SAMPLE INTRODUCTION INTERFACE FOR ON-CHIP NUCLEIC ACID-BASED ANALYSIS OF *HELICOBACTER PYLORI* FROM STOOL SAMPLES

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ABSTRACT

We present a sample introduction interface that allows direct on-chip processing of crude stool samples for the detection of *Helicobacter pylori* (*H. pylori*). Efficient cell lysis and DNA extraction were achieved using stored reagents, reconstituted upon sample addition. Clinical stool samples were analysed using the device and successful amplification of a *H. pylori* specific target was achieved.

KEYWORDS: Clinical Diagnostics, DNA, Magnetic Extraction, Sample Preparation

INTRODUCTION

It is estimated that approximately two-thirds of the population harbours *H. pylori,* a Gram-negative microorganism that colonises the gastric mucosa in the human stomach and has been shown to have a significant role in the pathogenesis of gastric cancer [1]. *H. pylori* is most commonly identified using commercially available immunoassays to detect the *H. pylori* antigen in stool samples. Clinical stool samples are particularly challenging for diagnostic analysis as they exhibit high variability in terms of sample consistency, contain PCR inhibitors and have low target analyte concentrations. Here, the principle of IFAST (Immiscible Filtration Assisted by Surface Tension) [2] was adapted in order to enable the direct processing of stool samples for the detection of *H. pylori*, eliminating the need to pre-treat the sample and reducing procedural times compared to conventional DNA kits. To achieve this, the device featured a number of novel features: (i) a large-volume sample chamber that enabled a 40-fold reduction in working volume, (ii) pre-loaded solid, detergent-free cell lysis and DNA binding agents that were reconstituted by the sample itself, and (iii) a real-world interface created using a septum-based sample introduction design.

EXPERIMENTAL

The microfluidic devices were produced from PDMS and included a septum-based sample introduction design (Fig. 1). Solid chaotropic salt (guanidine hydrochloride, GuHCl) and dry superparamagnetic particles (PMPs) were stored in the sample chamber on-chip and reconstituted upon addition of stool sample. Following cell lysis and DNA binding, the PMPs were pulled via a magnet through a washing chamber containing an immiscible oil solution and into an elution chamber where the DNA was released into aqueous media for subsequent analysis. Eluted DNA was examined for purity and concentration using a spectrophotometer, and amplified using the polymerase chain reaction for the detection of the a *H. pylori* specific target (UreC gene).

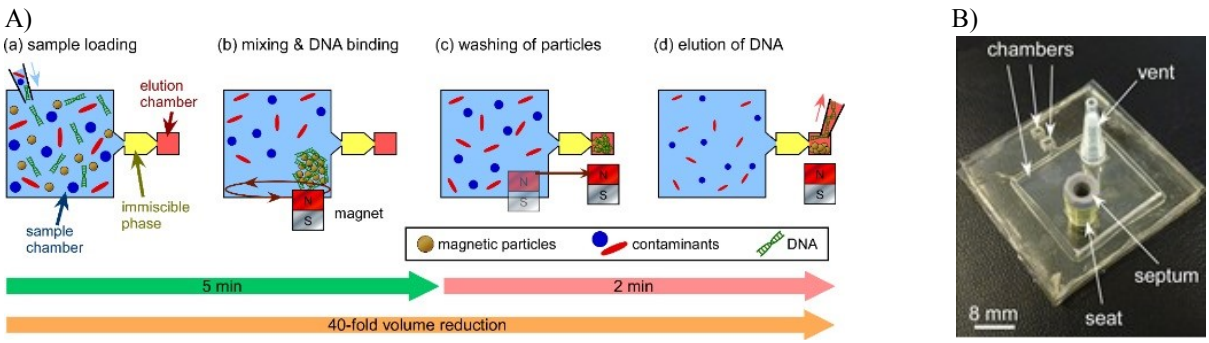

Figure 1: Schematic showing the design of the PDMS device and overview of the DNA extraction process, (a) sample loading & cell lysis, (b) mixing of PMPs with sample for DNA binding, (c) transfer of PMPs through the immiscible phase for washing, and (d) elution of DNA from the PMPs. Schematics not to scale. B) Real-world interface featuring a septum-based stool sample introduction port.

RESULTS AND DISCUSSION

Using *E.coli* as a model Gram-negative pathogenic target system, the on-chip cell lysis method, using stored reagents, was shown to be as effective ($p = 0.928$, ANOVA) as conventional off-chip lysis (Fig. 2) and reliable DNA extraction efficiencies were achieved across a wide range of added DNA concentrations (Fig. 3). Clinical stool samples were then analysed for DNA concentration and purity after processing on the IFAST device. As expected, the DNA concentrations obtained varied from patient to patient due to varying levels of infection (5.1 – 190 ng μL^{-1}, average 59.3 ng μL^{-1}). Comparison of the purity of the samples to their original appearance (based on the Bristol Stool Chart) showed a strong correlation ($R^2 = 0.96$ and $P<0.001$ Pearson's R), with more liquid samples (e.g. Types 6 and 7) producing higher extracted DNA purities (1.0 – 2.0 purity, average 1.5). Successful amplification of a *H. pylori* specific target (UreC gene – PCR product size 274 bp) was achieved on those stool samples extracted using the IFAST device.

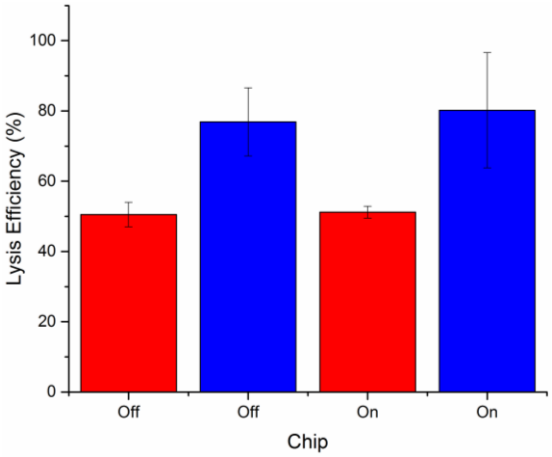

Figure 2: Lysis efficiency of the stored 5 M GuHCl reagent both on- and off-chip (n=6) for 2.54 x 10^6 (red) and 2.54 x 10^4 cells (blue).

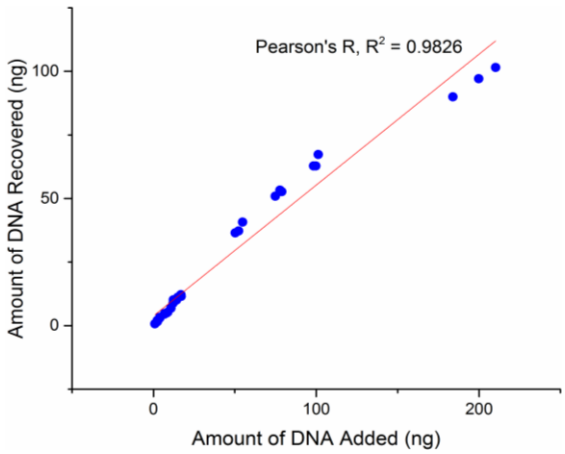

Figure 3: DNA extraction efficiency from E. coli *cells, showing a strong linear correlation between the amount of DNA added to the system (ng) and the amount of DNA recovered from the system (ng).*

CONCLUSION

This microfluidic approach offers advantages over commercial systems, such as Qiagen's QIAamp Stool DNA Mini Kits, in the time taken for analysis (a 7-fold reduction), further pre-concentration of target DNA by eluting in a volume of 10 µL compared to 200 µL and ease-of-use (e.g. multiple centrifugation steps are not required). The combination of a real-world interface for crude biological samples and rapid DNA extraction offers potential for the methodology to be used in point-of-care devices where rapid and efficient diagnosis of *H. pylori* could be important in eradicating the infection and reducing the risk of gastric cancer development.

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