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A Simple, Rapid, and Automatic Centrifugal Microfluidic System for Influenza A H1N1 Viral RNA Purification

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Molecular diagnostics consists of three processes, which are a sample pretreatment, a nucleic acid amplification, and an amplicon detection. Among three components, sample pretreatment is an important process in that it can increase the limit of detection by purifying nucleic acid in biological sample from contaminants that may interfere with the downstream genetic analysis such as nucleic acid amplification and detection. To achieve point-of-care virus detection system, the sample pretreatment process needs to be simple, rapid, and automatic. However, the commercial RNA extraction kits such as Rneasy (Qiagen) or MagnaPure (Roche) kit are highly labor-intensive and time-consuming due to numerous manual steps, and so it is not adequate for the on-site sample preparation. Herein, we have developed a rotary microfluidic system to extract and purify the RNA without necessity of external mechanical syringe pumps to allow flow control using microfluidic technology. We designed three reservoirs for sample, washing buffer, and elution buffer which were connected with different dimensional microfluidic channels. By controlling RPM, we could dispense a RNA sample solution, a washing buffer, and an elution buffer successively, so that the RNA was captured in the sol-gel solid phase, purified, and eluted in the downstream. Such a novel rotary sample preparation system eliminates some complicated hardwares and human intervention providing the opportunity to construct a fully integrated genetic analysis microsystem.

Keywords: centrifugal microfluidics, DNA/RNA purification, micro-total-analysis-system, Influenza A H1N1 virus

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Investigating the Morphology and Kinetics of Three-Dimensional Neuronal Networks on Electro-Spun Microstructured Scaffolds

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Petri dishes and glass slides have been widely used as general substrates for in vitro mammalian cell cultures due to their culture viability, optical transparency, experimental convenience, and relatively low cost. Despite the aforementioned benefit, however, the flat two-dimensional substrates exhibit limited capability in terms of realistically mimicking cellular polarization, intercellular interaction, and differentiation in the non-physiological culture environment. Here, we report a protocol of culturing embryonic rat hippocampal neurons on the electro-spun polymeric network and the results from examination of neuronal cell behavior and network formation on this culture platform. A combinatorial method of laser-scanning confocal fluorescence microscopy and live-cell imaging technique was employed to track axonal outgrowth and synaptic connectivity of the neuronal cells deposited on this model culture environment. The present microfiber-based scaffold supports the prolonged viability of three-dimensionally-formed neuronal networks and their microscopic geometric parameters (i.e., microfiber diameter) strongly influence the axonal outgrowth and synaptic connection pattern. These results implies that electro-spun fiber scaffolds with fine control over surface chemistry and nano/microscopic geometry may be used as an economic and general platform for three-dimensional mammalian culture systems, particularly, neuronal lineage and other network forming cell lines.

Keywords: electrospun, 3-D culture, 3-D imaging, hippocampus