITS) (Sigma-Aldrich), adjusted to *p*H 7.2 was replaced several times. After 5 to 6 days in culture a confluent monolayer of cells (syncytium) developed, which spontaneously contracted with stable frequency and the electrical signals of the cells were recorded. Therefore, the culture medium was replaced by extracellular solution (145 mM NaCl, 3 mM KCl, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 10 mM N-2–Hydroxyethylpiperazine-N′-2–ethanesulfonic acid (HEPES), 8 mM glucose, adjusted to *p*H 7.3 with NaOH). Figure 1 shows cardiac myocyte cells on the gates of the AlGaN/GaN transistors.

For electrical measurements, the potential of the electrolytic gate of the devices was defined by an Ag/AgCl-wire reference electrode, which was connected to ground potential. As cell action potentials occur at low frequencies, a low-pass filter at 3 kHz was used to reduce the noise level during signal recording. Figure 2 shows the transconductance of a single AlGaN/GaN-EGFET element as a function of the gate-source voltage (V_{GS}). Under usual operating conditions (drain-source voltage $V_{DS}=0.5$ V), the devices exhibited a maximum transconductance g_m of 0.2 mS at $V_{GS}=-1.8$ V. Under these conditions, the leakage current I_{GS} through the electrolytic gate was found to be negligible (<2 nA).

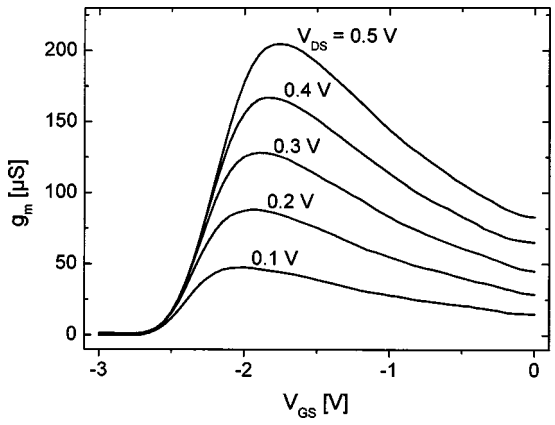

FIG. 2. Transconductance of a single AlGaN/GaN EGFET as a function of gate-source voltage for different drain-source voltages.

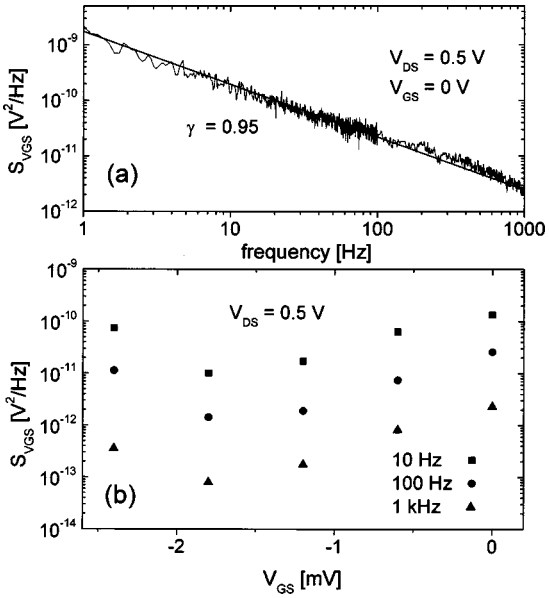

FIG. 3. (a) Typical noise power density spectrum of a single AlGaN/GaN EGFET element at $V_{GS}=0$ V. A $1/f^{\gamma}$ dependence with $\gamma=0.95$ is observed. (b) Variation of the noise power spectral density as a function of V_{GS} for different frequencies. Lowest noise levels were observed at the highest transconductance of the devices.

Low-frequency noise ($1\ \text{Hz}<f<1$ kHz) was measured in the common source configuration for different gate-source voltages at room temperature. From the observed drain-source current noise, the equivalent gate-input noise was calculated by $S_{VGS}=S_{IGS}/g_m^2$.

Figure 3(a) displays a typical noise power density spectrum, which shows a $1/f^{\gamma}$ frequency dependence. The exponent γ varies with V_{GS} between $\gamma=0.9$ and 1.3. The corresponding dimensionless Hooge parameter α (Ref. 23) was calculated to be 5×10^{-3} at $V_{GS}=0$ V from the relative source-drain current spectral density S_{IDS}/I_{DS}^2. The noise power spectral density exhibits a minimum for $V_{GS}=-1.8$ V, as shown in Fig. 3(b) for different frequencies. The equivalent gate-source voltage noise under these conditions presents a peak-to-peak amplitude of 15 μV, which is one order of magnitude smaller than for comparable silicon-based devices.[12,24]

The high signal-to-noise (S/N) ratio of our AlGaN/GaN EGFET device is demonstrated in Fig. 4, which presents the change in equivalent gate-source voltage calculated from the

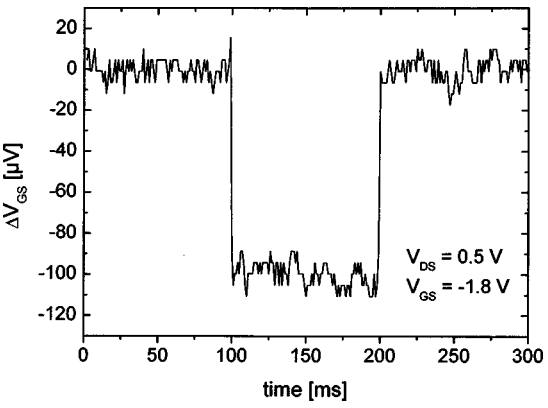

FIG. 4. Transient response of a single AlGaN/GaN EGFET element to a 100 μV pulse applied for 100 ms via the electrolyte gate.

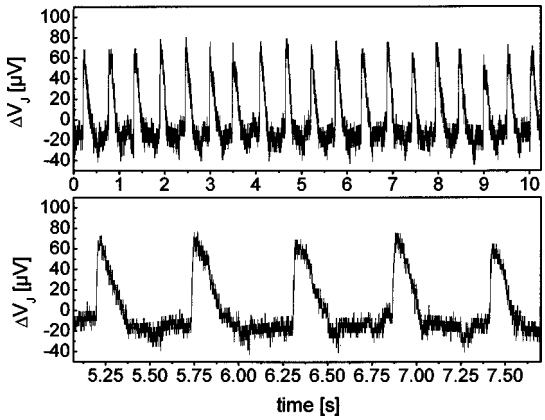

FIG. 5. Extracellular potential of a spontaneously beating cardiac myocyte syncytium recorded with an AlGaN/GaN EGFET. The cells were cultivated on the device surface.

change in the source-drain current upon application of a 100 μV test pulse to the electrolytic gate for a time interval of 100 ms. The signal is clearly resolved on the background noise with a S/N ratio of $>5:1$.

In Fig. 5, recordings of a spontaneously beating cardiac myocyte syncytium cultivated on the device surface are shown. The voltage V_J in the junction area between cell and transistor gate was recorded. Transistor signals were 100–150 ms in duration, firing at a stable frequency for several minutes. Signals with an amplitude of 70 μV were clearly detected because of the low background noise of the AlGaN/GaN transistors.

Action potentials of electrogenic cells are generated mainly by ionic fluxes of sodium, calcium, and potassium across the cellular membrane. The initial event is an influx of sodium ions, triggering a fast-rising action potential. In the case of cardiac myocytes, the action potential is retarded by an inward directed calcium flux leading to a plateau phase. The following potassium efflux leads to a repolarization of the cell. The shape of the action potentials recorded with planar sensors can be described in first approximation by a point-contact model,[12,25,26] which considers mainly two contributions to the electrical signal: capacitive currents across the attached part of the cellular membrane into the contact point caused by changes of the intracellular potential, and, ionic currents through ion channels in the attached membrane part, which are forced to flow along a small cleft formed by the cellular membrane and the sensor surface acting as an electrical resistor. Both currents lead to a voltage change at the sensor surface and have to be summed for the interpretation of the recorded signal shape.[13]

The signal shapes recorded by the AlGaN/GaN transistors (Fig. 5) seem to consist mainly out of the potassium signal part.[13,26] The exact reason for this signal shape remains to be clarified.

In conclusion, the recording of the spontaneous extracellular action potentials with AlGaN/GaN EGFET arrays has been demonstrated. The devices show stable operation under physiological conditions and, due to the low noise, a much higher signal resolution than currently used Si-based devices.

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