In vitro model of CNS neuronal pathway recovery using microfluidic chips

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Abstract. Development of biodegradable implants using new methods in biotechnology and neural engineering is one of the most perspective approaches in rehabilitation of central nervous system after injury and in neurodegenerative diseases. In this study we propose an experimental model of brain injury by growing two weakly coupled neuronal networks in three-chamber microfluidic chip. We modeled a functional recovery by plating a new dissociated cells in the place of weak synaptic connectivity. We showed that a weak synaptic unidirectional connections between two cultures of neuronal cells can be enhanced by integrating a new cell population into the growth site of axons. The proposed microfluidic chip design may be used to create a new type of scaffold which recovers realistic heterogeneous architecture of neuronal connectivity for rehabilitation of the brain injury.

1. Introduction

One of the promising methods of neurotransplantation in CNS injury recovery is a scaffold development and implantation. The scaffold serves as a basic structure made of biodegradable materials which carries induced pluripotent cells (IPSc) or stem cells. Such structure composed of biodegradable material (polymers, hydrogels, hyaluronic acid) provides integration of the cells in the brain with defined cellular density and after few days it dissolves leaving only the cells in the area of trauma [1-4]. In a perspective such structures will be implanted into the injury area of the brain and then the restore the lost functions [5].

The fundamental problem functional integration of various types of the cells in such technology consists of two tasks. First, the new cells should differentiate and form functional connectivity with the mature neural tissue. Such problem can be studied on a model of the neural tissue by growing clusters of dissociated neuronal cultures in microfluidic chips [6,7]. Several groups demonstrated that functional bidirectional connection form between primary neurons and stem cell-derived neurons [8,9] via chemical synapses and between human iPS cells using in vitro co-culture device [10]. Also, in our previous study [11] we showed that progenitor neurons can be co-cultured with mature neural network and form functional synaptic connectivity.

The second problem is that the structure of the scaffolds today is usually is homogeneous and can provide only homogeneous network of integrated cells, which can adversely affect the restoration of injuries in the brain. This problem can be solved studying different geometric shapes of the structure that shapes axons growth and network development [12]. In this study we used microfluidic devices with asymmetric microchannels design, which allow to isolate the cells and provide axon growth in
desired direction to form heterogeneous network. We propose and test a model of unidirectional functional connectivity recovery by plating a neurons into the chip with three chambers with asymmetric design.

2. Materials and methods.

2.1. Device Fabrication

Microfluidic chips were fabricated using silicone polymer PDMS (polydimethylsiloxane). Standard two-layer lithography was used for mould fabrication [12]. Liquid PDMS was poured onto the structured mold in a thin layer (3-5 mm) and put in dry-air sterilizer for 4 hours at 70 °C. Then the PDMS chips were carefully cut from mold and inserted in dry-air sterilizer for 12 hours at 100 °C. Then the chip was mounted on the sterile glass (figure 1(a)) or on the surface of a microelectrode array (MEA).

The chips consisted of three chambers: Source (1), Implant (2) and Target (3) which were connected by 2 sets of 8 asymmetric microchannels (figure 1(b)). The length of each microchannel was equal to 600 µm and consisted of three sections to provide unidirectional axon growth.

![Microfluidic chip with 3 compartments (chambers) to model CNS injury as uncoupled networks and restoring connectivity by integrating new cells. (a) Chip structure and dimensions; (b) Stages of the experiment: 1. growing two uncoupled cultures; 2. integration of the new cells by plating the culture in Implant chamber; 3. Confirmation of connectivity formation; (c) Photo of the chip.](image)

2.2. Cell Culture

Hippocampal neuronal cells were dissociated from embryonic mice (E18) and plated in the chambers of PDMS chips with initial density approximately 7,500 cells/mm2. First, the cells were plated in the Source and the Target chambers of the chip, the Implant chamber remained empty. The neurite outgrowth through the microchannels was monitored during 20 days using automated microscope system (Cell IQ, ChipMan Technologies, Finland).
2.3. Extracellular Recording and Stimulation

Bioelectrical activity of neuronal cultures was studied using microelectrode arrays with 60 electrodes (Multichannel Systems, Germany). We manually aligned the microfluidic chips with MEA so that 24 electrodes were placed in the microchannels between Source and Implant chambers (3 electrodes in each of 8 microchannels), 24 electrodes – in the microchannels between Implant and Target chambers, 8 electrodes - in Implant chamber. The recording of the bioelectrical activity was performed using a multichannel USB-MEA120-Inv-2-BC-System (Multichannel systems, Germany) at a sample rate of 20 kHz. The experiments were performed from 3 to 24 DIV after culture plating. Analysis of the recorded bioelectrical activity was performed with custom made scripts in Matlab [6]. The responses to electrical stimulation were recorded on 23 DIV. We used a low-frequency stimulation consisted of biphasic voltage pulses ±800 mV, 260 µs per phase, positive first, intervals between stimuli were 3 s. Series of 60 stimuli were applied through one electrode in the microchannel between Source and Implant chambers or between Implant and Target chambers.

3. Results and conclusion.

3.1. Connectivity between neuronal cultures

First, we developed a microfluidic chip which consisted of three chambers connected by asymmetric microchannels. Each microchannel had three sections with "traps" that did not allow the axons to grow in the direction from Implant to Source chamber and from Target to Implant chamber (figure 2). Hippocampal neurons were plated in the Source and the Target chambers. Within 48 h after plating the neurons started to grow neurites into the microchannels from the Source and reached the Implant chamber at least after 5 days. Neurites from the Target chamber grew in the traps of the microchannels and did not reach the middle Implant chamber. On 12 DIV the cells were plated in the Implant chamber and started to grow in the microchannels within 2 days forming connection with the axons from the Source and the dendrites from the Target.

We estimated a coupling of the cultures with neurites through the microchannels before and after plating the cells in the Implant chamber on 3, 9, 12, 14, and 18 DIV (n=10 chips) (figure 2 (b)). The percentage of the filled channels (MAD) between Source and Implant was high (76%) before planting, and reached 100% on the 1st day after new cells were plated into the Implant chamber. The first sections of microchannels between Implant and Target were partially filled (26.20% ± 12.67%) by axons from the Source. On 2 DIV after cells integration the MAD between Implant and Target was equal to 61.30% ±10.75% and reached 100% on 6 DIV.
3.2. Bioelectrical spiking activity
We recorded the spiking activity from the MEAs on 11, 17 and 24 DIV (n=3 cultures). On 11 DIV we observed spikes and sparse bursts from all microchannels (figure 3 (a), middle panel). There were no spikes in Implant chamber and in the first section of microchannels between Implant and Target. On 18 DIV (6 DIV after plating in Implant chamber) the axons passed through all microchannels and the bursts propagated from the Source to the end of the microchannels between the Implant and the Target chamber (figure 3 (a), bottom panel).

3.3. Stimulus evoked activity
Next, we applied a series of electrical stimuli to confirm that the spiking activity propagated from the Source to the Target chamber after new cells integration. The activity should pass through the microchannels reinforcing new cells in the Implant chamber, but not vice versa. The stimulus applied to the axons of the Source chamber (figure 3 (b), middle) induced the spiking response which was registered at the end of the microchannels between Implant and Target chamber. In contrast, the stimulus applied to the axons in the microchannels between Implant and Target chamber didn't evoke the spikes in the axons of the Source chamber (figure 3 (b), bottom).

Figure 3 (a,b,c). (a) Microfluidic chip coupled with a microelectrode array; (b) Bioelectrical spiking activity on 11 DIV and 24 DIV (12 days after plating the cells in Implant chamber). (c) Stimulus evoked spiking activity. Middle - stimulus applied to the axons between Source and Implant, bottom- applied to the axons between Implant and Target. Presented signals corresponded to the electrodes of MEA on the photo (top). Each plot presented the signals from all stimuli (1-60) and color of the signal coded the stimulus number in a sequence.

3.4. Conclusion
In this work we developed a method to form a new unidirectional synaptic connectivity between two populations of cultured networks using microfluidic devices. The results show that integration of new dissociated cells between two mature networks provide a new "bridge" for spiking activity propagation. The direction of activity pathways was defined by a structure of the asymmetric microchannels of the chip. Therefore, this co-culture system can be used in study of neurorehabilitation processes using stem or IPS cells [8] and in design of new type of the scaffolds which will provide proper morphological and functional repair of the neural tissue.

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