ENGINEERING A POINT-OF-CARE VIRAL CONCENTRATION DEVICE FOR RAPID MOLECULAR DIAGNOSTICS OF INFLUENZA IN HUMAN RESPIRATORY SPECIMENS

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ABSTRACT
We have developed a portable, simple to fabricate, and highly effective polymeric microfluidic sample concentration device to increase the speed and sensitivity of molecular diagnostics of influenza from patient respiratory samples. This method is more friendly than off-chip methods to in-line on chip processing since it obviates the need for a centrifuge. The disposable chip fabricated with xurography utilizes evaporation and interfacial dragging effects. Up to 18 times concentration for viral RNA from influenza A virions was achieved by evaporating 1 mL to 50 µL in 0.5 hour or less with an average recovery of 60.3% (+/-20.5%) from patient specimens.

KEYWORDS: Disposable Virus Sample Concentration, Molecular diagnostics, Evaporation, Xurography

INTRODUCTION
Viral titer in any given patient sample can and does vary widely. A low viral titer requires nucleic acid extraction of either a large volume of patient specimen, or a smaller volume of highly concentrated sample. Concentrating patient specimens from milliliters to microliters has the potential to increase process speed and lower the PCR limit of detection. Current methods to concentrate pathogens from patient samples involve physical centrifugation that require large and costly equipment, which cannot be implemented at the point of care.

Microfluidic methods such as electrophoresis and focusing, and mechanical filters or weirs suffer from complicated setup or fabrication, and often the addition of expensive accessories. Filter and weir methods are susceptible to clogging and sample retrieval problems.

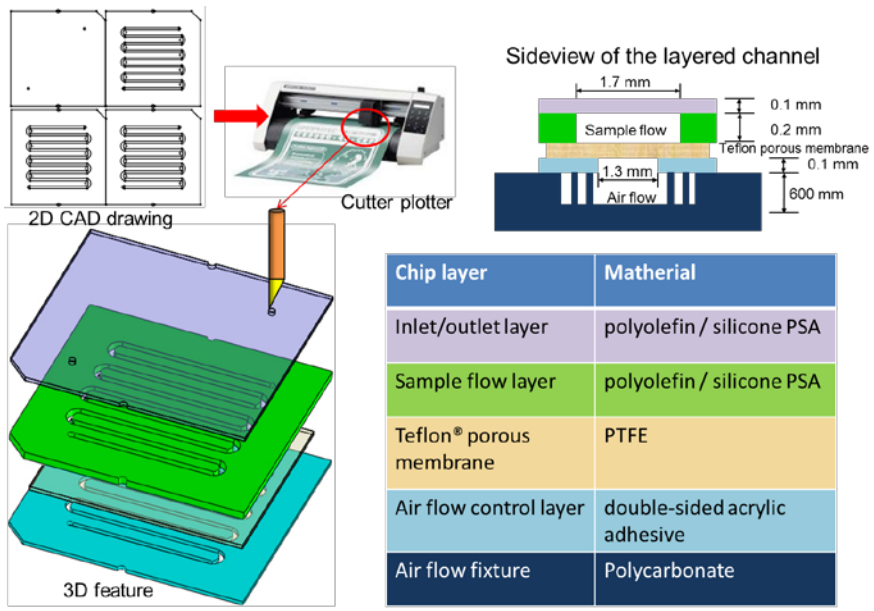
Overall, there is a need for a rapid, simple to fabricate and set up, versatile, and low cost microfluidic viral sample concentration device that can be integrated with on-chip nucleic acid extraction and PCR to improve and enable molecular diagnostics at the point-of-care.

THEORY
The micro-evaporation device includes a liquid sample layer and a gas flow layer, between which a hydrophobic porous membrane layer is sandwiched [1]. The convective gas flow reduces the pressure in the gas flow layer. As a result, there is a vapor pressure gradient across the liquid/gas interface at the porous membrane that drives evaporation of the liquid from the liquid stream to the gas stream. The analyte solution (containing the pathogens) is retained by a membrane non-permeable to the pathogens, while water is removed, forming a moving meniscus.

As meniscus moves towards the channel outlet, pathogen particles experience many body forces that govern their movement relative to the fluid. When the interfacial surface tension between the particles and the fluid dominates over non-specific adhesive van der Waal's and electrostatic forces, viral particles will be collected in the moving meniscus and driven towards a desired location while they are concentrated in a small volume.

EXPERIMENTAL
The device is fabricated with a fast 3D maskless xurography method [2, 3] using commercially available pressure sensitive adhesive polymeric materials and requires no lithography or cleanroom operations (Figure 1). The 2D CAD patterns on the fluid and the airflow control layers are cut, aligned, and adhered to the airflow chamber by applying pressure to the assembly. After testing, the concentrator is discarded in order to avoid cross contamination between samples, and the airflow chamber can be reused. The entire fabrication process takes less than 10 minutes. The completed device after assembly is the size of a credit card, as shown in Figure 2.

The device was tested using both cultured influenza A in cell culture supernatant and influenza A positive patient nasopharyngeal fluid resuspended in PBS. Specifically, 1 mL of viral sample is concentrated to 50 µL in less than 30 minutes. Both the concentrated output and the "un-concentrated" input sample are processed with a Qiagen QIAamp Viral RNA mini kit and the on-chip micro-solid phase extraction column (microSPE) [4] to extract the viral RNA. The extracted sample is amplified with rRT-PCR, from which the concentration factor is calculated as the RNA concentration in the output over the RNA in the input viral sample. The viral RNA recovery efficiency is the concentration factor divided by 20 to account for the volume reduction.



Chip layer	Matherial
Inlet/outlet layer	polyolefin / silicone PSA
Sample flow layer	polyolefin / silicone PSA
Teflon® porous membrane	PTFE
Air flow control layer	double-sided acrylic adhesive
Air flow fixture	Polycarbonate

Figure 1: Device fabrication using 3D maskless xurography. Left: fabrication process flow. Top right: sideview and geometry of the layered device. Bottom right: material of each layer.

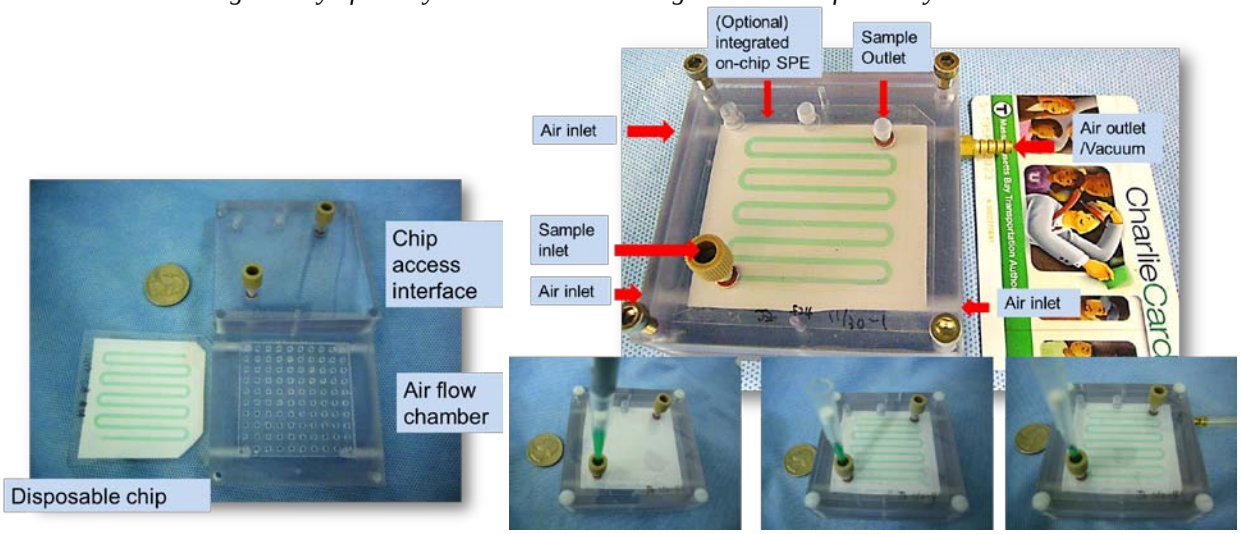


Figure 2: Completed chip, before (left) and after (right) assembly showing sample dispensing in the device in a filtered pipette tip. As evaporation occurs, sample volume decreases and sample concentration in the chip increases.

RESULTS AND DISCUSSION

The concentration effect for input viral concentration ranging from 1 to 10^4 PFU/mL is shown in Figure 3 and Table 1. The average RNA recovery efficiency was 60.3% (+/-20.5%) for patient respiratory samples and 57.3% (+/-16.5%) for cultured samples, with close to 80% efficiencies for the lowest input concentrations. The average concentration factor was 11.5 (+/- 3.3). These results imply that if the ratio between the input and the output sample volume is higher than 20, the concentration factor could be even higher. Samples that would have taken 6 to 7 hours to process can now be processed in 0.5 hour.

From Table 1, as input viral concentration decreased, the rRT-PCR readout of the viral RNA concentration also decreased, resulting in greater number of samples showing "undetermined" Ct values. After concentration by evaporation, the originally un-concentrated samples that were undetectable with PCR after on-chip microSPE, became detectable with a definitive RNA copies readout. The large discrepancy in the calculated recovery efficiency, from 21.6% for 10^4 PFU/mL, to 68.8% and 64.8% for 10^3 and 10^2 PFU/mL, was possibly due to the large variation in the extraction efficiency between different microSPE channels, therefore was not an accurate indicator of the performance of the concentration device. Nevertheless, this showed that for the same volume of viral samples, concentrated samples contained higher RNA content than un-concentrated samples. In other words, to extract the same amount of RNA copies from the un-concentrated viral samples would take a larger volume (up

to 15 times), thus a longer process time for the microSPE (instead of half an hour, 7.5 hours). In contrast, if the sample were pre-concentrated before microSPE, which took less than half an hour, a much smaller volume would be necessary for down-stream extraction and detection by PCR.

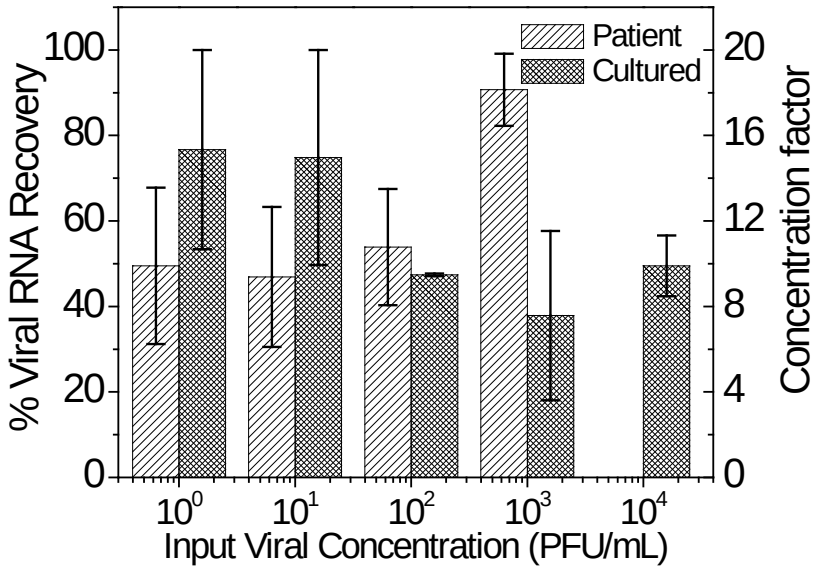


Figure 3: Viral RNA recovery and concentration factor for serial dilutions of both patient and cultured influenza samples. Both input and output samples from the concentrator are processed with the QIAamp Viral RNA mini kit.

Table 1. Viral RNA recovery for serial dilutions of patient influenza samples before and after viral concentration. Both input and output samples from the concentrator are processed with the on-chip microSPE

Input viral particles (PFU/mL)	Average Input RNA/mL	Average output RNA/mL	% RNA Recovery
1.00E+04	1.38E+07	5.95E+07	21.6%
1.00E+03	3.02E+05*	4.16E+06	68.8%
1.00E+02	4.84E+04**	6.28E+05*	64.8%
1.00E+01	N/A***	N/A***	N/A

*#of *=#of samples that had undetermined PCR (Ct>36), n =3*

CONCLUSION

The viral concentrator is efficient at concentrating influenza viral samples, either from cell culture or from human patients, and the concentrated sample contains higher RNA content in a much smaller volume for downstream nucleic acid extraction. There is tremendous potential in using this device for concentration of a variety of bacterial and viral pathogens in other human specimens besides respiratory samples. This device can also be integrated with any downstream on-chip sample preparation and detection sensor platforms, to achieve highly sensitive point-of-care detection of infectious diseases.

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