様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　元 年　 ６ 月 １７ 日現在

機関番号：１２６０１
研究種目：基盤研究(B)（一般）
研究期間：2016～2018
課題番号：１６Ｈ０４３４４

研究課題名（和文）Electrical TFT platform for fundamental understanding of neuromuscular communication in the aim of neuroprosthesis.

研究課題名（英文）Electrical TFT platform for fundamental understanding of neuromuscular communication in the aim of neuroprosthesis.

研究代表者

ティクシェ三田　アニエス（TIXIER-MITA, Agnes）

東京大学・先端科学技術研究センター・准教授

研究者番号：００３３４３６８

交付決定額（研究期間全体）：（直接経費）　　13,000,000円

研究成果の概要（和文）：TFT 電極アレイに基づく電気プラットフォームを開発した。デバイスの上に培養した神経細胞の光学観測と電気刺激、測定を同時に行うことを可能にしました。また、TFT 電極アレイを制御し、選択した電極の信号検出や刺激信号を加えるためのArduino コントロールシステムを開発した。スキャンすることを可能とし、神経培養の電気活動の2 次元マッピングが可能です。同時に、Central Pattern Generator(CPG)と接続し、神経細胞の培養、TFT電極アレイ、コントロールシステム、CPGが良好な適合性を示しました。皮質ニューロン、脳オルガノイド、心筋細胞の細胞培養に適用しました。

研究成果の学術的意義や社会的意義
The platform is the only existing tool for optical and electrical analyze of mix cells culture network of electrogenic cells (neurons, cardiomyocyte, muscle cells), at mesoscale. It will be used for fundamental understanding of communication in cells network, and investigation on related disease.

研究成果の概要（英文）：An electrical platform, based on TFT electrode array, was developed. It allows optical observation as well as electrical stimulation and sensing of neurons cells cultured on top of the device. An Arduino control system was developed in order to control the microelectrodes array and to program them for sensing, or to apply stimulation signal. It allows also scanning of the array, to obtain a 2D mapping of the cells culture electrical activity. At the same time, the platform was interfaced with a Central Pattern Generator (CPG) to realize a more biologically plausible stimulation of neurons and to investigate the interactions between artificial and biological entities. That platform was applied to several types of cells culture in order to enlarge the field of expertise of our group: cortical neurons, brain organoids and cardiomyocyte cells. Two new research projects have been built based on the results.

研究分野：バイオデバイス

キーワード：Bio-sensor array　Integrated bio-device　Thin-Film-Transistor　Spiking Neural Network　Neuroprosthesis　Cells network analysis

１．研究開始当初の背景

Activity of electrically excitable cells (neurons, skeletal, smooth or cardiac muscle cells) in culture are usually studied using microelectrodes processed on a glass substrate: these cells spontaneous electrical activity (neurons) or physical deformation with pulses (muscle cells) are suitable with electrical sensing. Multi-Electrode-Arrays systems (MEAs) are standard devices for that purpose. They allow stimulation or sensing, on a 1mm square surface, of the activity of these cells. The level above, in term of resolution, concerns CMOS-MEAs devices, which propose 2D mapping sensing possibility with very high resolution, smaller than single cell. However, despite that advantage to standard MEA devices, the sensing area remains very small (1mm square, for cells cultures which often reach cm square dimensions) and optical observation is difficult due to the non-transparency of the device. When uniform cells cultures are studied, MEAs and CMOS-MEAs are suitable tools. But when mix-cells cultures are studied, or large cells networks are analyzed, a new tool, with large sensing area and high resolution is needed, to address more realistic bio-medical questions concerning cells interaction or cells communication. This is that new tool that we proposed to develop in this project.

２．研究の目的

The purpose of this project is to develop an electrical platform, based on the Thin-Film-Transistor (TFT) glass substrate of TFT/LCD displays, for fundamental understanding of biological cells interactions. The aim of the platform is to mimic in vivo environment to develop biomedical solutions for neuroprosthesis. This platform gathers the possibility of simultaneous and real time electrical stimulation and sensing of biological cells, and optical observation. In addition, an artificial biomimetic neural system has been integrated to investigate on the interaction between biological cells and artificial signal, in the aim of developing neuroprosthesis. Studies from homogeneous to heterogenous types of cells are possible. This platform was applied to cortical neurons, brain organoids as well as heart cells culture.

３．研究の方法

The TFT glass substrate is part of displays of computers, iPads or other, and is used to control the pixels ON/OFF state of these display. It consists in a glass plate covered with a dense array of transparent Indium-Tin-Oxide microelectrodes, controlled by an array of switch Thin-Film-Transistors. That array of microelectrodes can be used as well for signal application (in our case, for cells stimulation), or for signal sensing (in our case, for cells activity sensing). The array of TFTs allows to switch ON or OFF microelectrodes in the array by programming. As a result, a large (cm size) sensing area, with resolution close to single cell can be obtained for cells culture investigation. Thanks to that substrate, it is possible to combine simultaneously optical observation, of cells cultured on top, to electrical sensing, thanks to the transparency of the device. In addition, in order to realize a more biologically plausible stimulation of neurons, compared to standard stimulation (usually square pulses), the platform with cells cultured on top will be interfaced to an artificial biomimetic neural network to investigate the interactions between artificial and biological entities.

In the project, four aspects were investigated first separately: ① TFT platform

development, ② microfluidic development to welcome cells culture, ③ culture of neurons and muscle cells, and ④ artificial biomimetic neural network. Then, they were combined together to reach an intermediate development of the platform, applied to 2 objectives: ⑤ multi-electrical stimulations and ⑥ electrical sensing, in addition to optical analyses. Finally, based on these developments and results, the ⑦ complete platform was realized and applied to various cells culture sensing.

４．研究成果

An electrical platform, based on TFT electrode array substrate, was realised. The control of the microelectrodes array was ensured by an Arduino control system, which was developed in order to select the microelectrodes in the array for sensing, or to apply stimulation signal. It allows also scanning of the array, to obtain a 2D mapping of the cells culture electrical activity. With that platform, neurons activity and stimulation were successfully obtained. At the same time, interfacing of the platform with a Central Pattern Generator (CPG) was done, for biomimetic stimulation of the cells culture, showing a good compatibility between neurons culture, the TFT microelectrodes array, the control system and the CPG. That platform was applied to several types of cells culture in order to enlarge the field of expertise of our group: cortical neurons, brain organoids and cardiomyocyte cells. Activity of the different cells culture was successfully measured, demonstrating that the TFT platform can be used for electrically active cells analyses. Based on these results and using the platform, fundamental bio-medical questions can now be addressed. Two new research projects have been built based on the results.

５．主な発表論文等

〔雑誌論文〕（計　3 件）

1. Faruk Azam Shaik, Grant Alexander Cathcart, Satoshi Ihida, Yoshiho Ikeuchi, Agnès Tixier-Mita and Hiroshi Toshiyoshi, “Electrical Stimulation, Recording and Impedance-Based Real-Time Position Detection of Cultured Neurons Using Thin-Film-Transistor Array”, Journal of Micromechanical Systems, pp. 1-10, (March 2018) DOI: 10.1109/JMEMS.2018.2810285
2. Faruk Azam Shaik, Grant Cathcart, Satoshi Ihida, Myriam Lereau-Bernier, Eric Leclerc, Yasuyuki Sakai, Hiroshi Toshiyoshi and Agnès Tixier-Mita, “Thin-film-transistor array: an exploratory attempt for high throughput cell manipulation using electrowetting principle”, Journal of Micromechanics and Microengineering, 27, 054001, (April 2017).
3. Eric Leclerc, Jean-Luc Duval, Christophe Egles, Satoshi Ihida, Hiroshi Toshiyoshi and Agnès Tixier-Mita, “In vitro cyto-biocompatibility study of thin-film transistors substrates using an organotypic culture method”, Journal of Materials Science: Materials in Medicine, **28(1)**, p. 4, (January 2017).

〔学会発表〕（計　17 件）

1. Grant Alexander Cathcart, Agnès Tixier-Mita, Satoshi Ihida, Faruk Azam Shaik, Hiroshi Toshiyoshi, “Algorithmic Open-Surface Identification and Location of Droplets on a Transparent TFT Substrate for Droplet-based Microfluidics”, The 22nd International Conference on Miniaturized Systems for Chemistry and Life Sciences (MICROTAS’2018), M074c, November 11th-15th November 2018, Kaohsiung, Taiwan.
2. Agnès Tixier-Mita, “Thin-Film-Transistor Technology: A Display Technology for Biological Applications”, VDEC D2T Symposium, September 26th 2018. (Invited)
3. Agnès Tixier-Mita, “Thin-Film-Transistor Technology used for Biological Applications”, 16th NAMIS Workshop on “Micro- and nanosystems, large area electronics and biofunctionalities towards novel integrated smart systems”, June 18th – 20th 2018. (Invited)
4. Agnès Tixier-Mita, “Bio-sensors Array Devices using Thin-Film-Transistor Technology”, International workshop on new micro technology and translational approaches for organ regeneration and cancer therapies, June 26th -27th 2018. (Invited)
5. Farad Khoyratee,Thomas Benneteau, Agnès Tixier-Mita, Sylvain Saïghi, Timothée Levi; “Bio-hybrid Experiments Using Tunable Real-time Biomimetic Neural Network”, in Proc. the 10th International Symposium on Microchemistry and Microsystems (ISMM'2018), June 19-21 2018, Busan, Korea.
6. Agnès Tixier-Mita, Grant Alexander Cathcart, Faruk Azam Shaik, Satoshi Ihida, Yoshiro Ikeuchi and Hiroshi Toshiyoshi, “Impedance Sensing with Thin-Film-Transistors (TFT) Array Biosensors”, 4th Conference on Impedance-Based Cellular Assays (IBCA'2018), Edinburgh, United-Kingdom, June 6th-8th 2018. (Invited).
7. Damien Blanchard, Pierre-Marie Faure, Satoshi Ihida, Takashi Kohno, Hiroshi Toshiyoshi, Timothée Lévi and Agnès Tixier-Mita, “Stimulation/Control System for TFT Array Biosensor – Application to Dielectrophoresis”, The 31st IEEE International Conference on Micro Electro Mechanical Systems (MEMS 2018), Belfast, United Kingdom, 21-25 January 2018. DOI: 10.1109/MEMSYS.2018.8346537
8. Faruk Azam Shaik, Grant Alexander Cathcart, Satoshi Ihida, Agnès Tixier-Mita, and Hiroshi Toshiyoshi, “HepG2 Cells Patterning by EWOD on Thin-Film-Transistor Aarray Devices- An Aanalysis to Prevent Cell Death”, The 21st International Conference on Miniaturized Systems for Chemistry and Life Sciences (MICROTAS 2017), Savannah, Georgia, USA, M073d, October 22-26, 2017.
9. Azam Shaik Faruk, Cathcart Grant Alexander, Satoshi Ihida, Toshiyoshi Hiroshi, Tixier-Mita Agnès, “A Thin-Film-Transistor (TFT) Array for Real Time Active EWOD Interface to Cell Culture”, **電気学会研究会資料**. CHS : The papers of technical meeting on chemical sensor, IEE Japan, 21-25, 2017-06-29. http://id.nii.ac.jp/1031/00107817/
10. Grant Alexander Cathcart, Agnès Tixier-Mita, Satoshi Ihida, Faruk Azam Shaik, Hiroshi Toshiyoshi, “Simultaneous Optical and Electrical Monitoring of Cells on a Transparent Thin Film Transistor Array”, The 19th International Conference on Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS' 2017), Kaohsiung, Taiwan, pp. 206-209, 18-22 June 2017. DOI: 10.1109/TRANSDUCERS.2017.7994386

11. Faruk Azam Shaik, Yoshiho Ikeuchi, Grant Cathcart, Satoshi Ihida, Hiroshi Toshiyoshi, Agnès Tixier-Mita, “Extracellular Neural Stimulation and Recording with a Thin-Film-Transistor (TFT) Array Device”, The 19th International Conference on Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS' 2017), Kaohsiung, Taiwan, pp. 206-209, 18-22 June 2017. DOI: 10.1109/TRANSDUCERS.2017.7994024
12. Agnès Tixier-Mita, Satoshi Ihida, Grant Cathcart, Faruk Azam Shaik, Hiroyuki Fujita,Yoshio Mita, Hiroshi Toshiyoshi, “A Test Structure to Characterize Transparent Electrode Array Platform with TFTs for Bio-chemical Applications”, 2017 International Conference of Microelectronic Test Structure (ICMTS), MINATEC, Grenoble, France, March 27-30 2017.
13. Faruk Shaik, Grant Cathcart, Satoshi Ihida, H.N. Unni, Hiroshi Toshiyoshi, AgnèsTixier-Mita, “A Comparison of the Continuous and Discrete Approach for Liquid Manipulation”, COMSOL Conference 2016, Bangalore, India, 20-21 October, 2016.
14. Hiroshi Toshiyoshi, Satoshi Ihida, and Agnès Tixier-Mita, “MEMS on TFT”, 2016 International Conference on Optical MEMS and Nanophotonics (OMN 2016), Singapore, 31 July‐04 August 2016, Mo4.1-1. (Invited)
15. Agnès Tixier-Mita, Satoshi Ihida, Grant A. Cathcart, Faruk Azam Shaik and Hiroshi Toshiyoshi, “More than Moore Applications with Thin-Film-Transistors Array Substrates from Liquid Cristal Display: New Devices for Biological Cells Analyses”, The 229th Electrochemical Society Meeting (229th ECS Meeting), San Diego, CA, May 29-June 2, 2016. (Invited)
16. Faruk Azam Shaik, Grant A. Cathcart, Satoshi Ihida, Jiro Kawada, Yoshiho Ikeuchi, Agnès Tixier-Mita and Hiroshi Toshiyoshi, “An exploratory attempt for Electrowetting on Thin-Film-Transistor array”, Asia-Pacific Conference of Transducers and Micro-Nano Technology 2016 (APCOT'2016), Kanazawa, Japan, June 26 - 29, 2016.
17. Grant A. Cathcart, Agnès Tixier-Mita, Satoshi Ihida, Faruk A. Shaik, and Hiroshi Toshiyoshi, “Transparent Thin Film Transistor Electrode Array for Real-Time In-Vitro Electrical Characterization of Cell Culture”, The 11th Annual IEEE International Conference on Nano/Micro Engineered and Molecular Systems (IEEE-NEMS 2016), Matsushima Bay and Sendai MEMS City, Japan, 17-20 April, 2016.

〔図書〕（計　0 件）

〔産業財産権〕
○出願状況（計　1 件）

名称：
発明者：
権利者：
種類：
番号：
出願年：
国内外の別：

○取得状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
取得年：
国内外の別：

〔その他〕
ホームページ等

６．研究組織

(1)研究分担者

研究分担者氏名：**年吉　洋**

ローマ字氏名：TOSHIYOSHI Hiroshi

所属研究機関名：**東京大学**

部局名 生産技術研究所

職名：教授
研究者番号（8 桁）：50282603

研究分担者氏名：**池内　与志穂**

ローマ字氏名：IKEUCHI Yoshiho

所属研究機関名：**東京大学**

部局名 生産技術研究所

職名：准教授
研究者番号（8 桁）：30740097