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Disposable environmental pathogen detection for cell phones

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Abstract

In this initiative we made considerable progress in developing a mobile sensor platform to detect environmental pathogens in water samples using cell phones.
We adapted and improved earlier designs of unidirectional valves, enabling sample and reagent handling within the unibody device. The new valve design, which consists of an introduced disruption of the adhesiveness of the sealing film, has considerable advantages on assembly efficiency and by eliminating undesired death volume.
The design allows reliable volume metering, which is a critical operation for any analytical procedure, including sample preparation steps for pathogen lysis.
We could show that our synthetic riboswitches have the potential to detect nucleotide sequences, optimization however is needed to eliminate “leakage” of the reporter protein. Achievable limit of detection with the present concept was calculated to be below clinical requirements yet, and additional amplification steps have to be integrated. Such solutions are available and could be smoothly integrated in the present platform.
The developed platform has great potential as a pathogen sensor outside the usual lab environment and the promising preliminary results, which could be achieved with the help of the Åforsk research grant, will be used to apply for further financing to complete the project.

Background:

This initiative explored the integration in autonomous Lab on a Chip devices (LOCs) for nucleotide sequence detection using cell-free synthetic biology principles (CFSB).CFSB can be a powerful biosensing concept that exploits the biochemical machinery in genetically engineered cells, without the disadvantages of keeping the cells alive or dealing with issues of biosafety.
Central part of our efforts was devoted to integrating sample handling in disposable, custom-made autonomous LOC devices conceived to optically couple to regular cell phones, as well as CFSB functionalization for quantitative evaluation. Candidates to establish the merits of our platform was the detection of analogues for the environmental pathogens calicivirus (norovirus) and *Campylobacter*.

Results:

Design, fabrication and characterization of LOC device

A field deployable LOC for pathogen detection should have the following components embedded:

- A pump to introduce the sample
- Reagents for sample treatment
- Unidirectional valves to isolate all reagents from the environment
- Detection region with CFSB sensor embedded
- Optics to allow detection of the signal with the front camera

- Optical coupler to work with diverse smart phones

We can build upon previous results on quantitative detection on autonomous LOCs[1], however have to revise the design to suit the specific characteristics of our envisioned platform. The molecular detection of pathogenic microorganisms, characterized through the expression of specific virulence genes, requires microbial cell disruption to release nucleic acids. Hence, an important step in sample preparation for nucleic acid testing is the lysis of the organism. Gram-negative cells, as *Campylobacter*, can not be lysed by lysozyme alone, as it is the case for Gram-positive bacteria, but need pre-treatment with a detergent (e.g. Triton X-100) or a cation chelating agent (as EDTA).[2] And for cleavage of the capsid protein VP1, that protects the ssRNA(+) genome of a caliciviridae as the norovirus, SDS buffer with EDTA could be used.

Fig 1a shows the set-up we envision for our sensor platform. The device will be using a finger pump in the form of a silicon tubing, with which it is easy to introduce liquid or air in combination with a check-valve. Valves and pumps are difficult to integrate in LOC-devices and previous examples included an additional elastic element, individually mounted into the device, which made the assembly inefficient, and introduced an undesired death volume[3]. Fig 1b shows the new concept we developed of a unidirectional manifold, connected to as many injector modules as necessary (different solutions for different sample preparation steps). It employs embedded 3D printed reservoirs and connectors, onto which silicon tubing can be plugged-in, which will work as a finger pump. Each injection module has its own check-valve, which is made by a 1mm diameter hole across the unibody bulk and a 500μm diameter trim of the manifold wall, which creates a controlled 250μm long barrier held by the sealing tape´s elasticity (Fig 1c).

[1] Comina, G., Suska, A., Filippini, A. (2015), “Autonomous chemical sensing interface for universal cell phone readout” Angew. Chem. 54, 8708–8712.

[2] Salazar, O. and Asenjo J.A.(2007), “Enzymatic lysis of microbial cells” Biotechnol. Lett. 29: 985–994

[3] G. Comina, A. Suska, D. Filippini, "3D printed unibody lab-on-a-chip: features survey and check-valves integration", Micromachines 6 (2015), 437-451.

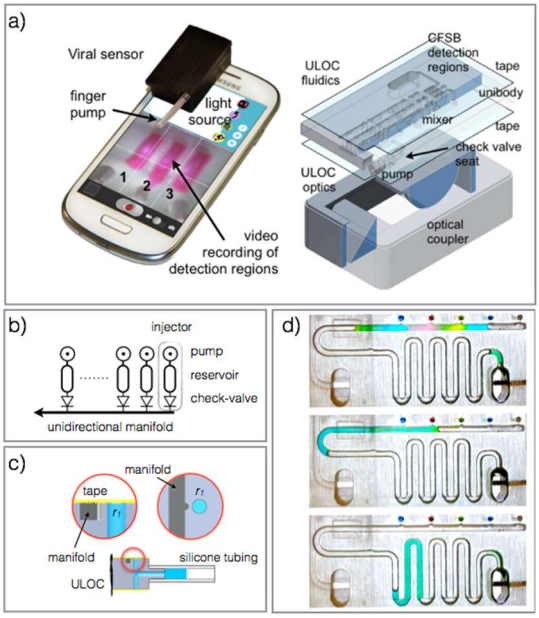

Fig. 1a): Envisioned design of the CFSB disposable sensor to be used as only accessory on cell phones. It includes 3D printed fluidics and optics components, allowing the expected type of readout captured through video recording. b) Scheme of the unidirectional manifold served by multiple injectors modules composed by a pumping element, a reservoir and a check-valve. c) Detail of the injector with the check-valve body showing the 250μm long barrier between the valve and the manifold, and the role of the sealing tape as elastic element to control unidirectional flow towards the manifold. d) Mixing performance after injection of 4 color solutions in the manifold and transport using an air injector.

The pumping triggers a short-term elastic deformation of the tape forcing the liquid across the 250μm long barrier into the manifold, and as soon as the pressure pulse has stopped, the tape reseals the barrier preventing a reflow.

In our flow regime, it possible to easily mix solutions, in contrast to classical microfluidics, which is prepared using photolithography techniques, where laminar flow regimes are dominating. This is due to the fact that our LOC channels can be several millimeters deep, and in our case also operate in transient regime, which facilitates mixing.
Successive injections of blue (10μL), red (5μL), yellow (5μL) and blue (5μL) volumes are injected in the manifold. Air transport at 7s, and 11s intervals, show mixture after this period, without additional mixing architectures required in the design (Fig. 1d).

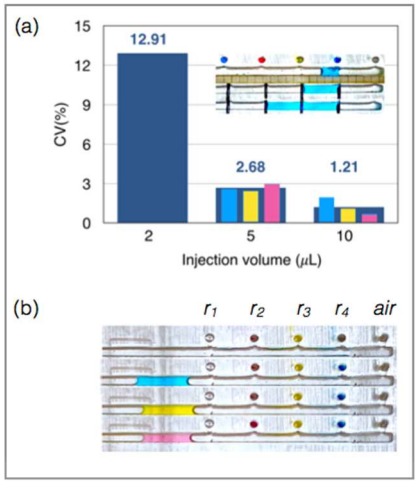

Fig. 2a) Reliability of injectors expressed as coefficient of variation for injection volumes between 2 and 10μL. b) Zoomed area on null dead volume check valves showing absence of contamination between injectors and manifold when 5μL volumes flow in the manifold.

Reliable volume metering is a critical operation for any analytical procedure, and is usually performed using pipettes and numerous disposable pipette tips. Fig. 2a shows the performance of the ULOC injectors delivering 2, 5 and 10μL aliquots respectively. The bar graph shows a collection of coefficient of variation (CV) of volumes measurements with injectors in a same device and across devices. As can be seen CV values vary between 13 and 1%, which is remarkably similar to commercial pipettes. The advantage is that once the device is loaded, dangerous or sensitive chemicals can be operated safely by untrained personnel without risking accidents, a prerequisite for a device envisioned for field use.

Crucial for the performance of this concept, is that inactive injectors should not contaminate or get contaminated from flow in the manifold. This condition was achieved by the null dead volume design of the check valves and is shown in Fig. 2b, showing one example of sequential injection of blue, yellow and red solutions, which when transported (air injector) in the manifold do not affect the color of the injector lines or becomes stained when passing the injectors locations.

Investigation of CFSB formulation and reference characterization

The CFSB formulation will be based on programmable synthetic riboregulators that control the translation of a reporter gene via the binding of a *trans*-acting trigger RNA. The switches contain a three-dimensional structure (hairpin) that blocks the ribosoms´ access to the ribosomal binding site (RBS) and the start codon. Only in presence of a complementary trigger RNA, which will be a short pathogen specific RNA fragment, sequestration of the RBS and start codon will be relieved, which will activate gene translation. To begin with, we focused our efforts to one of the two pathogens, i.e. the food poisoning pathogen *campylobacter jejuni*. Several reporter genes were purchased, allowing fluorescent as well as colorimetric read-out. These were mCherry (red fluorescent), EGFP (green fluorescent) and LacZ, an gene coding for the β-galactosidase enzyme which mediates the conversion of colorless X-gal (5-bromo-4-

chloro-3-indolyl-β-D-galactopyranoside) to a blue product (5,5'-dibromo-4,4'-dichloro-indigo) or chlorophenol red-β-D-galactopyranoside to the violet-red chlorophenol red.
Additionally, two reference plasmids were purchased, one containing a riboswitch selective for theophylline and one selective for zika-virus (Addgene, 75006), triggering the production of LacZ. These plasmids can be used as a template to genetically modify the sequence of the riboswitch in order to make it selective to defined pathogen signatures. Sequence analysis were performed using online tools at The National Center for Biotechnology Information[4] and combined with reference sequences[5].
Table 1 shows the sequences selected for the development of our campylobacter sensor using ZIKV_Sensor_27B_LacZ (Plasmid #75006, Addgene) as a backbone.

Table 1: Campylobacter (NCBI Reference Sequence: NC_002163.1), riboswitch assembly DNA

mRNA target subsequence:	AAACTCACGATAATTTCCATAACTACATCC
Sensor sequence (RNA)	uaauacgacucacuauagggCCUACAUCAAUACCUUUAAUAGCACUCAAAGGAC UUUAGAACAGAGGAGAUAAAGAUGUUUGAGUGUUACAACCUGGCGGCAG CGCAAAAGAUGCGUAAAaug
Sensor sequence (DNA)	taatacgactcactatagggCCTACATCAATACCTTTAATAGCACTCAAAGGACTTTA GAACAGAGGAGATAAAGATGTTTGAGTGTTACAACCTGGCGGCAGCGCAAAA GATGCGTAAAatg
switch reverse primer	CATCTTTATCTCCTCTGTTCTAAAGTCCTTTGAGTGCTATTAAAGG TATTGATGTAGG CCCTATAGTGAGT CGTATTAGCGC
switch forward primer	GGACTTTAGAACAGAGGAGATAAAGATGTTTGAGTGTTACAA CCTGGCGGCAGCGCA AAA GAT GCG TAAAatg

Reference plasmids were investigated and in the case for pST5832, LacZ response was selectively activated by theophylline (Fig. 03a). The reference plasmid 75006 (addgene.com) however showed a strong and immediate reporter build-up even without the presence of the analyte RNA sequence (Fig. 3b). The cell-free reaction in this control experiment used the S30 T7 High-Yield Protein Expression System (Promega) and consisted of: S30 Premix Plus (20 µl) and T7 S30 Extract, Circular (18 µl), chlorophenol red-b-D-galactopyranoside (5 µl of 0.6 mg/ml), RNase inhibitor (0.5%), and sensor DNA , i.e. circular plasmid with DNA constructs encoding the toehold sensors (1µg) in a total of 50 µl.

[4] https://www.ncbi.nlm.nih.gov/
[5] Pardee, K., Green, A. A., Ferrante, T., Cameron, D. E., DaleyKeyser, A., Yin, P., Collins, J. J., 2014. Paper-Based Synthetic Gene Networks, Cell 159, 940-954

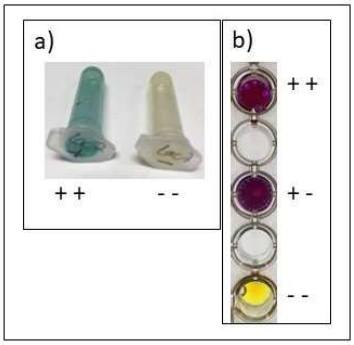

Fig.03: Positive control riboswitch with two different β-galactosidase substrates. LacZ response activated by theophylline (a) or RNA (b).+ + indicate switch and analyte, + - indicated switch without analyte, - - indicate without switch and analyte.

A possible reason for the presence of β-galactosidase enzyme activity in the negative control could be due to a strong leakage of LacZ translation, or a contamination of the plasmid preparation with β-galactosidase. The speed of the response (buildup of violet-red chlorophenol red could be seen immediately after adding the plasmid to the cell-free mixture) rather indicates the latter reason: the E.coli host, which was used to propagate the sensor plasmid, translated the encoded LacZ and plasmid extraction (GeneJET Plasmid Miniprep Kit ,Thermo Fisher) did not efficiently eliminate all the enzyme, which, together with the high sensitivity of the substrate could have lead to a strong build-up of reporter..

Outlook

Future preparations will use linearized, gel extracted sensor DNA, which will effectively exclude the possibility of enzyme contamination of the riboswitch DNA. Calculations and new insight in the riboswitch function[6] has led to the conclusion that in order to reach clinical relevant detection limits for pathogen nucleotides, an amplification mechanism must be integrated in the detection scheme. This can be achieved with the so-called nucleic acid sequence based amplification (NASBA), a method for RNA amplification, which can be smoothly integrated in the sensor chip.

Conclusion and acknowledgement

The funding has allowed us to initiate the development of disposable environmental pathogen detection for cell phones. We have been able to achieve very promising preliminary results, which will be valuable for an application beyond this seed money provided by the Åforsk Foundation, for which we were deeply grateful.

[6] Pardee, A.A. et al., (2016), "Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components", Cell 165, 1255–1266.