論文の内容の要旨

論文題目　Development and applications of a bio-hybrid platform for myocyte investigation using Thin-Film Transistor technology
（薄膜トランジスタ回路による筋細胞の培養・計測・
スクリーニング技術の構築）

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This research aims to develop a bio-hybrid platform using the Thin-Film Transistor (TFT) technology for investigation of excitable cells. In this research, the TFT platform is mainly used as an *in vitro* microelectrode array (MEA) to perform electrical recordings on muscle cells, also known as myocytes. The combination of microelectromechanical systems (MEMS) with the TFT technology makes it very attractive for the development of novel microsystems that can combine multiple electrical measurement techniques on one platform. The key contribution of this work is the responses it provides about the possibility of using TFT technology as a base for integrated MEMS to overcome the common difficulties faced with standard *in vitro* MEAs. Three main electrical techniques were considered in this research: (1) electrophysiology, (2) electrochemistry, and (3) dielectrophoresis. Indeed, these techniques are essential to characterize myocytes, evaluate their electrical and chemical properties and understand the mechanisms causing them.

In the past several decades, the wealth of recent research on applications of MEMS in the biomedical field have played an important role in the generation of new microsystems for bioanalysis. Among these new instruments, MEAs contain multiple electrodes through which electrical signals from excitable cells are obtained or delivered. These devices provide a non-invasive electrophysiology laboratory technique for *in vitro* studies of cell cultures and tissues. There are two major types of MEAs – passive and active. Passive MEAs contain electrodes firmly connected to a conducting track limiting the electrode density and the number of electrodes. Active MEAs based on complementary metal-oxide-semiconductor (CMOS) chips allow high electrode densities but are opaque, limited to small areas, and rigid. As a results, a challenging problem which arises in this domain is the difficulty to combine transparency, large number and high density of electrodes on a large surface, and multiple measurement techniques on one unique platform for a better understanding of the electrical and chemical communication in a cell network.

To overcome this problem, this research proposes the development and applications of an *in vitro* MEA using the TFT technology. TFT technology has been mainly used in the field of display technology with a wide range of applications including liquid crystal display televisions. Due to its large industrial applications, such microsystems can be fabricated with high yield. In this work, integrated TFTs are used for controlling an array of indium tin oxide (ITO) microelectrodes. The main challenge faced was to obtain reliable, repeatable, and reproducible results for each measurement techniques evaluated in this study. Indeed, the design and application of MEMS technologies for use in biological and biomedical areas generate a host of new problems and issues that require a broad understanding of aspects from material sciences, biological sciences and engineering. The objective of this Ph.D. was first to explore the possibility to perform electrical measurements with a TFT-MEA for biological applications, and then to improve each techniques for investigation of myocytes.

The outline of this research is described in **Figure 1**. The first part delivers the purpose of this work by providing preliminary background information that puts it into context, specifying the aims and objectives, and identifying the value it brings to the scientific research. The second part presents the theory behind the fundamental parts of this research by defining the structure, function, and electrical properties of cardiac muscle cells, the techniques for studying them, and the TFT technology. The third part describes the preparation of the TFT platform, and the experimental setups for each measurement technique: electrophysiology, electrochemistry, and dielectrophoresis. The fourth part presents the results obtained for each techniques, with a main focus on the measurement of the extracellular potentials of cardiac muscle cells. Then, the possibility to perform electrochemical measurements was considered by performing voltammetric and amperometric measurements with the TFT platform. Lastly, dielectrophoresis of skeletal muscle cells was explored for displacement of cells on specific areas of the TFT-MEA. Collecting the data obtained with electrical measurements, the fifth part discusses the analysis of the results and assess the strength and limitations of these techniques. Finally, the sixth and last part summarizes and concludes this work, and examines the possible areas for future research.

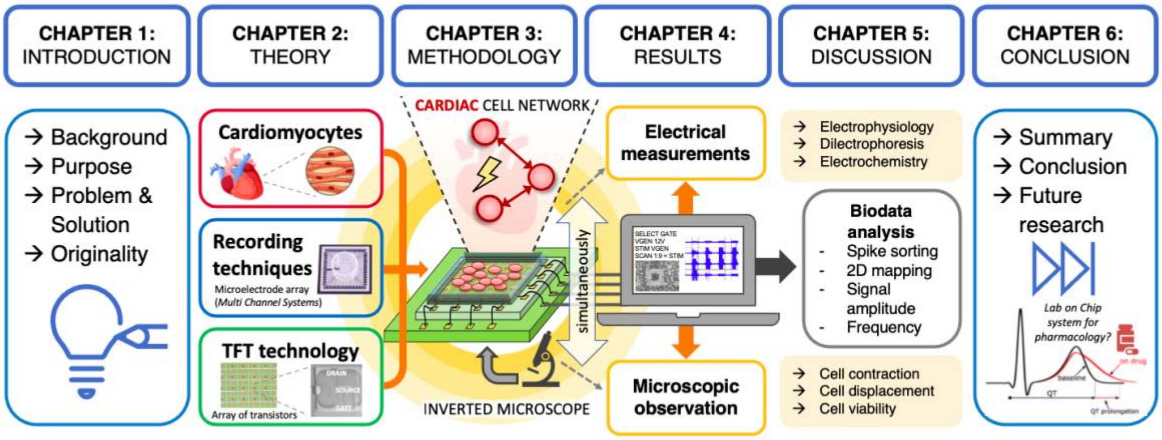
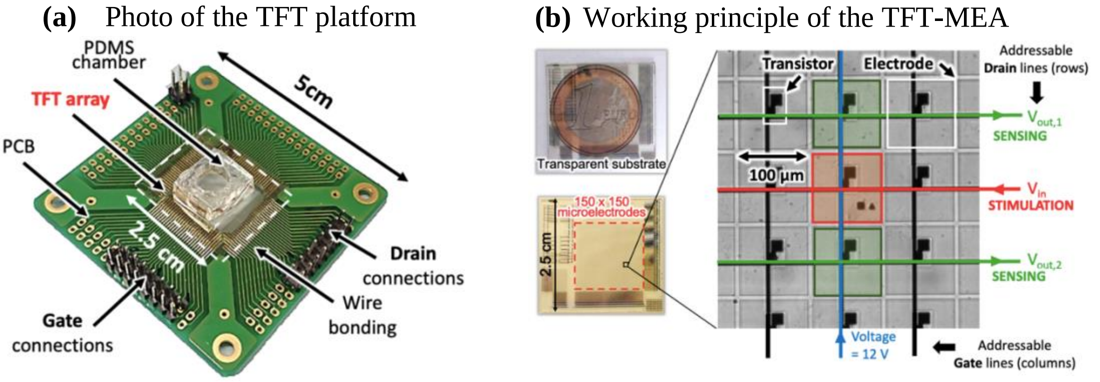


Figure 1: *Outline of this PhD research.*

The surface of the TFT platform is made of a large, dense array of transparent 100 µm × 100 µm ITO microelectrodes fabricated on top of a glass substrate. Each electrode is independently connected to an integrated TFT, which is used for switching ON/OFF the corresponding electrode. Then, the electrode can be used for sensing or applying electrical signals. **Figure 2** presents a photo of the TFT platform and a description of its working principle.



(a) Photo of the TFT platform **(b)** Working principle of the TFT-MEA

Figure 2: *Description of the TFT platform: (a) photo of the TFT platform after post processing fastens with a printed circuit board (PCB), and (b) its working principle.*

This research mainly focused on the application of this TFT platform to electrophysiology. TFT-MEAs with a 150 × 150 transparent microelectrode array were made for capturing the extracellular potentials across a culture of cardiac myocytes. For these experiments, TFT-MEAs of 28 × 28 microelectrodes (784 microelectrodes) were used. Beating cardiac cells were isolated from neonatal mouse hearts and cultured on the platform. Thanks to the transparency of the platform, contraction of cardiac cells was observed with an inverted microscope simultaneously with electrical measurements. **Figure 3** shows the electrophysiological results obtained after analysis with a spike sorting algorithm developed for that purpose.

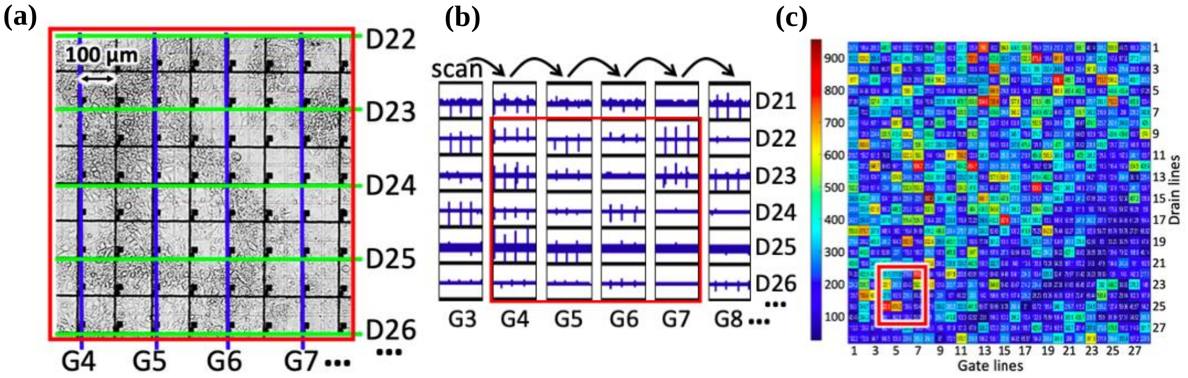
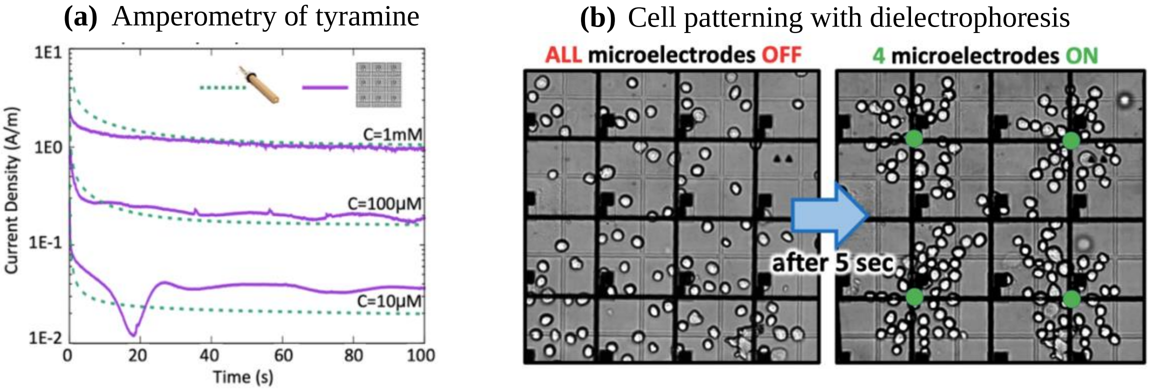


Figure 3: *Electrophysiology of cardiac cells with the TFT-MEAs: (a) observation of cardiac cell culture with inverted microscope; (b) electrical recordings with Multichannel System equipment before filtering, (c) 2D reconstruction of the average peak-to-peak amplitude of electrical cell signals on a 28 × 28 microelectrode array (from 100 µV to 1 mV).*

After that, the TFT platform was used as an electrochemical sensor for biochemical components. Standard gold working electrode (WE) was compared with ITO WE from TFTs. Also, the standard Ag/AgCl glass reference electrode (RE) was compared with an integrated Ag/AgCl RE on the TFT device. These experiments have qualitatively demonstrated that ITO microelectrodes can be used as WE and that Ag/AgCl ink can be used as an integrated RE on the TFT platform. Based on these results, amperometric experiments were performed with tyramine in PBS. Both integrated and non-integrated types of microelectrodes presented linear and similar variations of current with the concentration of tyramine, as show in in **Figure 4.a**. The current with ITO WE is lower than with standard gold WE, which is certainly due to the smaller size of the electrode surface: 100 μm^2 for the ITO WE, and 3 mm diameter for the standard gold WE.

Finally, dielectrophoresis of C2C12 skeletal muscle cells was performed with the TFT platform for cell transportation and patterning on the programmable surface. The glass substrate of the platform was previously coated with a 400 nm layer of Teflon to prevent damage of the electrodes when working with high voltage. Best results were observed when 20 V and 100 kHz were applied to the source lines of the TFT array, without damaging the electrodes. In this work, the skeletal cells were attracted to the electrodes and could successfully be moved on the TFT array substrate with Teflon coating. These results provide evidence for the possibility of patterning and motion of cells on the surface. **Figure 4.b** shows an example of cell displacement by dielectrophoresis.



(a) Amperometry of tyramine

(b) Cell patterning with dielectrophoresis

Figure 4: *(a) Electrochemistry: amperometry of tyramine - current versus time, at 10 μM, 100 μM and 1 mM, using a standard Au electrode or an ITO microelectrode of TFT device for the working electrode (scan rate: 0.05 V/s); (b) Dielectrophoresis of C2C12 skeletal cells: cell patterning using 4 microelectrodes after 5 seconds.*

The regulation of ion exchange through the phospholipid membrane of biological cells plays a crucial role in signal transmission between myocytes and is also essential for all vital functions of our muscles. The effect of new drug candidates, on the electrical activity of ion channels can be directly investigated by standard MEAs that provide high spatial resolution. However, they do not combine transparency, high density of microelectrodes on a large surface, and multiple measurement techniques. These characteristics are all met by adapting the TFT technology to the development of novel MEAs. On this basis, a TFT-MEAs has been developed and evaluated for the study of myocytes. TFT-MEAs can be used not only for electrophysiological measurements but also for electrochemistry and dielectrophoresis through appropriate functionalization. Future research involves the use of TFT-MEAs as a key tool for development of new lab on chip systems.