Talanta 145 (2015) 60–65

Contents lists available at ScienceDirect

Talanta

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

Paper-based device for separation and cultivation of single microalga

Chih-Chung Chen [a], Yi-Ju Liu [b], Da-Jeng Yao [a,*]

[a] Institute of NanoEngineering and MicroSystems, National Tsing Hua University, Hsinchu 300, Taiwan, ROC
[b] The Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu 300, Taiwan, ROC

ARTICLE INFO

Article history:
Received 21 January 2015
Received in revised form
10 March 2015
Accepted 6 April 2015
Available online 15 April 2015

Keywords:
Paper-based device for separation
Single-cell separation
Microalgae isolation
Laser printer

ABSTRACT

Single-cell separation is among the most useful techniques in biochemical research, diagnosis and various industrial applications. Microalgae species have great economic importance as industrial raw materials. Microalgae species collected from environment are typically a mixed and heterogeneous population of species that must be isolated and purified for examination and further application. Conventional methods, such as serial dilution and a streaking-plate method, are intensive of labor and inefficient. We developed a paper-based device for separation and cultivation of single microalga. The fabrication was simply conducted with a common laser printer and required only a few minutes without lithographic instruments and clean-room. The driving force of the paper device was simple capillarity without a complicated pump connection that is part of most devices for microfluidics. The open-structure design of the paper device makes it operable with a common laboratory micropipette for sample transfer and manipulation with a naked eye or adaptable to a robotic system with functionality of high-throughput retrieval and analysis. The efficiency of isolating a single cell from mixed microalgae species is seven times as great as with a conventional method involving serial dilution. The paper device can serve also as an incubator for microalgae growth on simply rinsing the paper with a growth medium. Many applications such as highly expressed cell selection and various single-cell analysis would be applicable.

1. Introduction

The ability to separate a single cell from a mixed heterogeneous cell population is among the most useful techniques for biochemical research, such as purification of stem cells, recombinant protein or antibody-producing clonal-cell screening, and selection of specific microalgae and microorganisms. The chemical components extracted from microalgae are valuable, and are widely applied in the fields of pharmaceuticals, cosmetics, functional foods and biofuels [1,2]. For example, microalgae can be cultivated to produce polyunsaturated fatty-acid oils, which are nutritional supplements of infant formulae [3,4]; microalgal carbohydrates serve as a source of carbon in the fermentation industry [5,6]; lipids extracted from microalgal biomass can provide a feedstock for biodiesel [1,7]. Varied proteins and pigments extracted from microalgae have been applied in the pharmaceutical industry [1,8]. The production of these microalgal chemicals and bioactive compounds typically requires a monoculture of specific microalgae species, for which the isolation of a single microalgae species is critically important. Microalgae species collected from the environment are typically a mixed population and species that must be isolated. Traditional methods of microalgae separation, such as serial dilution and a streaking-plate method, are both inefficient and intensive of labor.

Numerous microfluidic devices based on magnetic [9], optical [10], electrical [11], droplet [12] and mechanical structure [13] have been applied for single-cell separation [14]. Although these approaches show promising results, the challenges of stable flow control, complicated and expensive equipment required and a limited dynamic sorting range still exist in most devices for the separation of a single cell from mixed biological samples. As such, an easily handled and affordable device enabling the separation of a single cell is in high demand. The most direct method to isolate individual cells is mechanical separation within physical boundaries. A conventional plastic multi-well plate is widely used for parallel analysis of multiple samples in biochemical laboratories. A straightforward method to miniaturize a multi-well plate to a microwell structure is expected to increase its efficiency enormously because of the smaller scale. Many microwell-based devices are reported [14]. Materials such as glass, silicon, PDMS, SU8 and PEG are used to fabricate microwell devices for cell separation and analysis, but most fabrications require clean-room and photolithographic techniques, resulting in large cost and time consumption. Moreover, the driving force to isolate cells for most microwell

* Corresponding author.
E-mail address: djyao@mx.nthu.edu.tw (D.-J. Yao).

http://dx.doi.org/10.1016/j.talanta.2015.04.020
0039-9140/

devices is gravity, which increases the duration for cell separation and possibly causes cell damage due to the long process.

Paper has become utilized as a substrate to establish microfluidic devices for use in rapid diagnostic tests [15,16]. Paper-based devices enable the advantages of easy fabrication, disposable nature and least cost. Patterning paper into regions of hydrophilic channel created by hydrophobic barriers provides basic capabilities of the distribution of a sample by capillary action along the axes *X* and *Y* on the paper without external forces such as pumps. Most paper-based channels and patterning are made on printing hydrophobic paraffin wax onto paper. Here we report a microwell-patterning paper-based device for the application of separation of single cells on creating a regional capillary along the *Z*-axis of the paper-based device. Using a general laser printer to create a carbon-powder pattern on the filter paper, we created a microwell pattern area of which the uncovered carbon powder enables a flow rate greater than that where covered with carbon powder along the *Z*-axis of the filter; the flow can carry and localize cells into the well area separately (Fig. 1A and B). The fabrication is simply conducted with a common laser printer and takes only a few minutes. The open-structure design of the paper device makes it operable with a common laboratory micropipette for sample transfer and manipulation with the naked eye. The paper device can also function as an incubator for microalgae growth.

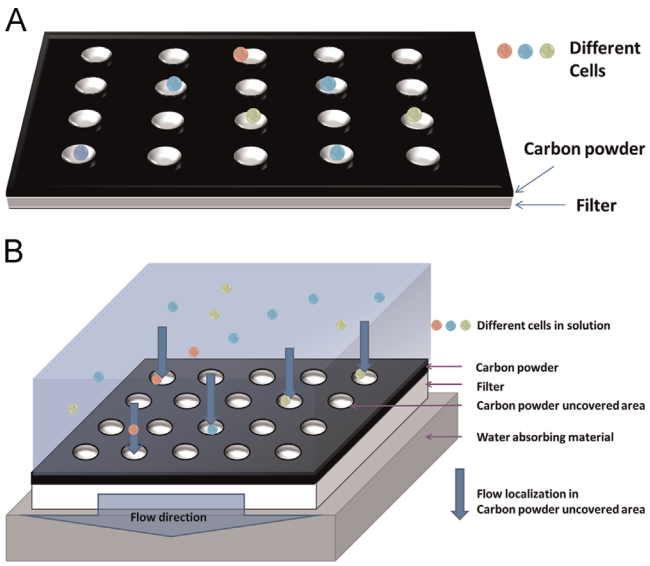

Fig. 1. Schematic illustration and operational principle of a paper-based device to separate single cell. (A) With a laser printer to create a pattern of carbon powder on filter paper, (B) by creating a container structure for sample solution loading and setting absorbing material under the paper device, the well area without covering of carbon powder is capable of a higher flow rate, which can carry the cell to the well area by capillarity.

2. Material and methods

2.1. Fabrication and operation

The microfluidic device was fabricated with a laser printer to create a carbon powder pattern of microwells on filter paper. The dish filter paper (3 μm pores, Adventech filter paper) was laminated with a general A4 paper and printed with a laser printer (Fig. 2A). The diameter of each microwell was 500 μm; the distance of each well was 1 mm. 96 microwells were designed as a unit to separate microalgae. The open-structure design of the paper device and the scale make it operable with a common laboratory micropipette manipulation with the naked eye (Fig. 2C). The paper device was sterilized with ethanol (70%) before use. Silicone was used to create a container on the paper device for sample loading (Fig. 2B). Mixed microalgae species (total volume 3 mL) at varied concentrations were prepared (descripted in Section 2.2) and loaded onto the paper device for separation. An absorbing material was placed under the paper device to create a capillary flow through the well

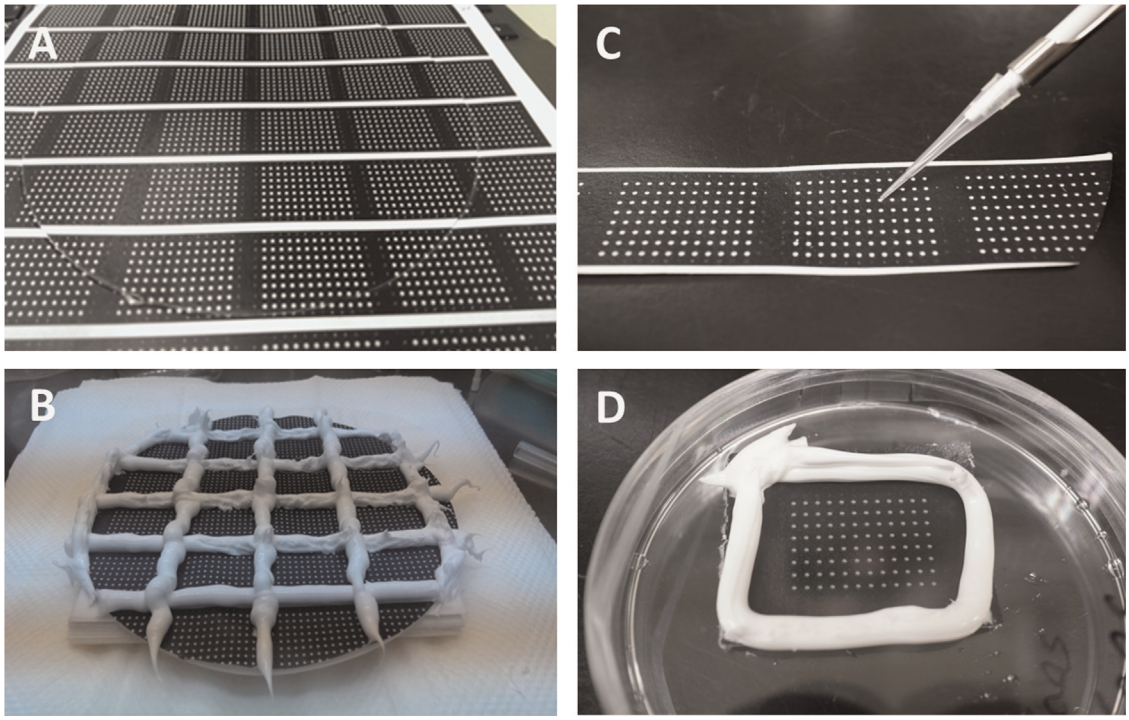

Fig. 2. Fabrication and operation: (A) The filter paper was laminated to general A4 paper and printed with a laser printer. (B) Using silicone to create a container structure for sample loading and setting absorbing material under the paper device. (C) The open-structure paper device can be operated with a common laboratory micropipette. (D) The paper device works as an incubator on rinsing the paper with a growth medium.