Biosensors and Bioelectronics 24 (2009) 3510–3516

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Electrotaxis of lung cancer cells in a multiple-electric-field chip

Ching-Wen Huang[a,b], Ji-Yen Cheng[b,c,d,*], Meng-Hua Yen[a,b], Tai-Horng Young[a]

[a] Institute of Biomedical Engineering, National Taiwan University, Taiwan
[b] Research Center for Applied Sciences, Academia Sinica Taiwan, Taiwan
[c] Department of Mechanical and Mechatronic Engineering, National Taiwan Ocean University, Taiwan
[d] Institute of Biophotonics, National Yang-Ming University, Taiwan

ARTICLE INFO

Article history:
Received 21 January 2009
Received in revised form 23 April 2009
Accepted 6 May 2009
Available online 13 May 2009

Keywords:
Electrotaxis
Microfluidic
Cancer metastasis
Lung cancer cell
Metastasis–electrotaxis

ABSTRACT

We report a microfluidic cell culture chip that was used for long-term electrotaxis study on a microscope. The cellular response under three different electric field strengths was studied in a single channel microfluidic chip. Electric field (EF) inside the microchamber was numerically simulated and compared to the measured value. Lung cancer cell lines with high and weak metastasis potential, CL1–5 and CL1–0, respectively, were used to demonstrate the function of the multi-field chip (MFC). The two cell lines exhibited greatly different response under the applied EF of $E = 74–375$ mV/mm. CL1–5 cells migrated toward the anode while CL1–0 cells did not show obvious response. Under the applied EF, cell orientation was observed accompanying the cell migration. Judging from the different temporal responses of the orientation and the migration, it is proposed that the two EF-induced responses may involve different signaling pathways.

1. Introduction

Electrotaxis, also known as galvanotaxis, is the induced directional movements of cells toward the cathode or the anode under direct current (DC) electric field (EF). Living cell migration in EF was discovered one century ago (Ingvar, 1920; Sato et al., 2007). The electrotactic responses of cancer cells are also reported in recent years (Djamgoz et al., 2001; Pu et al., 2007; Yan et al., 2009). Endogenous EF plays crucial physiological roles such as embryo development and nerve regeneration (McCaig et al., 2005). Endogenous DC EFs occur in the form of transepithelial potentials (TEP) *in vivo*, with the strengths of few mV/mm to a few hundreds of mV/mm. When cells transform, the TEP can be changed, thus generating a voltage gradient between the cancerous tissue and neighboring normal tissue (Djamgoz et al., 2001).

Conventionally, *in vitro* electrotaxis experiment is performed in an electrotactic chamber constructed by assembling cover glasses in a Petri dish where cells are cultured (Song et al., 2007). Culture medium evaporation is a potential problem because the connections among the components are not tightly sealed. Some modifications on the experimental setup have been reported. For example, Li and Kolega (2002) used 5 cm long and 10 mm thick flat agar slab as the salt bridge of the electrotactic chamber. Chamber sealing is achieved by vacuum grease to prevent medium evaporation.

Another drawback of the conventional setup is that an incubator is needed to maintain the temperature of the electrotactic chamber for long-term observation on a microscope. For example, in a previous design air curtain is used for maintaining the chamber temperature at $38 \pm 1\,^{\circ}C$ (Li and Kolega, 2002). The use of an incubator results in a bulky system.

Microfluidics are very effective in reducing the size of an experiment setup and in turn the reagent and sample consumption. Recently, chip-like electrotactic chambers are reported (Korohoda et al., 2000; Sato et al., 2007). Their designs reduce chamber dimension and hence Joule heating. However, the culturing medium is not tightly sealed against the ambient air in these designs.

Furthermore, the conventional electrotactic chamber provides only single EF strength in each experiment. Therefore observing the cell responses in different EF strengths is time consuming. Microfluidics may be a way to reduce the experiment time. Most of the microfluidic devices that utilize electric field are related to cell trapping by EF gradient (Muller et al., 1999; Gray et al., 2004; Hsiung et al., 2008) or to electroporation (Lu et al., 2006; Jain and Muthuswamy, 2007). Studies on cell electrotaxis have rarely been reported.

In this work a tightly sealed cell culture chamber (Cheng et al., 2008) and a temperature controlling heater (Cheng et al., 2007a,b, 2008) were integrated to construct an electrotactic chip. Rapid laser ablation technique was employed to fabricate the components of

* Corresponding author at: Research Center for Applied Sciences, 128 Sec.2 Academia Road, Taipei City 11529, Taiwan. Tel.: +886 2 27898000; fax: +886 2 2782 6680.
E-mail address: jycheng@gate.sinica.edu.tw (J.-Y. Cheng).

0956-5663/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2009.05.001

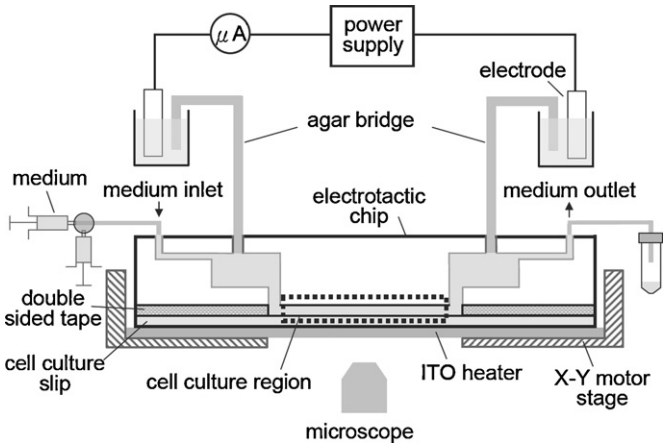

Fig. 1. System for electrotaxis study. Top view of the electrotactic chip is shown in S1-F1.

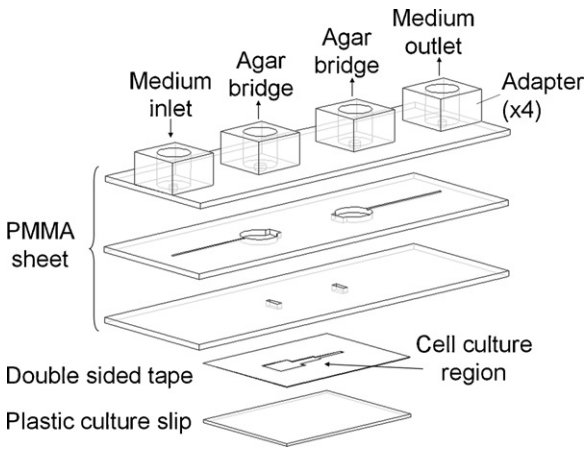

Fig. 2. Assembly drawing of the MFC. There are three EF segments in the microchannel. Higher EF strength was obtained in segment with smaller width. A photo picture showing the embodiment of the entire system is in S1-F2.

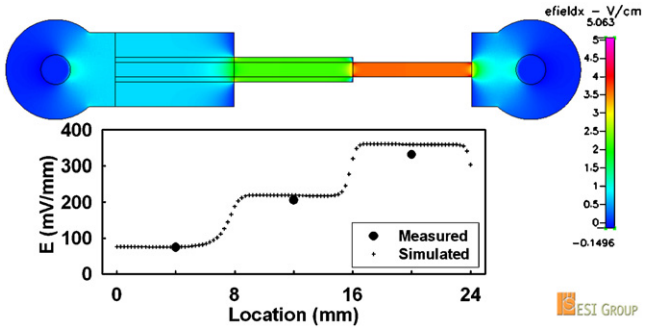

Fig. 3. (Upper) Simulated EF in the multi-field chip. (Lower) Simulated and measured E along the channel. The measured EF strength is 74 ± 0.1, 205 ± 0.8 and 331 ± 1.3 mV/mm. The error bars are too small to be seen in the figure.

the chip (Cheng et al., 2004, 2007a). Our electrotactic chip was evaporation-free and provided multiple EFs in a single microchannel. Multiple EF strengths were provided in a single experiment so that total experiment time was largely reduced. The microenvironment inside the chip can be better controlled than that in the traditional macro scale setup. The chip was applied for electrotaxis study of human lung adenocarcinoma cell lines, CL1–0 and CL1–5 cells.

CL1–5 is one of the sublines derived from the parent cell line, CL1–0, and has higher invasiveness than CL1–0 *in vitro* as well as *in vivo* (Chu et al., 1997; Chen et al., 2001; Shih et al., 2001). Gene expression differences among these sublines are revealed by global genomic analysis (Chen et al., 2001). It is therefore worthwhile to investigate the response of these sublines under physiological DC EF. In this work the electrotaxis response of these cancer cells with different metastasis potential was studied.

2. Materials and methods

2.1. System for electrotaxis study

Fig. 1 shows the configuration of the entire system. The system consisted of an electrotactic chip (single-field chip, SFC, or multi-field electrotactic chip, MFC), a home-built transparent ITO heater chip (Cheng et al., 2007a,b), agar salt bridges, a syringe pump (NE-1000, New Era Systems Inc.), a DC power supply (GPC-3030DQ, Instek), an ammeter (Model 189 Fluke), an X–Y motor stage, and an inverted microscope (CKX41, Olympus) equipped with digital camera (E-330, Olympus, not shown in Fig. 1). The preparation of the agar salt bridges is described in the literature (Song et al., 2007). The X–Y stage was controlled by a computer to move the electrotactic chip (see below).

2.2. SFC and MFC design and fabrication

As shown in Fig. 2, the MFC has connecting holes for the medium inlet/outlet and the agar bridges. Top-view of the MFC is shown in the supplementary material S1-F1. Cells were cultured in a microchannel (the cell culture region) for the electrotaxis study. Three segments with widths of 5000, 1667, and 1000 μm were sequentially aligned in the microchannel. The length of the cell culture region was 24 mm. For single field electrotaxis study, an SFC was used. The SFC has similar configuration with that of the MFC but with a simple channel that has width and height of 3000 and 70 μm, respectively. The length of the cell culture region is 15 mm.

Based on Ohm's law, the electric field strength through a bulk material is $E = I/(\sigma W h)$, where I is the electric current flowing through the bulk material, the culture medium in the electrotactic chamber; σ is the conductivity of the culture medium; W and h are the width and depth of the electrotactic chamber, respectively. Therefore, the EF strength is inversely proportional to the width, W, while all the other parameters remain unchanged. The EF strength in each segment in Fig. 3 is expected to be different while the EF in each segment is homogeneous (E = constant). Numerical simulation was done to confirm this (see below).

CO_2 laser ablation was used to fabricate the microchannel in the chip (Cheng et al., 2004). Briefly, the pattern of the designed channel was drawn by computer software AutoCAD (Autodesk). The pattern was then transferred to a laser scriber (M-300, Universal Laser Systems or ILS-II, LTT Corp.) to ablate trenches on a piece of polymethylmethacrylate (PMMA) substrate and double sided tape (8018, 3 M). The PMMA substrate with the trenches was then bonded with a blank cell culture slip (Thermanox Plastic Coverslips, NUNC) using the double sided tape to form the electrotactic chip that contains the sealed microchannel. The thickness of the double sided tape defines the depth of the electrotactic chamber. Cytotoxicity of the double sided tape on CL1–5 was examined. The result does not show observable cell viability change (Fig. S2-F1).

2.3. Electric field simulation and measurement

Simulations of the EF in the SFC and the MFC were performed using commercial software CFD-ACE+ (CFD Research Corp., Huntsville, AL). The electric module based on Gauss's Law was used.