Sensors and Actuators A 172 (2011) 54–60

Contents lists available at ScienceDirect

Sensors and Actuators A: Physical

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

Micro and nano-platforms for biological cell analysis

W.E. Svendsen*, J. Castillo-León, J.M. Lange, L. Sasso, M.H. Olsen, M. Abaddi, L. Andresen, S. Levinsen, P. Shah, I. Vedarethinam, M. Dimaki

DTU Nanotech, Technical University of Denmark, Building 345 East, 2800 Kgs, Lyngby, Denmark

ARTICLE INFO

Article history:
Available online 30 March 2011

Keywords:
Cell culturing
Cell sorting
Nanoelectrodes
Peptide nanotubes

ABSTRACT

In this paper some technological platforms developed for biological cell analysis will be presented and compared to existing systems. In brief, we present a novel micro cell culture chamber based on diffusion feeding of cells, into which cells can be introduced and extracted after culturing using normal pipettes, thus making it readily usable for clinical laboratories. To enhance the functionality of such a chamber we have been investigating the use of active or passive 3D surface modifications. Active modifications involve miniature electrodes able to record electrical or electrochemical signals from the cells, while passive modifications involve the presence of a peptide nanotube based scaffold for the cell culturing that mimics the *in vivo* environment. Two applications involving fluorescent in situ hybridization (FISH) analysis and cancer cell sorting are presented, as examples of further analysis that can be done after cell culturing. A platform able to automate the entire process from cell culturing to cell analysis by means of simple plug and play of various self-contained, individually fabricated modules is finally described.

1. Introduction

In vitro cellular analysis has paved the way to major breakthroughs in the history of biology and drug discovery. However, no matter how good *in vitro* experiments are, they will never be able to represent the *in vivo* situation fully. But since *in vivo* experiments are expensive and sometimes unethical, researchers strive towards developing modifications of lab-on-a-chip systems so that they better mimic the environment experienced by cells inside the body. Micro- and nanotechnology are two very promising technologies for achieving *in vivo* like conditions. A recent example is the mimicking of the human kidney using microfluidic chambers [1]. In this paper we will illustrate the advantage of microfluidic handling of cells in combination with nano- and microsensing platforms.

For cellular analysis it is often necessary to culture the cells. The standard methods used for this, e.g. culture flasks, do not in any way represent the *in vivo* situation. Here we will demonstrate a microfluidic culturing system for adherent and non adherent cells, with controlled environment and diffusion based feeding, but without flushing away important signalling chemicals. Then a discussion of the surface of the culturing system is given, where a novel 3D nanopattern method is investigated to mimic *in vivo* conditions, where no smooth glass or plastic surfaces exist.

Probing intercellular dynamics and intercellular signalling is at the research frontiers for understanding cellular function. Therefore, new technologies to address these issues are developing fast. It has been demonstrated that cells can survive penetration by nanoscale structures [2]. The obvious next step is to utilize this fact for probing the dynamics of the interior of living cells. Novel methods for fabricating 3D micro- and nanoelectrodes for this purpose will be presented.

Sorting of different types of cells is also an interesting research area and one that has received a fair amount of attention. Several methods have been used, both physical, based on cell dimensions alone, e.g. bumper arrays [3], biological, based on functionalizing substrates with proper markers binding on specific cell types [4], and electrical, based on differences in the dielectric properties of cells, e.g. dielectrophoresis [5] or electrorotation [6]. We here present the use of dielectrophoresis in order to sort cells expressing stress markers on their surfaces from cells without stress markers.

As a final point, the concept of a microfluidic motherboard, equipped with valves, simple microfluidic devices, e.g. mixers, and sockets for accepting microfluidic chips for specific applications will be presented. This motherboard can by plug-and-play integrate any chip that fits a standard general specification, irrespective of material and fabrication process.

2. Cell culture microfluidic bioreactor for adherent and non-adherent cells

Microfluidics enables cell culturing mimicking *in vivo* conditions [7,8], i.e. physical, biochemical and physicochemical properties such as pH, gas concentration and temperature, unlike the conventional culturing methods. Chemical gradients represent a very

* Corresponding author.
E-mail address: winnie.svendsen@nanotech.dtu.dk (W.E. Svendsen).

0924-4247/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.sna.2011.02.027

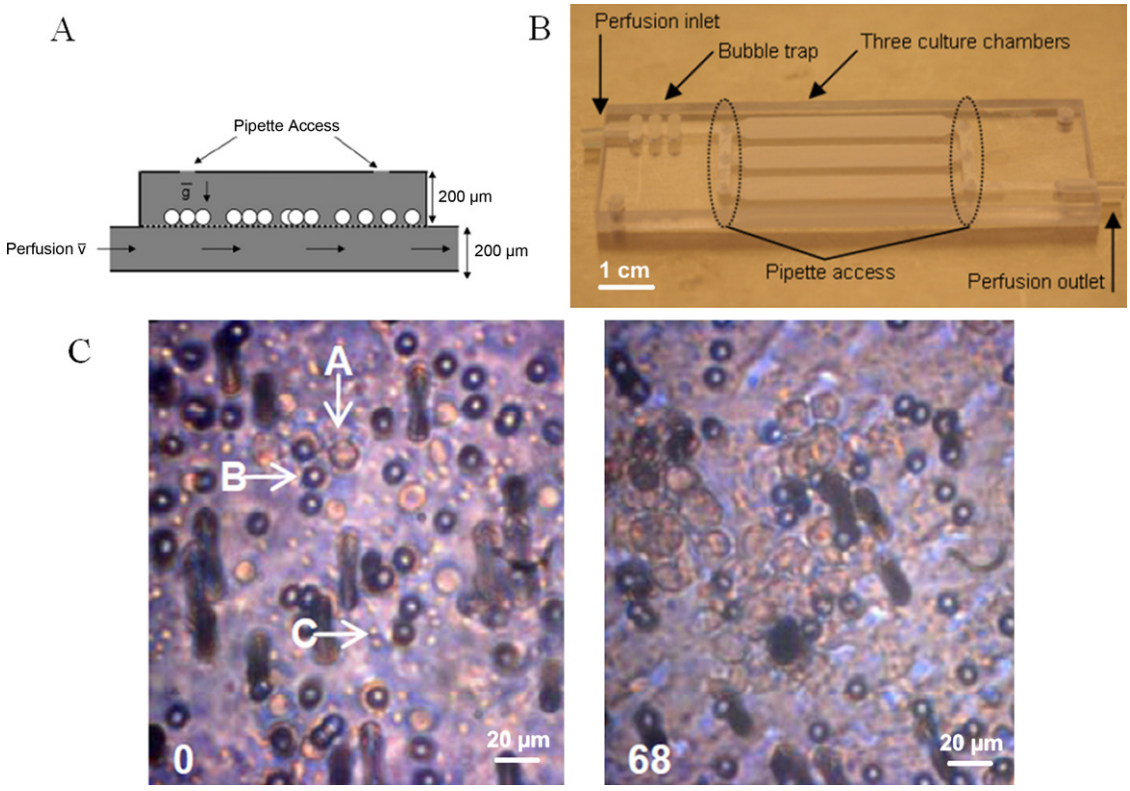

Fig. 1. (A) A schematic of the membrane reactor with pipetting access holes. (B) The membrane reactor with three chambers and microfluidic inlets and outlets. (C) The lymphocytes resting on the membrane at start, i.e. 0 h (left) and after 68 h of post activation with phytohemagglutinin (right). Just after loading into the chamber, the lymphocytes indicated by arrow A are small with minimum cytoplasm, only slightly larger than the pores shown at arrow B. A number of platelets can also be observed at arrow C. After 68 h the platelets have disappeared and the lymphocytes have expanded their cytoplasm and are now growing into aggregates.

important factor that regulates the cell's function [9]. These gradients are obtained in the cell microenvironment by diffusion. This can easily be adapted in a microfluidic setup by adjusting the geometrical conditions of the chamber, which also helps to stabilize the gradients in the microenvironment [10,11]. A microfluidic system featuring continuous perfusion helps provide fresh media as culturing progresses and the components are metabolized. By performing cell culture in the laminar flow regime offered in microfluidic systems, precise control of pH, oxygen, nutrition and temperature can be obtained [12].

The majority of micro cell culture reactors are designed for adherent cells [13–17], as these are the dominant cell types used in biological studies. The handling and perfusion of fresh media for culturing of such cell types is much easier. Here a cell culturing microfluidic reactor is presented, where both adherent and non adherent cells can be cultured, maintaining precise control of pH, oxygen, nutrition and temperature, and sustaining the biochemical microenvironment of the cells, while supplying nutrients to the cells by diffusion controlled processes.

The membrane cell culturing bioreactor is illustrated in Fig. 1A and B. It is composed of two chambers: an upper cell culturing chamber and a lower perfusion chamber. Both are fabricated using micromilling and are bonded together, with a 7 μm thick polycarbonate membrane with a porosity of 0.14 and pore size of 5 μm sandwiched between them. The cells are loaded and kept in the upper chamber by pipetting or microfluidic inlet. The medium is perfused in the bottom chamber and the cells are fed by diffusion through a micromembrane. In Fig. 1C the culturing of lymphocytes is illustrated. In this fashion it is possible to monitor the effects of drugs on cells continuously.

There are several advantages of the membrane based cell culture chamber. Firstly, perfusion can immediately be started without waiting for the loaded cells to settle. Secondly, the cells can be protected from air bubbles that will inevitably arise in the microfluidic channels, as the flow is not directly in contact with the cells. Thirdly, the cells can be protected from any kind of agitation, as the perfusion chamber and culture chamber are separately controlled.

To illustrate the usefulness of the chamber, a full protocol for fluorescent in situ hybridization (FISH) was carried out, mapping chromosomes translocations. The results were compared with those achieved by applying the standard protocol on microscope slides, as is the case for a routine FISH analysis, as shown in Fig. 2. No striking differences between the fluorescent images in the two cases were observed.

3. Self-assembled 3D nanowire structure pattern for cellular growth and sensing applications

Cell and tissue cultures have traditionally been done in 2D flat plastic or glass surfaces which are far from the topological representations of the more complex 3D environment found in the extracellular matrix. The suggestion that 3D porous nanotopographies that mimic physiological conditions represent a more accurate approximation for the growth of cells and tissue used in a long list of studies (e.g. cell migration and adherence, signal transduction) and biomedical applications (e.g. drug assays, regenerative medicine) has been supported by recent studies [18,19].

Epitaxial gallium phosphide nanowire arrays were used in cellular force and cell survival investigations [2,20–23]. These studies demonstrated that cell adhesion and survival was better on nanowire arrays than on planar substrates. However, the toxic nature of the substrate material used for the fabrication of the nanowires is a big limitation for the use of these nanostructures in biomedical applications or in *in vivo* studies [24,25].