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Polymer Cantilever Integrated with 3D Micro-well Structure and Electrical Stimulation for Enhanced Cardiac Toxicity Screening Application

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Key Words: Polymer cantilever, Micro pattern, Electrical stimulation, Cardiomyocyte

Abstract

This paper described a polymer cantilever device integrated with a 3D microwell structure and an electrical stimulation. By employing the proposed cantilever device, the number of cardiac cells required for the experiment was significantly reduced and the sensitivity of the cantilever sensor was increased about 2 times by optimizing the thickness using the well structure. The cantilever device consisted of two parallel microelectrodes and 3D micropatterns inside the well formed on the cantilever. The microelectrodes were utilized in order to maximize the efficient of maturation for cardiac cells. The optimized design with lower spring constant and less initial bending greatly improved cantilever displacement caused by the contraction force. The highly sensitive cantilever structure allows for precise analysis of changes in beating duration and contractility. After basic experiment, the fabricated cantilever device was successfully employed for monitoring mechanical response of cardiac cell treated with drugs. Human iPSC-driven cardiomyocytes were also employed to improve the accuracy of drug screening.

1. Introduction

The heart plays integral role in circulation blood throughout the body by repeated contraction and relaxation. Various studies have been conducted to identify the cause frequent occurrence of abnormal symptoms such as arrhythmia caused by drug side effects (1). Among those studies, the patch clamp assay is one of the good method that used for electrophysiological measurements.

The method assesses drug toxicity by measuring the changes in the transmembrane ions of cardiomyocytes. However this method is difficult to evaluate cardiac tissue a lot of cost and time in evaluation. In an effort to compensate these drawbacks, a number of methods to directly observe the physiology of the heart's contraction force have been proposed. However, further miniaturization and mass production are still difficult because the cantilevers are usually made from polydimethylsiloxane (PDMS) (2). On the other hand, the using of animal cells often causes a serious problem in screening result. In the present work, 3D micro well structure is patterned a top of the photo sensitive polymer based cantilever surface to minimize a unused cell number. Moreover, cantilever surface patterned two pair metal

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electrodes are provide a synchronize beating frequency and support a maturation of cardiac cell. Because a maturation of the cardiac cells on the cantilever is also important factor to improve the accuracy in drug screening.

2. Result

In order to improve the efficiency of cardiac cell experiments, we were proposed to use a microwell structure on the cantilever and employed various electrical stimulation to improve the maturation of cardiac cells.

Fig. 1 shows optical images of human induced pluripotent stem cell (hiPSc) grown on the cantilever with and without the well structure. In the Fig. 1a, cultured cells are spreading outside of cantilever surface. In contrast, with micro well structured cantilever surface (Fig. 1b), proposed number of cells are growing inside of well structure.

Moreover, the cantilever bending displacement is greatly increased. This enhances support a sensitivity of the cantilevers to study cardiac drug toxicity. In further, the hiPSc can be culturing long-term and involving experimentally induced changes in gene and protein expression.

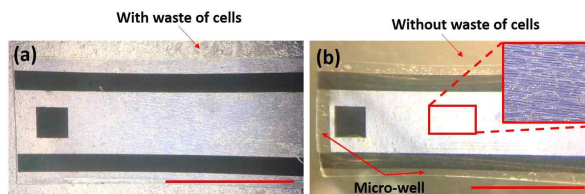


Figure 1. hiPSc growing on the different cantilever surface. (a) Conventional and (b) micro well structured cantilever surface, (scale bar: 2 mm).

As shown in Fig. 2 human induced pluripotent stem cell utilized to study toxicity with various concentration of Verapamil. Verapamil is the L-type calcium channel blocker that is decreases the contraction force as a function of the drug concentration. The experiment result shown that hiPSc affected by various concentration of drug and

decreased contraction force. The hiPSc experiment has a potential for patient specified drug screening application.

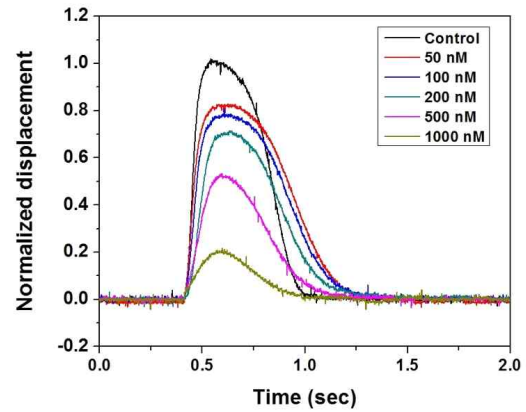


Figure 2. hiPSc drug toxicity result of Verapamil

In this research, a 3D micro well structure is fabricated on the cantilever surface to decrease of hiPSc cell consumption. The rectangular two metal electrodes are utilized to apply electrical stimulation to provide cell maturation after long term electrical stimulation. In further, the real time monitoring of the response of cardiac muscle cells to Verapamil. The proposed 3D micro well structured polymer cantilever system has well capability to monitoring the mechanical changes of cardiac cells.

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