ASPEN

ASPEN 2017

The 7th International Conference of Asian Society for Precision Engineering and Nanotechnology Program

November 14 Tue.
- 17 Fri., 2017

COEX, Seoul, Korea

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Novemver 15 Wed.

Time	Paper ID	Title / Speaker / Affiliation

MEMS/NEMS — Room 3(E3)

Chair Takasi Nisisako(Tokyo institute of technology)

09:30~09:50	MEM-O-01	***Hands-off Particles Separation using a Deterministic Lateral Displacement Microfluidic Device*** Naotomo Tottori(Tokyo Institute of Technology), Takasi Nisisako(Tokyo Institute of Technology)
09:50~10:10	MEM-O-02	***A Soft and Disposable Step-emulsification Device for Generating Monodisperse Emulsions and Particles*** Seungman Choi(Tokyo Institute of Technology), Naotomo Tottori(Tokyo Institute of Technology), Rui Zhang(Tsinghua University), Takasi Nisisako(Tokyo Institute of Technology)
10:10~10:30	MEM-O-03	***Stage-top-incubator System for Mechanical Stimulation Real-time Monitoring of Growing Cells*** Yun-Jin Jeong(Chonnam National University), Dong-Weon Lee(Chonnam National University)

Micro/Nano Fabrication Processes 1 — Room 3(E3)

Chair Futoshi Iwata(Shizuoka University)
Gyu-Man Kim(Kyungpook National University)

13:50~14:10	MNF-O-01	***Topography Modeling of 3D Elliptical Vibration-assisted Micro-texture Turning*** Chen Zhang(Nanjing University of Aeronautics and Astronautics), Yun Song(Nanjing University of Aeronautics and Astronautics)
14:10~14:30	MNF-O-02	***Ultra-precision Machining of Greyscale Micro Image on Metal Surface Conprised of Inverted Piramid Features*** Rui Huang(National University of Singapore), Xinquan Zhang(Singapore Institute of Manufacturing Technology), A. Senthil Kumar(National University of Singapore), Weekeong Neo (National University of Singapore), Kui Liu(Singapore Institute of Manufacturing Technology)
14:30~14:50	MNF-O-03	***Effects of Stamp Geometry on Transfer-printing of Au Thin-film*** Atsushi Kawahata(Tokyo Metropolitan University), Kazuto Nagahashi(Tokyo Metropolitan University), Arata Kaneko(Tokyo Metropolitan University)

Stage-top-incubator system for mechanical stimulation real-time monitoring of growing cells

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KEYWORDS: Bioreactor, incubator, mechanical stimulation, cardiomyocytes.

In this research work, we have developed a small incubator which is capable of culturing the cells. The real time morphology and maturity level of the incubated growing cells can be directly monitored through the microscope. The developed stage top incubator system consists of a controller, a humidifier, and a 5% CO_2 gas cylinder. The internal humidity and CO_2 concentration of the incubator chamber are kept constant by injecting the 5% carbon dioxide gas. The CO_2 flow rate is controlled through the electromagnetic valve inside the controller into the incubator chamber through the humidifier. The incubator chamber is made of aluminum alloy with an excellent thermal conductivity and the inside temperature of the incubator is maintained constant by using the hot water circulation. The temperature sensor, a humidity sensor and a CO_2 sensor are integrated inside the incubator for monitoring the real time environmental conditions of the incubator. The sensors are controlled and monitored by using the GUI (graphical user interface, written in C language) program. The inside temperature distribution of the developed incubator is 37 ℃ ± 0.7 ℃ and the temperature stabilization time is ~60 min. The cardiomyocytes were cultured inside the stage top incubator system on the PDMS substrate with micro grooves structures. The morphology of the cells is observed and analyzed through the microscope and the findings are reveals that the cells are aligned in the micro groove direction while growing. The experimental results further demonstrates the real time cell culture feasibility of the developed stage top incubator system.

Manuscript received: August XX, 201X / Revised: August XX, 201X / Accepted: August XX, 201X

1. Introduction

Cell culture is a technique used for culture the cells which is secluded from the tissues of an organism under artificial conditions. Since the cells are ultra-sensitive to the surrounding environment, cell cultivation is carried out in an incubator that provides optimal conditions for cell growth such as temperature, humidity, and carbon dioxide concentration. Although the conventional incubators is an ideal device for cultivate cells, it cannot be used to observe the maturity levels of the cultured cells or tissues in real time. Also the conventional incubator required large-sized space for installation.

Recently, several research efforts have been devoted for matured cells using biomimetic technology to improve the inaccuracy due to the use of immature cells in accordance to disease modeling and drug toxicity test. In general the cells are experiencing various kinds of mechanical forces within the body. The concept of cell stretching by using biomimetic techniques is categorized according to various operating techniques involved in cell stretching.

Among the several methods to induce the cell stretching, piezoelectric micro and nano manipulation has received significant attention owing to its high accuracy, versatile controllable in strains, and high displacement resolution which provides an active tool for governing loads during cell stretching process. However, some piezoelectric actuators require direct physical contact with the cell, which limits its use to elongating the cell [1].

Electromagnetic actuators is an another sophisticated alternative to induce mechanical stress on the cell. In this method controlled electromagnetic motors have been used to achieve the desired stretch effect. The major drawback of this technique is heating and pollution from the lubrication. However, because of the relatively simple set-up of static and dynamic loads, such as high-precision and intuitive programming, cell elongation using electromagnetic actuators is an attractive feature [2].

Pneumatic actuators have been used extensively in vitro to induce mechanical stress or strain on cells. This operating concept has important advantages in avoiding contamination such as simple setup, homogenous deformation, and no direct contact with the cell or medium. Most devices using pneumatic actuators are based on deformation of thin membranes with controlled operating pressure. Cells are cultured directly on this membrane [3].

In this study, we developed a bioreactor which is capable of mechanical stimulation using a pneumatic actuator to the mature cells, and a stage top incubator capable of real-time analysis using a

microscope and automated control of cell culture conditions (temperature, humidity, and CO_2 concentration).

2. Design and fabrication

2.1 Bioreactor for mechanical stimulation

Figure 1 illustrates the schematic diagram of a bioreactor capable of mechanical stimulation using a pneumatic actuator. The Bioreactor consists of a cover, a top plate, a bottom plate, and a PDMS diaphragm structure made up of glass and polydimethylsiloxane (PDMS). The length, width, and thickness of the external dimension of the designed bioreactor is 35 mm × 35 mm × 12 mm and the cell growth area is 1.4 cm^2. The PDMS diaphragm structure is fabricated by forming a hole in a glass wafer using a sand blasting process and bonding a PDMS membrane using an O_2 plasma process. The thickness of the PDMS diaphragm is 10 μm and the diameter is 3 mm. One well was designed and fabricated to include four PDMS diaphragms. The fabricated bioreactor is deformed by applying a pressure using a pneumatic actuator and the deformation of the diaphragm also induces the cells stretching.

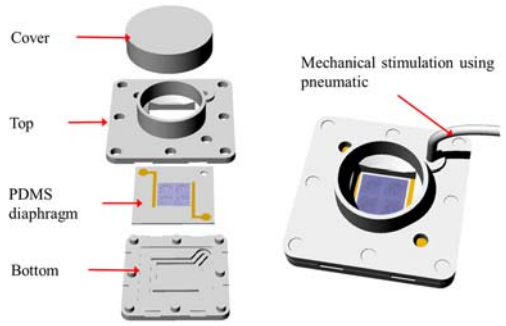

Fig. 1 Schematic of bioreactor capable of mechanical stimulation of cells using pneumatic

2.1.1 Stage top incubator

Figure 2 illustrates the schematic diagram of the fabricated stage top incubator. The designed stage top incubator consists of an incubator chamber, incubator controller, humidifier, water tank and CO_2 gas cylinder. Humidity and CO_2 concentration inside the incubator are kept constant by injecting the 5% carbon dioxide gas. The electromagnetic valve is used to regulated the CO_2 flow rate. The hot water inside the water tank is circulated through the incubator chamber using the pump to regulate the temperature inside the incubator. In order to effectively increase the temperature inside the incubator using hot water, the incubator chamber was made of aluminum alloy.

The real time environmental conditions inside the incubator is monitored by the temperature sensor, a humidity sensor and a CO_2 sensor which was integrated inside the incubator. The sensors are controlled and monitored by using the GUI (graphical user interface, written in C language) program. The internal temperature dissemination of the fabricated incubator is 37 ℃ ± 0.7 ℃ and the temperature steadying time is ~60 min (Fig. 3).

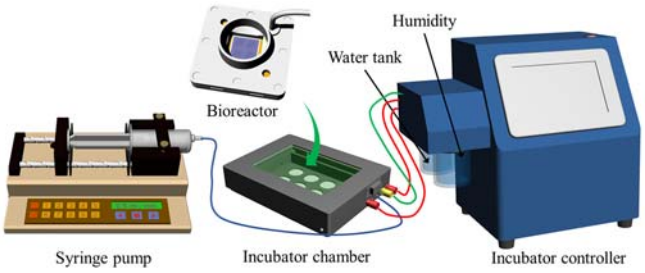

Fig. 2 Schematic of stage top incubator system

3. Conclusions

In this study, we developed a bioreactor which can be used for mechanical stimulation of cells by using pneumatic pressure, and a stage top incubator system capable of real time maturity level observation by using a microscope and an automatic control of cell culture environment. It is expected that cells will be able to mature through mechanical stimulation and can be used for real-time observation of cultured cells, so that they can be effectively used in disease modeling and drug screening applications.

ACKNOWLEDGEMENT

This research was supported by the Commercializations Promotion Agency for R&D Outcomes(COMPA) funded by the Ministry of Science, ICT and Future Planning(MSIP)(No.2016K000209) and the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIP) (No.2015R1A2A2A05001405).

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