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Regulating the differentiation of PC12 by acoustic fluid stimulation

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***Abstract*—Regulating the differentiation and regeneration of nerve cells has been proved effective for treating neurological disorders diseases. Although there have been several studies related to neuromodulation, these studies have suffered from invasiveness or low spatial resolution. Herein, a hypersound acoustic stimulation on nerve cells was explored and the results demonstrated this novel method had powerful effect on targeted nerve cell regulation. A bulk acoustic wave resonator fabricated with MEMS process was used to generate hypersound. The acoustic fluid (AF) effect was produced with mechanical pressure when hypersound transmitted with attention in the solution. Results indicated that exposing PC12 cells to AF stimulation, small protrusions would appear within 10 minutes. And differentiation ratio of the AF stimulated cells was 16% higher than that of cells cultured with nerve growth factor (NGF). This original and effective method is compatible with conventional cell culture and had potential in single nerve cells regulation.**

Keywords—bulk acoustic wave resonator, hypersound, acoustic fluid effect, differentiate

I. INTRODUCTION

Neuromodulation is becoming an attractive method to treat nervous system disease, like Parkinson's disease, depressive disorder et al [1]. Several neuromodulation methods have already been utilized in clinic treatment , but they are limited by invasiveness or low spatial resolution. Such as deep brain stimulation (DBS) [2], which requires invasive surgical operation to plant the electrode in the deep brain region, also accompanied with immunoreaction to foreign materials, the later surgical for maintenance [3]. Trans-cranial direct current stimulation (tDCS) and trans-cranial magnetic stimulation (TMS) are emerging technology for the non-invasive modulation, but confined by the low spatial resolution and the depth of penetration[4-7]. Optogenetics is allowing for fine modulation of nerve cell activity [8]. But the optical stimulation cannot reach deep brain region and the fiber delivered into tissue with surgical operation may cause inflammation [9-10].

Acoustic stimulation has become more attractive over past decades for its superiority of noninvasive and capability of the deep tissue regulating [4]. But low frequency ultrasound coupled with microbubbles hardly arrive at the object cells precisely limited its further application [11]. Hypersound is defined as an acoustic wave with gigahertz frequency and sub-micrometer wavelength. This technology has been used in poration[12] and drug delivery [13] of cells as the mechanical stress significantly increased compared with conventional ultrasound method.

In this work, a hypersound acoustic stimulation on nerve cells was explored systematically and the results demonstrated that this novel method has the potential in a few and single cell modulation.

II. METHODS

A. Cell line and NGF

PC12 cells, derived from a pheochromocytoma of rat, were used as a model cell which could differentiate with the regulation of nerve growth factor (NGF). PC12 cells (ATCC, US) were cultured in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated horse serum (Gibco, US) and 5% (v/v) fetal bovine serum (Gibco, US) in a humidified incubator at 37℃ with 5% CO2. Coverslips with Poly-L-Lysine (PLL) were applied in acoustic fluid (AF) stimulation experiments. Cells with AF stimulation were cultured in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum with or without NGF (Sigma, US).

B. Bulk acoustic wave resonator

A bulk acoustic wave resonator fabricated by standard microelectromechanical system (MEMS) process was utilized to generate the hypersound with frequency of 1.58 GHz. The sinusoidal signal of 1.58 GHz generated by a signal generator was transmitted to the resonator with amplified by an amplifier and the hypersound took placed with the resonance oscillation. When hypersound attenuated in the solution, micro-vortices created with mechanical pressure which called acoustic fluid (AF) effect.

C. Microchip-PC12 cells regulation system

The microchip-PC12 cells regulation system was consist of a bulk acoustic wave resonator fixed on EVB board, polydimethylsiloxane (PDMS) chamber and a coverslip with

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PC12 cells (Fig.1). Cells were stimulated by AF every 24 hours with duration of 10 minutes, the cells morphology was observed by microscope (Olympus IX53, Japan) 24 hours after each time stimulation.

III. RESULTS AND DISCUSSION

A. PC12 cells respond to AF stimulation

To test the effect of AF stimulation on PC12 cells , we stimulated cultured cells with AF for 10 minutes, then we observed the cellular morphology change with microscope immediately. As shown in Fig. 2, cells have grown out small protrusions after exposed to AF stimulation, but the control experiments of cells without AF stimulation did not have such changes. This confirmed that PC12 cells responded to the AF stimulation rapidly and had the differentiation potential under this stimulation.

B. PC12 cells differentiation with AF stimulation

In the next, we observed cells morphologic change for a long-term culture. Three experimental groups were designed, cells stimulated by AF, AF combined with NGF, NGF respectively. Cells cultured 24 hours before stimulated with AF, the interval of each AF stimulation was set as 24 hours. For NGF treated group, the NGF cultured with cells all the time. From Fig. 3, we discovered that stimulated cells have grown out protrusions, especially of AF stimulation method culturing cells up to 96 hours, the cells body of AF stimulation groups have grown bigger than others.

The ratio of cells differentiation were also studied. Fig. 4 gave the differentiation ratio of cells stimulated with AF three times. As it showed that the differentiation ratio of cells stimulated by AF could reach nearly 70% after 96h culture, which is 16% higher than that of cells exposed to NGF. Meanwhile when cells stimulated by AF combined with NGF, higher cell differentiation ratio has achieved than the single treatment. These data indicated that PC12 cells respond to AF stimulation efficiently. In addition, this novel AF stimulation method do not need any foreign reagent introduced, which is advantageous of avoiding external interference with drugs.

IV. CONCLUSIONS

In summary, we set up a microchip based PC12 cells regulation system, results demonstrated that cells could differentiate effectively under the AF stimulation. Cells could grow out small protrusions after exposed AF 10 minutes, and the differentiation ratio with AF stimulation was higher than cells cultured with traditional NGF stimulation. By contrast, cells differentiation ratio apparently higher when stimulated by AF combined with NGF than a single treatment. This method was more effective and safe than the drug stimulation. Compared with conventional ultrasound stimulation, AF stimulation can focus on hundred micrometers areas for single or a few cell modulation, which will be of great significance in noninvasive and accurate treatment of nerves system diseases.

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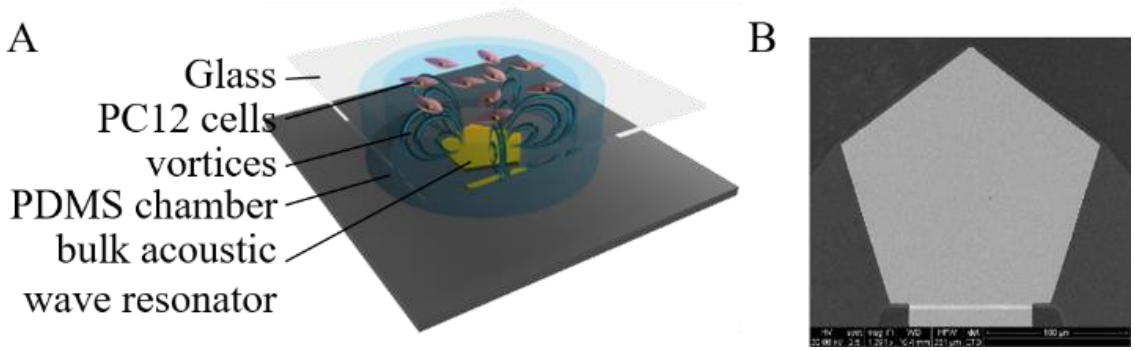

Fig. 1. (A) Schematic of microchip-PC12 cells regulation system. (B) Scanning electron microscope (SEM) photo of resonator, scale bar represents 100um.

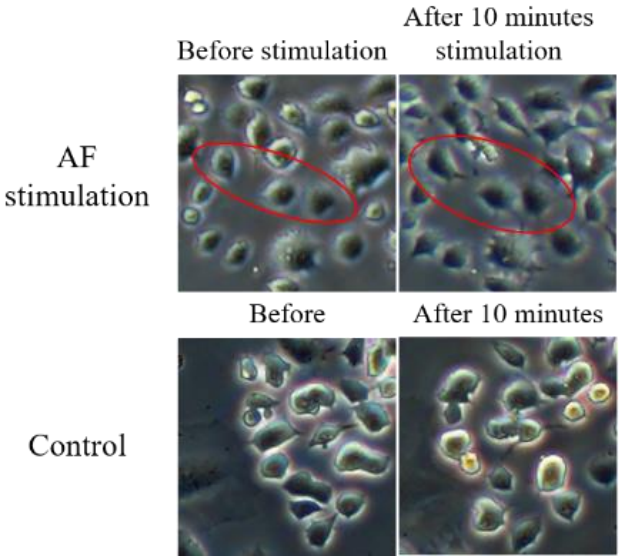

Fig. 2. The microphotographs of PC12 cells before and after stimulated with (AF stimulation) and without (control) acoustic fluid.

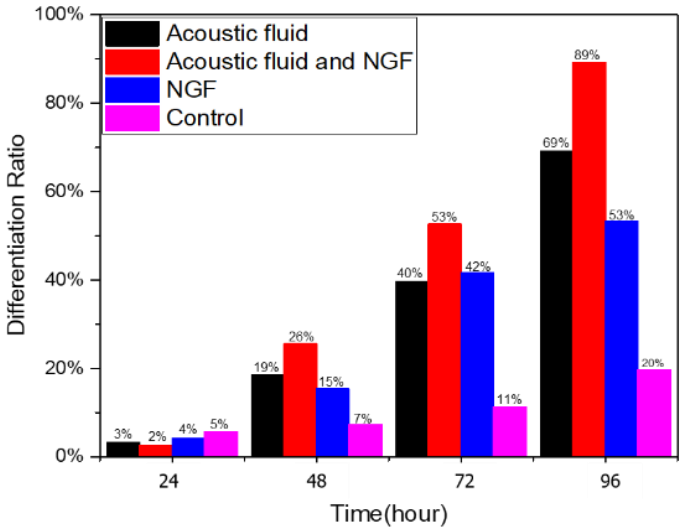

Fig. 4. Differentiation ratio of PC12 cells before stimulation(24h), and 24h culture after each time AF stimulation. Three experimental groups: AF, AF combined with NGF, NGF, the NGF was always existed. The control group without any stimulation was cultured for the same time as other groups.

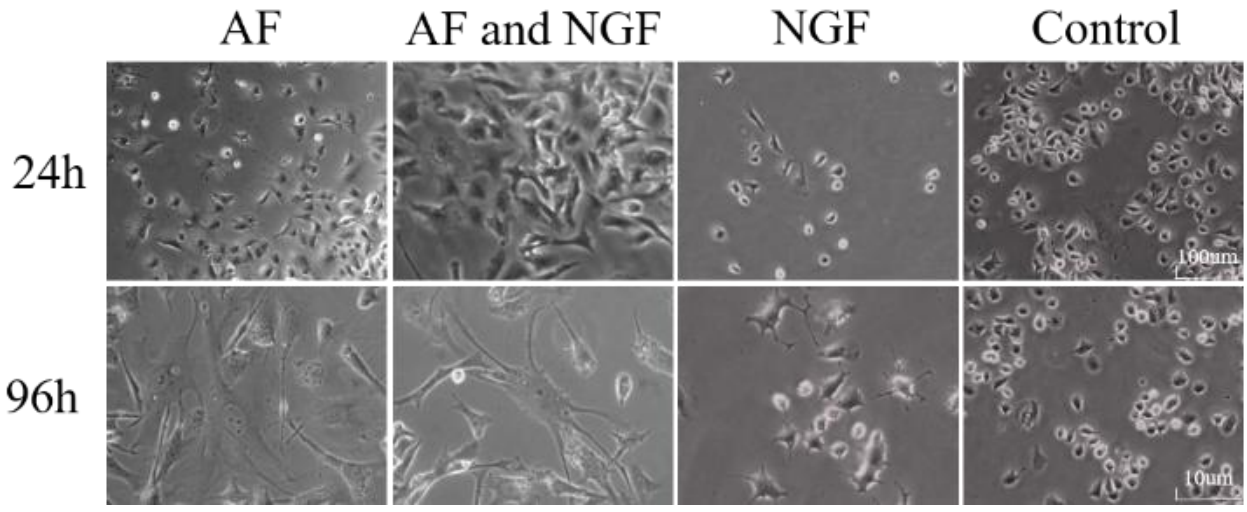

Fig. 3. The microphotographs of PC12 cells stimulated by AF, AF combined with NGF, NGF and without stimulation; scale bar represents 10um and 100um.