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Improved sensitivity and limit-of-detection of lateral flow devices using spatial constrictions of the flow-path

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ABSTRACT

We report on the use of a laser-direct write (LDW) technique that allows the fabrication of lateral flow devices with enhanced sensitivity and limit of detection. This manufacturing technique comprises the dispensing of a liquid photopolymer at specific regions of a nitrocellulose membrane and its subsequent photopolymerisation to create impermeable walls inside the volume of the membrane. These polymerised structures are intentionally designed to create fluidic channels which are constricted over a specific length that spans the test zone within which the sample interacts with pre-deposited reagents. Experiments were conducted to show how these constrictions alter the fluid flow rate and the test zone area within the constricted channel geometries. The slower flow rate and smaller test zone area result in the increased sensitivity and lowered limit of detection for these devices. We have quantified these via the improved performance of a C-Reactive Protein (CRP) sandwich assay on our lateral flow devices with constricted flow paths which demonstrate an improvement in its sensitivity by 62x and in its limit of detection by 30x when compared to a standard lateral flow CRP device.

1. Introduction

Point-of-care (POC) rapid diagnostics have seen tremendous development in recent years, as the emphasis of care is shifting towards prevention and early detection of diseases. Such testing gives immediate results in non-laboratory settings, does not require any technical skills from the user and is relatively inexpensive, making it ideal for use not only in poorly-resourced, remote areas of developing countries, but also in hospitals and at the bedside within well-developed nations. One example of such POC diagnostics is paper-based fluidic devices, that were first proposed by the Whiteside's group in 2007 (Martinez et al., 2007), after which the field has been progressing rapidly (Cate et al., 2015). A subcategory of POC diagnostics, lateral flow devices (LFDs), are currently the primary means for the detection of a variety of analytes and the most common example of such devices is the pregnancy test that can be easily bought over the counter at a pharmacy or local shop. These are POC devices in their simplest form and even though their range of applications has been widely expanded to detection of other diseases or conditions, there has not been any other major advancement in their capabilities, which therefore limits their wider clinical uptake. LFDs possess many intrinsic advantages such as being low-cost, mass-producible, disposable, equipment free, and requiring no external power to operate.

LFDs are constructed using porous media such as paper and other types of materials, namely nitrocellulose, that facilitate various tasks, one being the immobilisation of the capture antibodies to constitute the test and control lines of the LFD. There is no need for external equipment as the sample and other reagents are transported by capillary action within these porous materials. LFDs enable implementation of two commonly used assay formats, namely the sandwich and the competitive assay (Koczula and Gallotta, 2016). In the sandwich format, which is used in this report, the analyte in the sample binds to a labelled detection antibody, usually conjugated with gold nanoparticles, and the complex is transported along the strip until it encounters a capture antibody which is specific to the analyte and is locally immobilised on the nitrocellulose membrane in the form of a test line or test spot. The capture-analyte-detection antibody complex is then visible as a coloured test line (or spot) that can be seen by eye or can be measured using a detector such as a scanner or camera. After the test line there is the control line which is used to confirm that the assay is working properly and has immobilised antibodies that are specific instead to the labelled detection antibody. Capture of these labelled detection antibodies at the control line again produces a colour change that verifies the correct completion of the assay. Surplus reagents, buffers, and assay fluids are further transported toward the absorbent pad that collects unwanted/unused waste.

One of the main disadvantages of LFDs is their relatively low sensitivities and poor limit of detection, which primarily restricts their use

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to detection of samples with highly abundant analytes (Sharma et al., 2015),(Posthuma-Trumpie et al., 2009). A critical factor that determines the limit of detection and sensitivity of a lateral flow assay is the immunoreaction time between the capture antibodies pre-deposited at a test/control line and the analyte, which therefore depends on the migration time of the sample over the test line (Bahadır and Sezgintürk, 2016).

There has been significant research aimed at developing better LFDs, both with increased sensitivity and lowered limit of detection, and several different strategies have been followed. To enhance the signal output of LFDs, many new labels have been trialled recently such as silver enhancement technology (Anfossi et al., 2013), enzymatic amplification (Parolo et al., 2013a) and magnetic nanoparticles (Tang et al., 2009). Other proposed strategies include changing the architecture of an LFD to improve its sensitivity such as increasing the size of the sample and conjugate pad (Parolo et al., 2013b) or introducing wax pillars in the flow path in order to reduce the flow rate (Rivas et al., 2014). In this current work, we present a novel strategy of using spatial constrictions in the flow path as a route to increasing the sensitivity and lowering the limit of detection of LFDs. The novelty of the technique presented here is that it allows the enhancement of the lateral flow devices regardless of the assay used, while maintaining the same materials and the same size of the device, therefore making the technique ideal for adaptation in a manufacturing process.

To demonstrate the advantages of specifically designed flow-paths for an improved LFD performance, in this report we have chosen to implement the detection of C-reactive protein (CRP). A schematic of our proposed constricted flow LFD used to demonstrate enhanced performance can be seen in Fig. 1, which provides a visual comparison of its design with that of a standard LFD.

CRP was chosen as the target analyte as it is a well understood inflammation marker,(Ahn et al., 2003) heart attack marker (Leung et al., 2005) and is also an excellent indicator for whether an infection is caused by a viral or a bacterial pathogen (Putto et al., 1985). CRP is normally present in very low concentrations in healthy individuals ($< 5\,\mu g/ml$) but rises significantly for unwell patients to values greater than $500\,\mu g/ml$ (Black et al., 2004). High concentrations of CRP in the bloodstream are not indicative of a specific disease, but quantitative measurements can provide valuable information for the diagnosis and treatment of a patient, and this is why CRP detection is broadly used in the primary care setting in conjunction with testing for other biomarkers.

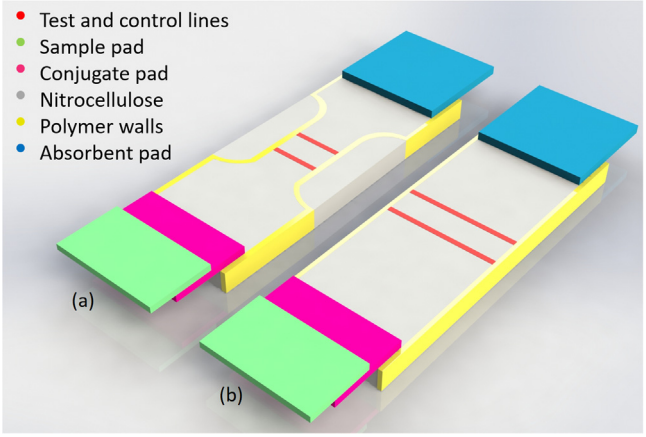

Fig. 1. Schematic of (a) the constricted flow device and (b) a standard lateral flow device.

2. Materials and methods

2.1. LDW manufacturing technique

In our previous reports, we have demonstrated the usefulness and versatility of a laser direct-write (LDW) approach in the manufacturing of diagnostic devices in porous materials such as cellulose (He et al., 2015a; Sones et al., 2014) and nitrocellulose (He et al., 2015b), as well as in creating 3d structures in such membranes (He et al., 2016). Several other approaches for patterning membranes that have also been reported are photolithography (Martinez et al., 2008), wax printing (Carrilho et al., 2009), inkjet printing (Li et al., 2010), laser cutting (Fu et al., 2010), plasma treatment (Li et al., 2008) and flexographic printing (Olkkonen et al., 2010).

Compared to these alternative techniques, LDW has some intrinsic advantages: it is a non-lithographic approach with high flexibility, and furthermore has small upfront equipment costs and no special laboratory and material requirements and hence has the potential to be upscaled for mass-production of paper-based devices at affordable costs.

Our previously reported LDW manufacturing technique relied on the immersion of the membrane in a photopolymer solution, the subsequent photopolymerisation with a laser beam, followed by the immersion of the membrane in a solvent to remove any unpolymerised residue. A decrease in the total number of fabrication-steps would however not only make this LDW process more efficient as a consequence of reduced fabrication times, but would also make it more cost-effective because of the reduced usage of expensive reagent, translating it into a truly mature technique appropriate for commercial manufacture. To optimise our original technique, we have included a deposition tool that allows localised deposition of the photopolymer only at specific locations on the paper where the fluid containing wall/structures need to be formed within the porous substrates to define the microfluidic device. This selective photopolymer deposition eliminates the (global) soaking step required to impregnate the photopolymer within the paper, prior to the laser illumination step, and furthermore also makes redundant the subsequent solvent developing step inherent in our original technique.

The LDW setup that allows the implementation of this improvised methodology is illustrated in Fig. 2. As shown in the schematic, the liquid photopolymer is locally deposited onto the paper substrate with a deposition nozzle at locations pre-defined by the user's device design. A laser beam subsequently follows the deposition head and illuminates the deposited patterns thereby inducing photo-polymerisation of the polymer, therefore transforming it into a solid state that is impermeable to liquids. The polymerised patterns define the fluidic walls that serve as demarcation barriers that confine and transport the liquids within the paper device.

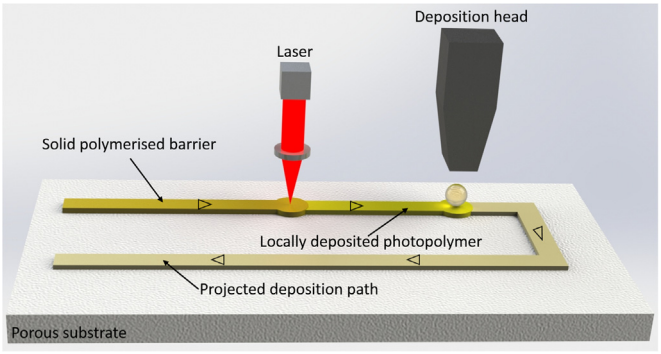

Fig. 2. Schematic of the modified laser-based direct-write procedure, which shows the inclusion of a deposition head for localised deposition of a photopolymer for creating polymerised hydrophobic structures in the porous substrate.