ELECTROPHYSIOLOGICAL RECORDINGS USING SPATIALLY ARRANGED MICROELECTRODE PROBES EMBEDDED INTO 3–D NEURONAL CULTURES

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ABSTRACT

This paper presents spatially arranged microelectrode probes to allow electrophysiological recordings inside 3–D neuronal cultures. Out-of-plane microelectrode probes standing on a substrate have gradation in height. Wire-bonding–based probe technology [1] makes it possible to provide the flexible probes. We would understand the dependent alteration of cellular activities caused by the construction process of 3–D artificial neuronal networks, because the developed probes can directly and spatially record cellular activities inside 3–D cell cultures. This paper demonstrates electrophysiological activities inside 3–D neuronal cultures could be successfully recorded using the embedded probes.

KEYWORDS: 3–D cell culture, Spatially arranged microelectrode probes, Wire–bonding–based probe technologies

INTRODUCTION

Characterizations of three–dimensional (3–D) in vitro cell cultures have been performed using microdevices and Lab-on–a–chip systems in recent years [1]. Compared to the traditional monolayer cultures, the 3–D cultures have several advantages such as: closer approximation to biological tissues, higher complexity, better physical properties and increased cell survival. Studies that would determine the difference of cell function between 2–D and 3–D environments would be of great benefit to biomedical engineering efforts.

This study focuses on spatial and low–invasive extracellular recordings inside 3–D artificial neuronal networks. J.J. Hickman et al. reported electrophysiological characterizations of hippocampal neurons cultured in a 3–D collagen hydrogel [2]. These characterizations using patch–clamp technique has some drawbacks as follows: (i) cell damage during the penetration of glass electrode into the gel, (ii) low–throughput and (iii) tendency of the gel to float. To overcome these issues, we propose a novel microelectrode probe arrays with a suction hole for gel clamping. The out-of–plane microelectrodes are embedded into 3–D neuronal cultures toward low–invasiveness and high–throughput recordings.

In this paper, spatially arranged microelectrode probes to allow electrophysiological recordings inside 3–D neuronal cultures are described along with the design and fabrication using our wire–bonding–based probe technology. Successful extracellular recordings inside 3–D cultures by the developed microelectrode probes is also reported.

FABRICATION

Spatially arranged microelectrode probes embedded into 3–D neuronal cultures are illustrated in Fig. 1. We have reported the fabrication process of spatially arranged microelectrode probes utilizing wire–bonding–based probe technology for spatially distributed chemical sensing [3]. A upper structure consists of a suction hole (Φ 30 µm), a suction channel and planar microelectrode arrays. A lower structure is a silicon chip coated by parylene–C. After the upper and lower structures are bonded, spatially arranged microelectrodes are formed by wire–bonding–based probe technology. Au wires coated insulator are converted to out–of–plane probes by cutting the bridge of wire using a laser machining. 4 × 4 isolated probes are stood successfully on planar microelectrode arrays (see Fig. 2 (a)). The difference of dummy substrates thickness and laser cutting position provides various heights of probes. The tip of Au wire (Φ 25 µm) coated parylene–C as an insulator was exposed by the laser cutting (see Fig. 2 (b)).

It is important to correspond recorded data to 3–D position of each microelectrode tip for spatial sensing. The 3–D position coordinates of each probe tip was measured by using a digital microscope (KH–7700, HiROX Corp.) in order to calibrate recorded data. The digital microscope was equipped with a 3–D surface analysis soft (SurfLab/KH, Mitani Corp.) and XYZ–electric driven stage (SHX–100, HiROX Corp.). The 3–D graphical representation based on the measured position coordinates of microelectrode tips was shown in Fig. 3 (b). The suction hole in the center of device was defined as the origin of coordinates. The highest and lowest heights of fabricated microelectrodes were 560 µm and 200 µm, respectively. The height of probe is limited by the thickness of dummy substrates ( Z ≤ tD + hW ) as shown in Fig. 3 (a). The thickness of dummy substrates was 625 µm in this work.

Figure 1: A schematic drawing of electrophysiological recordings using spatially arranged microelectrode probes embedded into 3–D neuronal cultures.
RESULTS AND DISCUSSION

We employed primary neurons which were obtained through enzymatic dissociation of rat fetal cerebral hemisphere tissue. The collagen–cell suspension (cell density: 350,000–500,000 cells/ml) was dispensed onto the spatially arranged microelectrode probes (see Fig. 4 (a)), and placed in an incubator at 37 °C and 5 % CO₂ for 45 min in order to allow gel formation for 3-D cultures (see Fig. 4 (b)). Finally, culture medium was poured onto the gel (see Fig. 4 (c)).

Milli–Mark™ FluoroPan Neuronal Marker was used to stain all parts of cultured neurons. The 3-D cultures were fixed with 4 % paraformaldehyde in PBS for 30 min at room temperature (RT). After washing with PBS, the 3-D cultures were blocked with blocking buffer (1 % BSA, 5 % Serum, 0.2 % Triton X in PBS) for 1 h at RT. Consequently, the 3-D cultures were incubated with the neuronal marker (Mouse IgG conjugated with Alexa 488) for 2 h at RT. After rinsing with PBS, the 3-D cultures were photographed using a confocal microscope. As shown in Fig. 5, most neuronal processes grew out of the plane of the cell body and extended into the collagen matrix after 9 days of culture.

Figure 4: Collagen gel embedded culture method on spatially arranged microelectrode probes: (a) Dispensing of collagen–cell suspension, (b) Incubation for gel formation, (c) 3-D cultures embedded the microelectrode probes.

Figure 5: A confocal image of 3-D cultured neurons entrapped in a collagen gel after 9 days of culture.

Figure 6: Electrophysiological recording systems of the probes embedded into 3-D cultured neurons.

Reference (Ag/AgCl)
Head amp.
Extraction electrode
Warm plate
Culture chamber
Embedded probes
20 mm

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Figure 6 shows extracellular recording systems composed of the microelectrode probes embedded into the 3-D cultures and 2 ch patch–clamp amplifier (gain: 20 k, band–path filtering: 10 Hz–5 kHz, sampling rate: 40 kHz). The highest (channel 1: 560 µm) and lowest (channel 2: 200 µm) microelectrode probes could successfully record spontaneous spike potentials of cultured neurons entrapped in the gel during 28 days of culture, as shown in Fig. 7.

We could observe that electrophysiological properties of the firing frequency and signal intensity depending on the process of 3-D neuronal culture's growth. Our results indicated that most of the electrophysiological characteristics of the 3-D culture neurons were similar to those of traditional 2-D cultures using MEAs (Microelectrode arrays), especially the activities temporarily calm down after 14 days of culture.
CONCLUSION

Embedded spatially arranged microelectrode probes to allow electrophysiological monitoring of a growth process inside 3–D neuronal cultures was described in this paper. Wire–bonding–based probe technology made it possible to provide out–of–plane flexible microelectrodes. The tip of each metal wire ($\Phi$ 25 µm) coated parylene–C as an insulator was exposed by the laser cutting. The highest and lowest height of fabricated microelectrode probes could be obtained 560 µm and 200 µm, respectively. The differences of dummy substrate thickness and laser cutting position provided various heights of structures for a spatial microelectrode.

In extracellular recordings of 3–D cultures which was more likely to mimic physiological tissue environments, spatially arranged microelectrode probes embedded into the 3–D cultures could successfully record spontaneous spike potentials of neurons entrapped in the gel during 28 days of culture. Our results indicated that most of the electrophysiological characteristics of the 3–D culture neurons were similar to those of traditional 2–D cultures, especially the activities temporarily calm down after 14 days of culture.

Given the results described here, further electrophysiological characterizations of 3–D neuronal cultures using our spatially arranged microelectrode probes are underway toward bioengineering applications such as: disease models for the study of regeneration and repair of damaged nervous systems, specifically Alzheimer's disease.

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REFERENCES


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