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MEMS-based Cell Manipulation System for Cellomics

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This paper, among the various applications of microfabrication techniques for cellomics, focuses on single cell manipulation system and cell separation system which are widely required in cell biology. Two systems use AC electrokinetics-electroation and dielectrophoresis-, which is versatile and biologically noninvasive force and can be easily integrated with other manipulation technique. For single cell manipulation, an integrated cell processor is designed to carry out the routine and cumbersome, but essential process for biomanipulation. It includes microfludic channels for transporting, PPy valves for isolation, electrorotation for orientation, and aspiration through microhole for immobilization. Compact and cost effective fabrication process for the presented device was considered. The integrated functions are successfully demonstrated with mouse (B6CBA) embryo cells. For cell separation system, microfludics and dielctrophoreitic force are anlayzed to increase the sensitivity about cells. Based on analytic and numerical solution, 3D asymmetric microelectrode system for cell separation was designed. Using multi-physics simulation and experimental studies with biological samples, the efficiency of performance is verified and tested. The techniques developed in this paper will provide an automated platform for manipulating a single cell without losing the viability of the cell and can be used to separate the specific cells, like a stem cell, which is difficult to be discriminated in typical method.

I. Introduction

After the human genome project finished faster than the predicted time, the biological paradigm is being shifted rapidly. Researchers are interested in post-genomic area, such as postgenomics, proteomics, and cellomics. Among them, recently, the cellomics have attracted many researchers. In addition, since microfabrication technology offers the opportunities to cellomics area, which the typical bulky macrosystem can not offer easily, such as cell samping and separation, culture devices, highthroughput drug screening tools, single cell manipulation and analysis, fundamental cell biological studies, and tissue engineering, MEMS-based microsystem has attracted increased attention.

Ⅱ. Cell Sampling and Separation

Modern methods in molecular biology, drug screening, diagnostics, and cell replacement therapy (CRT) often require the separation and analysis of cells. For examples, it has been estimated that 90% of the cost and 95% of the time needed to obtain molecular diagnostic data today is associated with sample separation, collection, and preparation (Gascoyne, 2004). The inability to effectively prepare sample is perhaps the major shortcoming in contemporary molecular analysis systems. Fig. 1 shows the block diagram showing the general sample preparation procedure for molecular diagnostics (Huang, 2002). Stem cell separation from the undifferentiated cells or other cells is urgently required in cell replacement therapy, of which market size is estimated as $30billion in 2010 (Bassett, 2003). MEMS-based devices have great potential to separate cells, especially valuable or rare cells like stem cells, since its downscaling can result in reduction of cells and reagent consumption as well as time of the operation (Müller, 2003; Voldman, 2001). Since, effective cell separation method has been required in biotechnology for a long time, there are a lot of researches about cell sampling and separation using MEMS techniques. They used various physical phenomenons, which can be implemented in microdevices, such as mechanical, electric, magnetic, acoustic, and light origin. The more detail examples of cell separation will be presented in conference site.

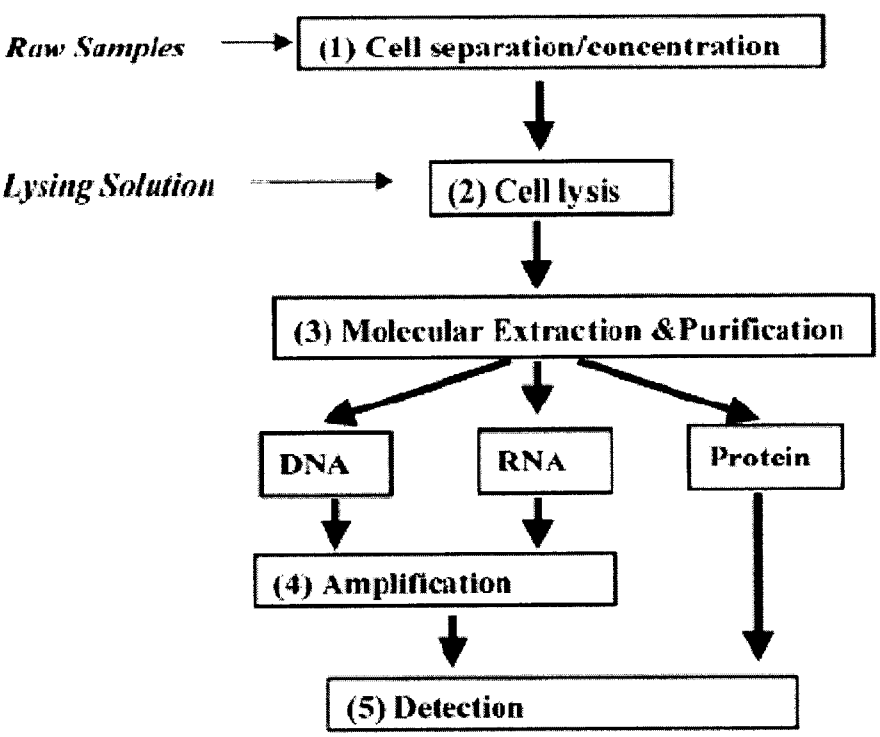

Fig. 1. Block diagram showing the general sample preparation procedure for molecular diagnostics (Huang, 2002).

Ⅲ. Single Cell Manipulation and Analysis

Recently, the characterization and manipulation of individual embryo cells has become a challenging issue in biomedical applications such as cloning, gene expression analysis, and cell replacement therapy (CRT) (Sun, 2002: Tan, 2001: Yanagida, 1998: Nakayama, 1998). Those techniques are essential in the agricultural industry (Glasgow, 2001), individual-cell-based diagnosis, and pharmaceutical testing (Jager, 2002). In spite of great interest in the manipulation of single cells, most cell manipulation tasks, such as gene injection, *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI), are carried out manually by a skilful operator using a microscope (Tan, 2001; Nakayama, 1998). Glasow designed and tested a microfludic system capable of transporting individual mouse embryos through a network of channels to selected locations (Glasgow, 2001). Arai presented 3D biomanipulation system for autonomous single cell manipulation, which integraed vision system, electrorotation, and micromanipulator (Arai, 1998). Chip-based patch clamping to understand the role of ion channels and to develop therapeutics was presented by Schmidt et al (Schmidt, 2000). Currently, there are several companies developing chip-based patch clamp solutions, such as Sophion Biosciences, Axon Instruments, Cytion, AVIVA Biosciences etc. Single cell manipulation and analysis using microdevices make it possible to perform analyses which other methods would not be possible due to system complexity.

This paper, among the various applications of microfabrication techniques for cellomics, focuses on single embryonic cell manipulation system and cell separation system which are widely required in cell biology. The goal of this study is design and analysis of effective and versatile MEMS-based cell manipulation system for these applications. I will also discussed the principle and analysis of dielectrophoreis and microfludics, which are basic physics used in this study, and how they are applied in our systems.

Ⅳ. Single Embryonic Cell Manipulation

Single mouse embryo manipulation using an integrated cell processor(Fig. 2) is described for effective and automated manipulation. Excellent experimental results with mouse (B6CBA) embryo cells showed that this device could substitute for routine and cumbersome manual work.

An MEMS-based integrated cell processor for single cell manipulation will be also introduced. The integrated processor can perform various functions such as cell transport, isolation, orientation, and immobilization. These functions are indispensable and frequently used for the manipulation of single cells, but can only be carried out by a skillful operator. The purpose of this study was the integration and automation of these functions for effective cell manipulation, using a MEMS approach. The isolation of a cell was performed using polypyrrole (PPy) valves in a microchannel into which cells were transported. The orientation of cells was controlled by electrorotation (ER), and the target cell was immobilized by suction from a microhole. All of these functions were seamlessly realized on a single chip. Excellent

experimental results with mouse (B6CBA) embryo cells showed that this device could substitute for routine and cumbersome manual work. It is expected that the integrated chip will contribute significantly to faster and more reliable manipulation of cells.

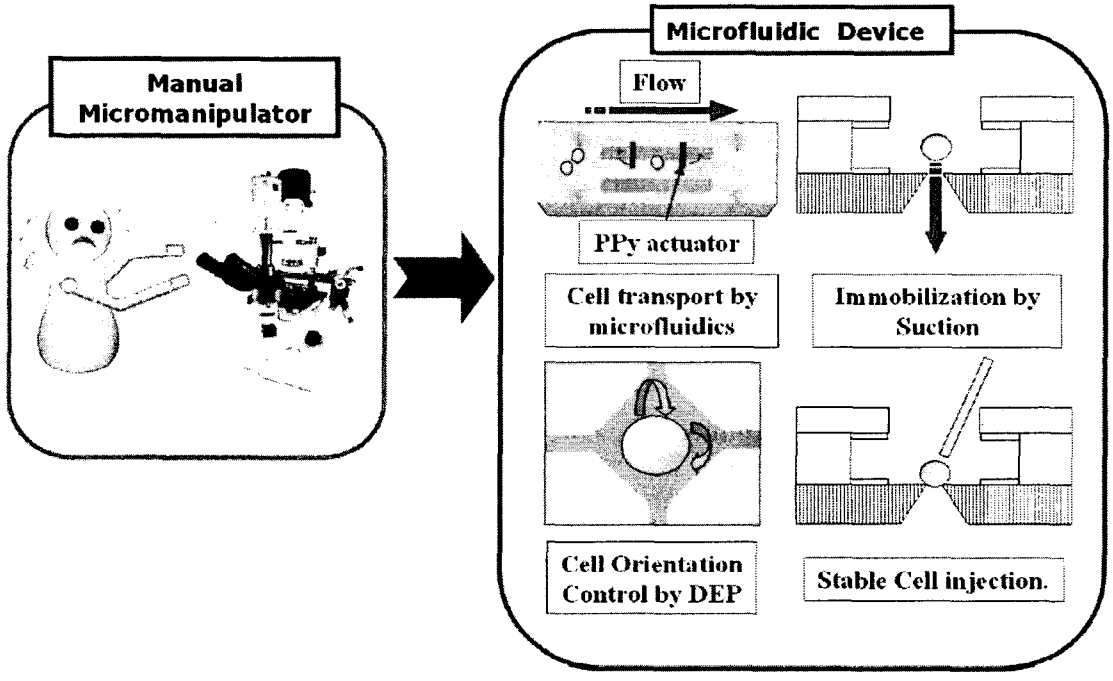

Fig. 2 The functions of the cell processor.

V. Cell Manipulation for Cell Replacement Therapy

Cell separation using a 3D asymmetric microelectrode system for enhancing the sensitivity about cells is examined. The theory and the state of the art in AC electrokinetics will be described that is the physical principle mainly used. The physics and versatility of this phenomenon is shortly introduced. Various previous researches using it in microsystem and their merit and limitations are explained.

MEMS-based devices have great potential to separate cells, especially valuable or rare cells like stem cells, since its downscaling can result in reduction of cells and reagent consumption as well as time of the operation. In this study, an optimized 3D-asymmeric microelectrodes have been designed, which are based on the analytic solution and numerical analysis for electrokinetics and microfluidics. In order to increase the discriminative power about cells, a continuous variable electric field from the 3D-asymmetric microelectrode is generated in the microchannel which is formed by isotropic wet etching on pyrex wafer. Since this electric field is varied according to the position in the microchannel, the force induced by the negative dielectrophoresis is also varied according to the position in the microchannel. Therefore, the movement of cells in the microchannel is influenced by the varied

dielectrophoretic force. Analytic solution about dielectrophoretic force according to the shape of the microchannel and the width of microelectrode is performed. 3D-asymmetric microelectrodes based on the analytic solution are designed. The effectiveness of the presented 3-D asymmetric microelectrode system compared to typical 3D-parallel microelectrode system which depends on the edge effect in the boundary in electrode was analyzed with numerical analysis. Based on numerical analysis for the 3Dasymmetric microelectrode, I proved the sensitivity to cells was significantly improved and the feasibility of this device was shown by experimental study (Fig. 3). Ultimately, the presented system has the potential to separate specific cells from mixed cells, which are difficult in typical method.

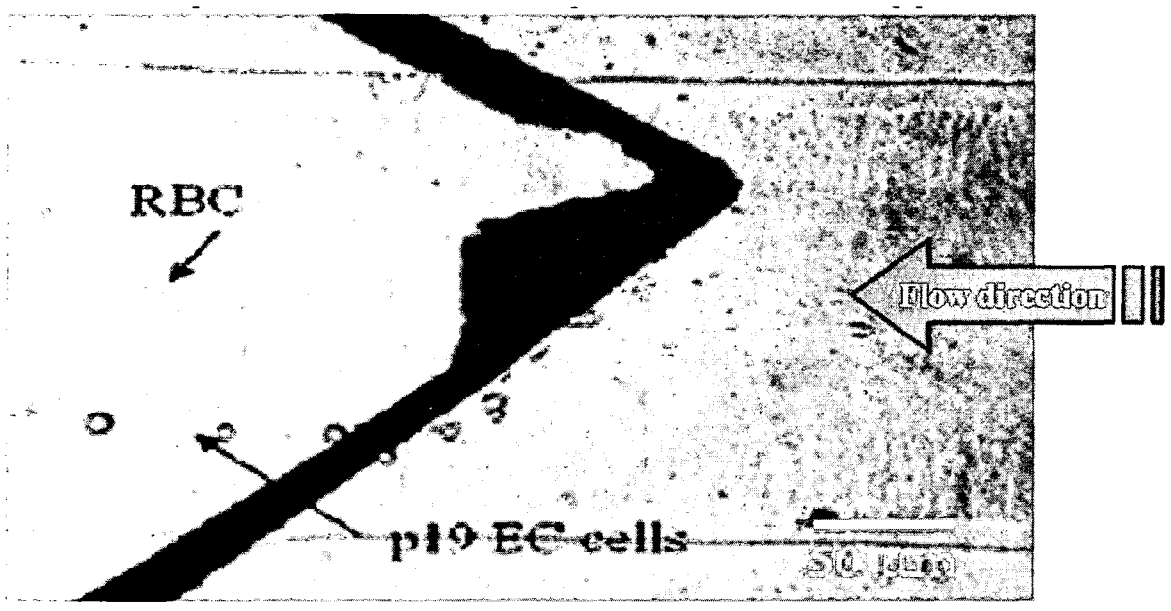

Fig. 3. Video clips showing the experimental results.

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