TITLE: Fabricating Neural and Cardiomyogenic Stem Cell Structures by a Novel Rapid Prototyping--the Inkjet Printing Method

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Fabricating Neural and Cardiomyogenic Stem Cell Structures by a Novel Rapid Prototyping--the Inkjet Printing Method

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ABSTRACT

Direct printing of living cardiomyogenic stem cells and embryonic cortical neurons to generate complex cellular patterns and structures of such cells was demonstrated in the study. Furthermore, the immunostaining analyses and the whole-cell patch clamp recordings showed the cortical neurons grown in the printed cellular patterns and structures maintained their basic cellular functions, including neuronal phenotypes and electrophysiological properties. These results and findings may greatly prompt the inkjet printing method evolving into a viable and cost-effective approach for engineering human neural and cardiac tissues or even organs.

INTRODUCTION

Rapid fabrication of tissue or organ analogs with well-defined structures and functions is a main challenge in regenerative medicine. In particular, there is an urgent need to produce neural and cardiac cell-based structures for dealing with serious clinical conditions of neural and cardiac systems, such as spinal cord injury, Parkinson’s disease, and congestive heart failure, in which functional neural or cardiac cells are often lost or damaged within the nervous or cardiac system, respectively [1, 2]. Since most of neural cells and cardiac myocytes have a very limited ability to regenerate, currently one promising therapeutic strategy is the implantation of healthy, in vitro-grown neural or muscle tissues fabricated with vital cells, including exogenous neural and cardiac cells and their progenitor and stem cells[3]. To perform the ambitious task, rapid tissue fabrication techniques are needed to produce functional neural and cardiac tissues.

Recently we have developed a novel rapid prototyping, the inkjet printing method to readily manipulate living cells and biomaterials[4, 5]. Furthermore, an effective bottom-up strategy to generate 3D tissues has been proposed by printing alternate layers of cells and biomaterials into their exact positions thus assembling anatomical structures[6]. Before this strategy can be used the assemble functional neural and cardiac tissues, studies to assess the influence of the printing process on the cells, including the phenotypes and functions of printed neural and cardiac cells must be conducted. Although the possibility of inkjet printing of neurons and the viability of printed cells have been verified [4], whether the neurons after being printed still maintain their neuronal phenotypes and electrophysiological functions is not clear yet. Moreover, direct printing of cardiomyogenic stem cells into a specific pattern has not been studied. All of those issues will be discussed in this study.
EXPERIMENTAL DETAILS

Embryonic rat cortical cells were obtained through enzymatic dispersion of day 18 fetal tissue from pregnant Sprague-Dawley rats as previously described [7]. QCE6 cell line was obtained as a gift from C. Eisenberg and cultured with the use of the previously described method.[7] The QCE-6 cell line has been transfected to express green fluorescent proteins. To prepare the cell print suspensions, the neurons and stem cells were resuspend into 3x PBS after they were collected as cell pellets under centrifugation. The bio-papers used were prepared from rat-tail Type I collagen gels by using the previously reported protocol. [4]

HP Desktop 550 printers were modified to accommodate the delivery of the living neurons and stem cells. A pattern that consisted of rows of circles was designed using Microsoft PowerPoint software. Moreover, a black gradient pattern was created for printing QCE-6 cell density gradient.

The cell print suspensions were loaded into the cartridge and the bio-papers were placed onto a printing stage. The cells were delivered onto the bio-paper following the designed pattern. After printing, the bio-papers with the printed cells were placed into dishes and moved to the standard cell culture incubator. The samples printed with cortical neurons were fixed with 4% formaldehyde. After blocking printed cortical cells on the collagen gels were exposed to the primary antibody of mouse monoclonal anti-MAP2 (Chemicon, Temecula, CA). Fluorescence-labeled secondary antibodies were applied. The stained samples were photographed using Zeiss LSM-5 confocal microscope.

Voltage clamp and current clamp experiments were performed with a Multiclamp 700A (Axon, Union City, CA) amplifier. Signals were filtered at 2 kHz and digitized at 20 kHz with an Axon Digidata 1322A interface. Sodium and potassium currents were measured in voltage clamp mode using voltage steps from -85 mV holding. Repetitive firing was measured with 1 s depolarizing current injections from -85 mV holding.

RESULTS AND DISCUSSION

Printed stem cell patterns

QCE-6 stem cells were derived from precardiac mesoderm of the Japanese quail and it was demonstrated the stem cell are able to differentiate into diverse cardiac cell type.[7] The ring patterns of QCE-6 stem cells were formed after printing, as shown in Figure 1A. The morphologies of printed stem cells within the ring pattern are seen in Figure 1B. Furthermore, microstructures of individual printed QCE-6 stem cells at high magnification were revealed by SEM images shown in Figure 1C. As shown in Figure 2, stem cell gradient patterns were produced by directly printing QCE-6 stem cells in a thin rectangle that fades in color continuously from black to white. On the low-density end of the gradient, few or no cells were seen, while on the high-density end, most cells in number accumulated and formed cell aggregates. From the left end to the right end of the pattern, the cell density decreased gradually with high fidelity to the pre-designed pattern.

Printed neuronal patterns and immunostaining

The completed ring of printed rat embryonic cortical was also formed, as shown in Figure 3A. After incubating with anti-MAP-2 antibodies, a neuron-specific marker, the processes and cell bodies of printed cortical neurons exhibited immuno-reactivity with the antibodies and emitted
green fluorescence under epi-fluorescent microscope, as shown in Figure 3B. The results strongly suggested the cortical neurons maintained their neuronal phenotypes after being printed.

Figure 1. (A) The QCE-6 cell ring was observed under a fluorescent microscope after 5 days of culture. (B) Fluorescent microscope image of morphologies of printed QCE-6 cells after 9 days of culture. (C) SEM image of individual printed QCE-6 cell on the surface of collagen gel after 21 days of culture. Original magnifications are as follows: 40X (A), 200X (B), and 8,000X (C).

Figure 2. Cell density gradient of QCE-6 cells at day 1. (A) Black color gradient pattern designed using Microsoft PowerPoint software. (B) Fluorescent microscopy image of QCE-6 cell density gradient created by direct inkjet printing of QCE-6 stem cells. Original magnification: 40x

Figure 3. (A) Light microscopy image of printed embryonic rat cortical neuronal ring at day 1. (B) Confocal microscopy image of printed cortical neurons stained with MAP-2 neuronal markers after 15 days of culture. Original magnifications are as follows: 40x (A) and 200x (B).
Electrophysiology of printed cortical neurons

As shown in Figure 4A, voltage-gated outward K⁺ currents and inward Na⁺ currents were found from the cortical neurons in the printed patterns at day 15, and these currents could be consistently evoked by 10 mV steps from a hold potential of -85 mV. These recordings suggested that the printed cortical cells were mature neurons with voltage-gated potassium and sodium channels on their membranes. Moreover, as shown in Figure 4B, the sustained repetitive firings of action potentials were also observed from the printed cortical cells, which indicated the printed cortical cells were still excitable and capable of firing action potentials. Our data reported here proved the embryonic cortical neurons still retained their basic electrical functions after being printed.

Figure 4. (A) Representative recording of Na⁺ and K⁺ currents and (B) Maximum action potential firing rates both obtained form a day 15 printed cortical neuron.

CONCLUSIONS

To the best of our knowledge, this study was the first to explore the possibility of inkjet printing of cardiomyogenic stem cells into pre-defined patterns and to evaluate the function fidelity of neurons, such as neuronal phenotypes and electrophysiology, after being printed through the firing nozzles. The results presented here showed that controlled patterns and structures of cardiomyogenic stem cells and cortical neurons can be fabricated by the novel inkjet printing method. Furthermore, the maintenance of neuronal phenotypes and basic electrical functions of the cortical neurons in the printed patterns was verified. This method, when combined with the 3D tissue printing method we have demonstrated previously[8], offers the capability of the modified inkjet printers to fabricate 3D functional neural and cardiac tissues, which may have potential in the regenerative treatments for serious neural and cardiac injuries and diseases.

REFERENCES


18