A programmable culture platform for stimulation and in situ sensing of lung epithelial cells

A programmable culture platform for stimulation and *in situ* sensing of lung epithelial cells

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Abstract

A programmable dynamic cell culture chamber compatible with a standard multi-well plate was designed and characterized. The system is integrated with an array of OECT biosensors, in view of an in-situ monitoring of the dynamic cultures

Introduction

Lung cancer is one of the leading causes of death in industrialized countries.[1] Development of cancer therapeutics requires improvements of the traditional static cell cultures, and is moving towards dynamic models in which the organ-specific microenvironment is recreated. Despite the development of a wide variety of sophisticated systems trying to replicate the conditions inside the human alveoli has been demonstrated.[2] there is a lack of versatile ones fully compatible with standard cell culture protocols. Moreover, the growth of cultures inside these systems limits the possibility to use multi-well standard detection methods. An in situ sensing set-up is also needed in order to monitor critical biological, chemical and physical events during the dynamic cell fetal development to better understand the underlying mechanisms of normal lung organogenesis,[3,4] such as miRNA delivering to neighbouring cells or direct in-vitro monitoring of cell necrosis and early/late apoptosis. In the last few decades, organic thin film transistors (OTFT) are the most studied novel devices in the field of biosensing, promising advantages such as high sensitivity, high reliability, and low cost.[5] In this work, we present a programmable dynamic cell culture platform designed to work in a CO_2 incubator, compatible with a standard multi-well plate (Figure 1). An epithelial lung cell culture can be mechanically stimulated with a cyclic increase of the hydrostatic pressure inside the culture chamber, trying to mimic the human respiration rate. In the second phase of this work, a live-monitoring platform based on Organic ElectroChemical Transistors (OECT) was designed, fabricated and characterized, in order to merge in a compact platform both the dynamic culture technology and the biosensing one.

Materials and Methods

An airtight sealed PMMA chamber was first designed using a 3D CAD tool (RhinocerosÒ, Robert McNeel & Associates) and fabricated by means of a Milling Machine. The pressure inside the chamber is increased by means of a diaphragm pump, while a solenoid valve brings it back to its initial value. Both the final differential pressure reached in each cycle and their frequency can be selected by software using an Arduino Nano® microcontroller unit, in the range between 1 and 12 kPa. The system can work both in overpressure and in depressurization condition. A dedicated electronic circuit was designed and simulated using LT SpiceÒ, and a printed circuit board was designed and assembled by means of Kicad open source software. A pressure sensor and two temperature sensors were also implemented to characterize the system. The OECTs were fabricated on a SiO_2 finished p-type silicon wafer, using a 200 nm thick PEDOT:PSS layer as active material.

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Results

Tests were carried out on the tightness of the chamber, on the repeatability of the pressure cycles, and on the constancy of the temperature between the inside and outside of the chamber (Figure 2). Preliminary proliferation tests with HACAT and HFF1 cell lines were performed in order to investigate the biocompatibility of the system. Both cell lines showed an identical proliferation

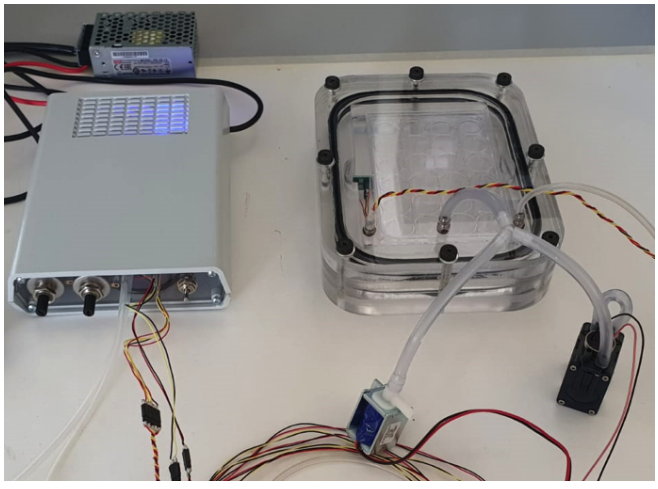

Figure 1. System assembled: electronic dedicated control unit (left), culture chamber (right).

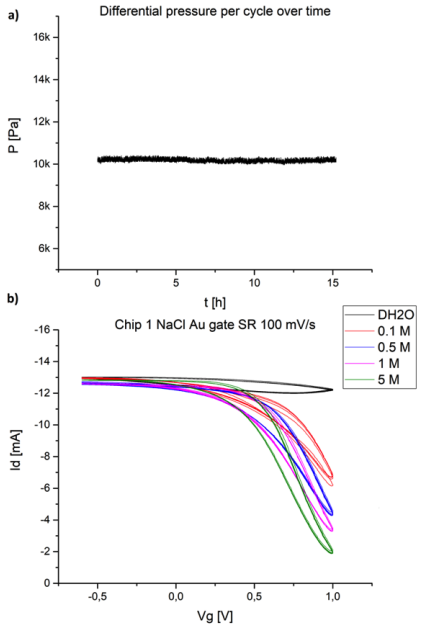

Figure 2. a) pressure reached at each cycle over time b) transfer characteristics of OECT in NaCl with different concentrations, Au gate electrode.

behavior inside the dynamic culture chamber, and outside of it. Tests were performed on OECTs using a NaCl solution with different concentrations from 0.1 to 5 M, using Ag/AgCl and Au gate electrodes, to evaluate the electrical response of the devices. Further investigations has been carried out with the same gate electrodes using a PBS solution and cell colture media (RPMI 1640), in order to demonstrate the device's reliability in a cell culture environment. All the transfer curves of the OECTs were performed with V_{DS}= -600 mV and V_G starting from -600 mV to 1 V. The devices showed a transconductance up to 45 mS.

Discussion and Conclusions

This work showed the possibility of growing a dynamic lung cell culture stimulated by a cyclic hydrostatic pressure. An array of OECTs has been fabricated and tested successfully with culture media. Further investigations will be carried out with A549 lung cancer cell line.

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