様　式　Ｃ－１９、Ｆ－１９、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Direct study of neurotransmitter/Action potential correlation with a CMOS integrated carbon nanotube sensors arrays

研究課題名（英文）Direct study of neurotransmitter/Action potential correlation with a CMOS integrated carbon nanotube sensors arrays

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：1024本カーボンナノチューブセンサの密な配列を作成して、特定の酵素による官能化後の神経伝達物質としてセンシングの用途に使用できることを証明しています。 この条件の中センサの上にニューロン細胞培養の増殖できました。カーボンナノチューブセンサの製造のための新しい統合方法を作成しました。この方法は1024デバイス上の小束の約80%の収率で電場操作を用いました。液体構成を使用することによりセンサは刺激に反応すると証明しています。この成果は、電気的な記録で使用することができる培地中の細胞培養の放出を研究するための新しいツールの開発できました。

研究成果の概要（英文）：We have created a high-density array of carbon nanotube field effect transistor sensors (CNT-FET) and we have proved that they can be used for the sensing of neurotransmitters after functionalization with specific enzyme. We have demonstrated the possibility of developing culture of neurons on those sensors and we have successfully records under those conditions. More specifically, we have created new type of CNT-FET sensors using electric field manipulation with yield of about 80% of small bundle on 1024 devices. By using liquid gate configuration, we have proved the high-sensitivity of our sensors. We have use non-covalent functionalization methods to bind glutamate oxidase to CNT-FET and we have shown that such biosensors are sensitive to glutamate. This accomplishment represents the fabrication of a new tool to investigate chemical release in culture medium which can be co-integrated with electrical recording.

研究分野：　Nanobiotechnology

キーワード：　Neurotransmitter　CMOS　Microelectrode Array　Carbon nanotube　sensors

１．研究開始当初の背景

The study of brain activity at the single neuron scale is one of the challenges in bio-nanotechnology. Large scientific projects have been launched to understand the neuron-neuron interactions, such as action potential propagation or chemical interaction via neurotransmitters exchanges.

The use of nano-electronic recording system can provide direct information on the electrical activities of neuronal network at subcellular scale. Nano-wires or carbon nanotube (CNT) can therefore be used as high precision electrodes for extra or intra-cellular signal recording. In this project we will use carbon nanotube sensors to detect chemical activity of neuron and compare it to the electrical activity in real time.

２．研究の目的

In this project we want to combine high-density micro electrode array technology and high-resolution CNT biosensing. The simultaneous real-time monitoring of both electrical activity via CNT electrode array and neurotransmitter release detection at high-resolution via CNT sensors is an original and promising way to advance our understanding of the complex communication scheme in neuronal tissues

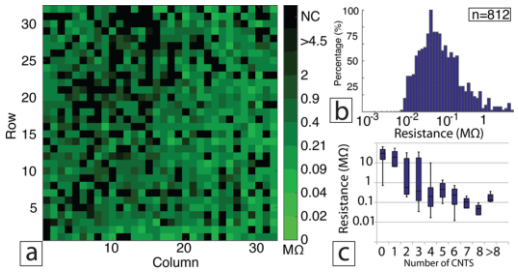
３．研究の方法

We want to investigate a direct measure of the presynaptic glutamate release and to directly observe glutamate release fatigue and/or recovery and understand the glutamate vesicles dynamics under different conditions. A new CNT nano-sensors matrix will be developed for this purpose. CNT have already been tested successfully for several new and sensitive sensing applications such as intercellular action potential recording and neurotransmitter sensing. With this project we aim to use advanced integration techniques to achieved a density of 10,000 independent CNT-FET micro-devices/mm^2

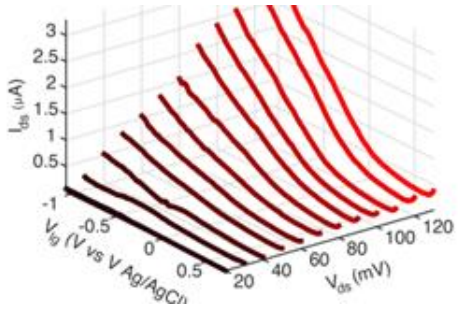
４．研究成果

We have created a dense array of 1024 carbon nanotube sensors and we have proved that they can be used for sensing application such as neurotransmitters after functionalization with specific enzyme. We have demonstrated the possibility of developing culture of neurons on those sensors and we have successfully records under those conditions. We have created a new integration method for fabrication of carbon nanotube sensors using electric field manipulation with yield of about 80% of small bundle on 1024 devices.

Our CMOS system allows for the sequential current readout of all 1024 CNT devices via 32 simultaneous signal amplification channels. To characterize each device, the potential of the drain electrode (Vd) was fixed and the source potential (Vs) was swept relatively from -0.2 V to 0.2 V. The current between the source and drain (Ids) has been measured for every pixel via one of the readout channels. The corresponding resistance has been calculated by linear regression of the Ids/Vds characteristic of our devices between -20 mV and 20 mV. A representation of the spatial distribution of the devices resistance on a typical CNT sensors array is shown Figure 1a. Each pixel in this image corresponds to a single CNT device with its resistance represented by a color scale. The different resistances values are usually homogeneously dispersed on the array. The histogram of the corresponding resistances has been plotted in Figure 1b to present the distribution ranging from a few tens of kΩ to a few MΩ. To understand the distribution better, a subset of device resistances has been compared to the number of CNTs approximated by SEM for each device (Figure 1c).

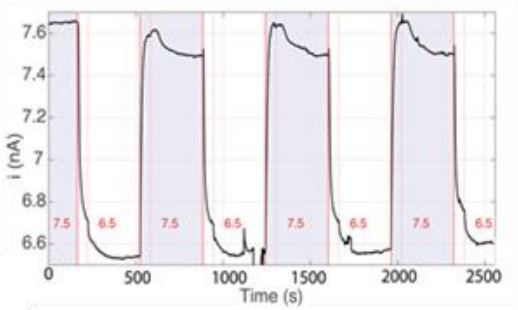


We have design and fabricate a second version of our system with about 11000 sensors. By using liquid gate configuration, we have proved the sensitivity of our sensors. A typical behavior of a selected CNT device recorded via the CMOS interface is presented Figure 2. In this case, the variation of Ids for Vlg ranging from -1 V to 0.7 V has been plotted at different Vds ranging from 20 mV to 120 mV.



We have use non-covalent functionalization methods to bind glutamate oxidase and we have shown that such biosensors are sensitive to glutamate. This experience leads to promising but uncomplete results. The sensitivity of our sensors and repeatability is demonstrated on the following picture using different pH buffer as model. We can see the repeatability and stability of our nanosensors.

We demonstrate new fabrication



methods which open new possibility for the future of neurosciences. Despite a lack of detection of small concentration of glutamate in complex medium, we have been able to prove that CNT sensors can be integrated in a density bigger than achieve before on substrate that can be used in the culture medium of neural cells. Moreover, we have demonstrated that the integration of those sensors in a full microfluidic system allows for the measurement of successive and fast change in the medium. This is necessary for use in the detection of rapid release of neurotransmitter. We have started the development of a second generation of CNT sensors integrated chip with more than 9000 devices. We hope that this new approach will allow for a more complete mapping of chemical (higher spatial resolution). Finally, the integration in the same microchip of both electrode and CNT sensors will lead to the full achievement of our goal. This project open the road of new ambition in the field of nanotechnology apply to brain study.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計１件）
Per review conference proceding

① Seichepine, F., Rothe, J., Dudina, A., Hierlemann, A., Frey, U. CMOS-integrated high-density arrays of carbon nanotube sensors (2014) 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS 2014, pp. 1835-1837.

〔学会発表〕（計４件）

① F. Seichepine, Array of 1024 LG-CNTFET Biosensors Integrated on a CMOS system, NT15, 2015-06-29, Nagoya University, Nagoya, Japan

② F. Seichepine, CMOS-integrated high-density arrays of CNT sensors, MicroTas 2014, 2014-10-28, San Antonio, United Stated

③ F. Seichepine, Large-scale integration of carbon nanotube devices on switch-matrix based CMOS chip, MNE2014, 2014-09-24, Lausanne, Switzerland

④ F. Seichepine, Integration of DWNT sensors on CMOS plateform, 47th

FNTG 2014, 2014-09-03 Nagoya University, Nagoya, Japan

〔図書〕（計 0 件）

〔産業財産権〕
○出願状況（計 0 件）

○取得状況（計 0 件）

〔その他〕
ホームページ等

６．研究組織
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