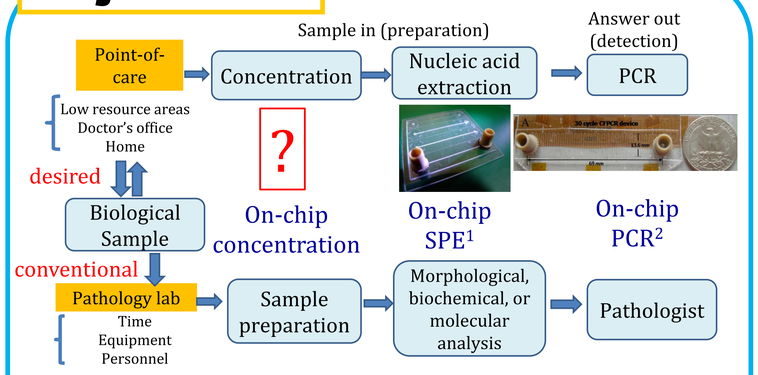
ENGINEERING A POINT-OF-CARE VIRAL CONCENTRATION DEVICE FOR RAPID MOLECULAR DIAGNOSTICS OF INFLUENZA IN HUMAN RESPIRATORY SPECIMENS

J. Y. Zhang, M. Mahalanabis, L. Liu, J. Chang, J. Do, and C. M. Klapperich
Department of Biomedical Engineering, Boston University, USA

BOSTON UNIVERSITY

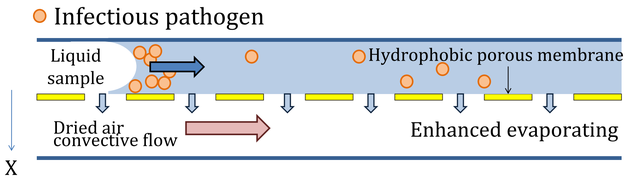
Objectives



? **To design a microfluidic sample concentrator to increases solid phase extraction (SPE) process speed, and to lower on-chip PCR limit of detection**

Background

1. Fluid mass transfer[3]

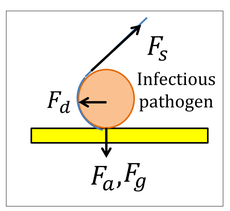


$$\dot{m} = -\bar{h}_D A \frac{\partial P}{\partial x}$$

$\dot{m}$: mass decrease due to evaporation
A: the area of the exchange surface
h_D: convective mass transfer coefficient
dP/dx: pressure difference at the liquid/gas interface

- Water vapor moves along partial pressure gradient
- Fluid flows to compensate for volume reduction, creating the moving contact line / meniscus

2. Particle mass transfer

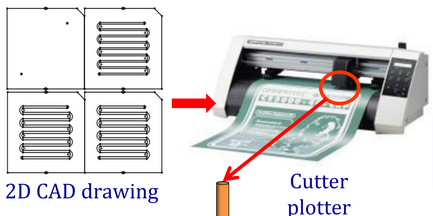


Forces controlling particle motion:
- Interfacial tension: F_s
- Adhesion force: $F_a = F_w + F_e$
  F_w: van der Waal's
  F_e: electrostatic
- Other body and drag forces: F_g, F_d

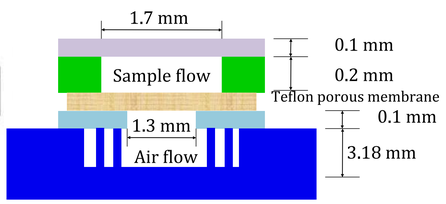
When Fs dominates, particles move along by interfacial tension

Methods

Chip fabrication with 3D Maskless Xurography

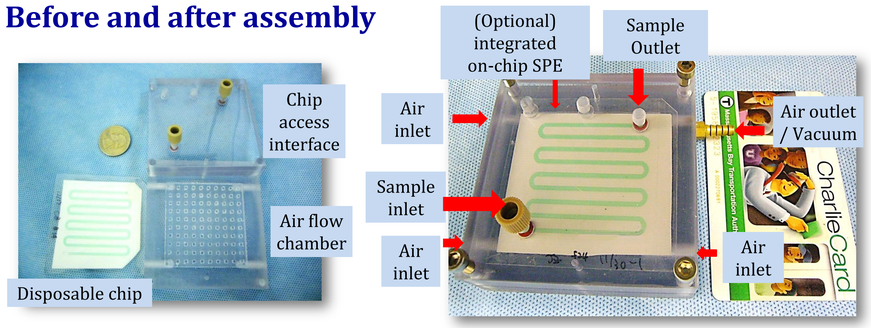


Layered chip design



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

Before and after assembly



Experimental flow and analysis

Surface treatment: incubating BSA 30 min then remove

Sample input: 1mL ATCC A/PR/8/34, or patient nasal swab dilution in PBS; 1 – 10^5 PFU/mL

Evaporate at: • 39°C • Output: 50uL

RNA Extraction: Qiagen QIAamp Viral RNA mini kit

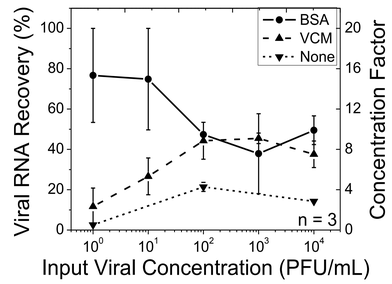
rRT-PCR: Invitrogen SuperScript® III Platinum® One-Step qRT-PCR Kit w/ROX • Applied Biosystems 7300PCR

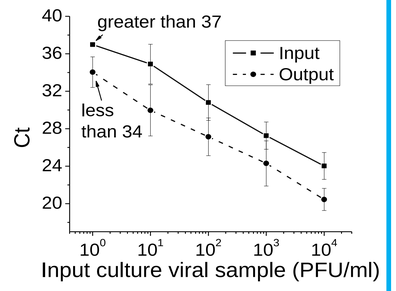
$$\% \text{ Recovery (efficiency)} = \frac{\text{Output RNA copies}}{\text{input RNA copies}}$$

$$\text{Concentration factor} = \%\text{Recovery} \times \frac{\text{input volume}}{\text{output volume}}$$

Results

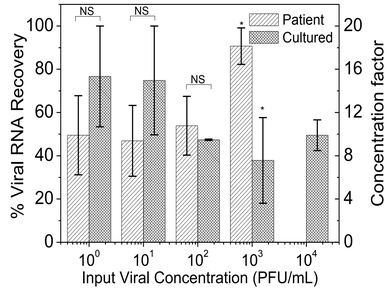
ATCC influenza A/PR/8/34 samples from cell culture

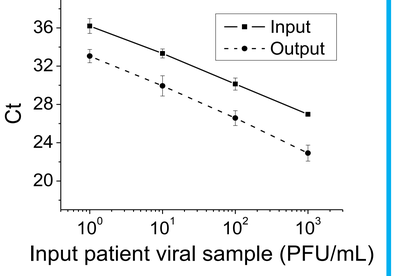




- Surface treatment improves viral RNA recovery
- For low input concentrations, BSA-treated chips had an average recovery of nearly 80%. The output sample was 16 times more concentrated than the input sample.
- Ct was consistently lower in concentrated samples, allowing detection of false negative samples with Ct beyond 37.

Comparison between cultured and patient specimens





- No significant difference between cultured and patient specimens.
- Overall, the viral RNA recovery for all concentrations was 60.3%, resulting in a concentration factor of over 12 times.
- Cultured: n = 2. Patient: n >=3. NS: not significant; *P<0.05; two sample unpaired two-tailed Student's *t*-test.

Conclusions

- Achieved a simple to fabricate, disposable, easy to operate polymeric viral concentration device
- Demonstrated influenza concentration to over 16 times within 30 minutes for improved PCR sensitivity

1. Kulinski, M.D.*, Mahalanabis*, M., Gillers, S., Zhang, J.Y., Singh, S., and Klapperich, C.M., "*Sample preparation module for bacterial lysis and isolation of DNA from human urine*". Biomedical Microdevices, DOI:10.1007/s10544-008-9277-1, (2009).
2. Cao, Q., Kim, M.-C., and Klapperich, C.M., "*Plastic microfluidic chip for continuous-flow polymerase chain reaction: Simulations and experiments*". Biotechnology Journal. 6(2): p. 177-184.
3. Zhang, J.Y.*, Do, J*, Premasiri, W. R., Ziegler, L.D. and Klapperich, C.M., "*Rapid point-of-care concentration of bacteria in a disposable microfluidic device using meniscus dragging effect*". Lab on a Chip. 10(23): p. 3265-3270.