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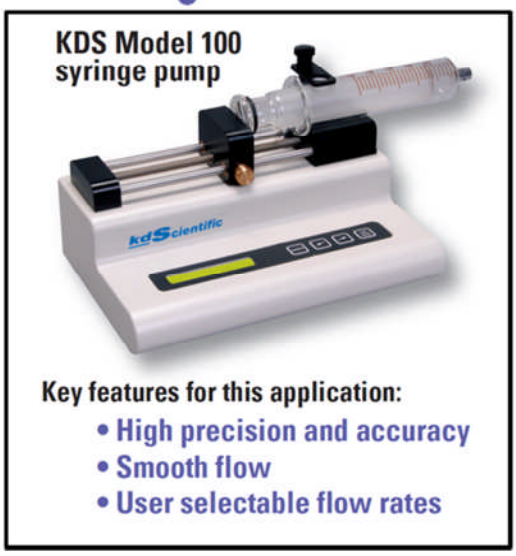
INFLOWMATION CHRONICLES

HIGHLIGHTS OF INTERESTING SCIENTIFIC APPLICATIONS



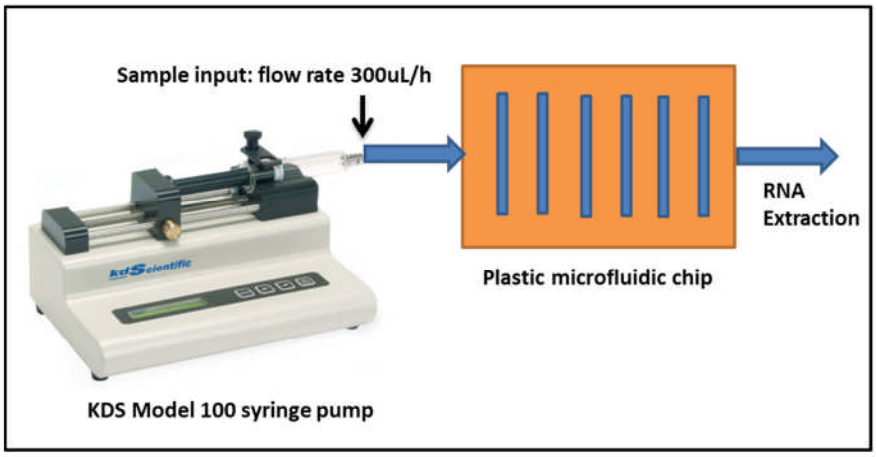


Figure 1: Schematic diagram of experimental setup for RNA extraction using a custom plastic microfluidic chip

What is Microfluidics-based extraction?

Microfluidics deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter scale. Typically, micro refers to small volumes (nL, pL, fL), small size, low energy consumption or effects of the micro domain.

Often processes which are normally carried out in a lab are miniaturized on a single chip in order to enhance efficiency and mobility as well as reducing sample volumes and testing costs. This paper provides an interesting research application for infectious diseases using the lab-on a chip technology.

Microfluidics-based extraction of viral RNA from infected mammalian cells for disposable molecular diagnostics

Introduction

In this study, a disposable plastic microfluidic device capable of extracting viral RNA from complex biological mixtures has been developed as a step towards building low-cost, mass produced molecular diagnostic systems for infectious diseases. The device offers a robust, easy-to-use sample preparation platform for rapid processing of clinical samples for nucleic acid-based diagnostics for pathogens. This platform model has successfully isolated viral RNA from mammalian cells infected with influenza A (H1N1) virus. The microfluidic chip was fabricated in cyclic polyolefin by hot-embossing with a nickel–cobalt electroformed master mold. The isolation of total RNA was done on-chip with a solid-phase extraction (SPE) system (MicroSPE) formed by trapping silica particles in a porous polymer monolith. Separation of RNA is achieved through reversible binding of the nucleic acids to the silica particles in the monolith. The SPE system allows isolation of intact total RNA from a mixture of infected whole cell lysates and serum supplemented cell culture supernatant. The system requires minimal user-handling, so the isolated RNA sample has low risk of degradation. The sample preparation platform presented here uses a very compact design that can be easily coupled with downstream amplification and detection modules to form a fully integrated lab-on-a-chip for nucleic acid analysis.

Lab on Chip Test Bed and Experiment

The chips were fabricated in Zeonex®, which is a medical grade cyclic polyolefin. The optical properties of Zeonex are essential for achieving in situ photopolymerization and the integration of an on-chip fluorescence detection module in the future to read out PCR amplification results. A high Tg is essential for high temperatures needed during PCR. The microchips were fabricated by hot-embossing with a nickel–cobalt alloy electroformed master mold. To seal the channels, another piece of Zeonex of the same dimensions was thermally bonded (136 ◦C, 250 psi, 2 min) on top in a hot press.

MDCK cells were cultured according to the guidelines in the "WHO Manual on Animal Influenza Diagnosis and Surveillance". The MDCK cells were inoculated with influenza A when the cells were between 60 and 80% confluent. After 5 days in culture following inoculation, cell rounding was observed which indicated the onset of cytopathic effect. A hemagglutination (HA) assay was run to determine the number of virus particles present in the culture. The highest dilution of the virus that causes complete hemagglutination is the HA titration endpoint. An HA titer of 4 HA units/mL was measured for the assay, which indicated that the virus was present in large amounts in the culture.

Viral RNA Isolation

Both the pelleted cells and cell culture supernatant were collected to test the microfluidic solid phase extraction columns. The sample was mixed with GuSCN (guanidium thiocyanate) containing lysis buffer in 1:1 ratio and flowed over the solid phase extraction columns. The purification process was carried out at room temperature. The sample, wash solutions and elution buffer were passed through the microchannels at a flow rate of 300 L/h with a **KDS100 syringe pump (manufactured by KD Scientific, Holliston, MA).** 15 L of the lysed sample was loaded into a SPE channel for 3 min. The channel was then washed with 70% ethanol for 2 min, followed by a wash with 100% ethanol for another 2 min. Finally, 15 L of RNase free water was flowed through the channel for 3 min to elute the extracted RNA.

All the samples were run in triplicate to minimize pipetting errors. The viral RNA extraction results from the SPE columns were compared against a commercially available viral RNA extraction kit (QIAamp®, Qiagen Inc., Valencia, CA), which is considered a "gold standard" method.

Results and Discussion

This study has demonstrated the nucleic acid extraction efficiency of the polymer monolith/silica column from several increasingly complex solutions on-chip. The extraction efficiency was found to be as high as 70±3%, which is comparable with existing methods. The infected cell lysate plus the cell culture supernatant was used to ascertain the viral RNA extraction efficiency. Four separate experimental runs with the sample to verify consistent results. The fluorescence signal intensity (Rn) was plotted as a function of the thermocycle number for the amplification of the M1 gene found in influenza A. Fig. 2 shows a representative real-time RT-PCR amplification plot for samples obtained using the Qiagen kit and the MicroSPE method.

Conclusion

The PCR results verify that the SPE system allowed for successful extraction and elution of viral RNA in the polymeric microchip, and the eluted RNA sample was PCR amplifiable. Fig. 3 shows the threshold cycle value (CT) for samples obtained using the two different RNA extraction methods (Qiagen versus MicroSPE methods). The mean CT value for the two methods was 26.65±1.11 and 29.58±2.28 for Qiagen and MicroSPE method, respectively. There was an average CT difference of 2.93 between the two methods, which corresponds to a 22.93 = 7.62- fold drop in substrate between the two extraction methods.

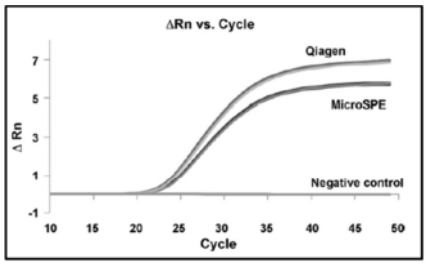


Figure 2: Real-time PCR amplification of influenza A M1 gene comparing the Qiagen column with the microfluidic (MircoSPE) lab on a chip method.

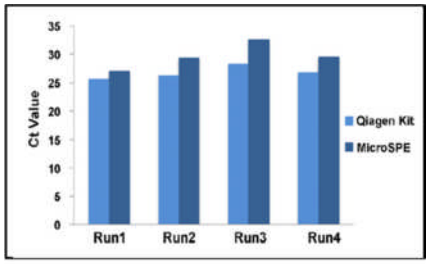


Figure 3: Threshold cycle (CT) values obtained with the Qiagen kit compared with the microfluidic (MircoSPE) lab on a chip method.

Reference

Bhattacharyya & Klapperich
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