FLOCK-BASED MICROFLUIDIC DEVICES WITH FLOW CONTROL, REAGENT INTEGRATION AND MULTIPLEXING FOR SIMPLE ASSAYS

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ABSTRACT

Electroflocked microfibers provide a versatile material for self-driven microfluidic devices. We recently investigated laboratory-based methods for patterning microfluidic flowpaths in flocked materials and characterized capillary-driven flow along these flowpaths. Here, we use flocked devices that were fabricated using an industrial production process. We extend the concept of flock-based microfluidics by introducing ways to integrate and release reagents in flocked devices, by realizing stop-and-go flow with normally-closed valves and finally, by performing a multiplexed bioassay to detect glucose in artificial urine with positive and negative controls.

KEYWORDS: Electrostatic Flocking, Microfibers, Self-Driven Microfluidics, Glucose Detection

INTRODUCTION

There is an interest in novel materials for low-cost and simple-to-fabricate diagnostic devices. Recent research focused on paper-like materials that are patterned or cut, and assembled into microfluidic devices.[1,2] Microfibers can be fabricated at low cost, from many materials, and can be functionalized in large amounts (Fig.1a). Such fibers are commonly used in the packaging and textile industries, where they are deposited by electroflocking to produce velour-like surfaces (Fig.1b). In research, flocked materials have been used in reinforced polymer composites[3] or as scaffold for cartilage tissue.[4] Our group previously explored the wicking characteristics of custom made flocked materials in 2D and 3D flowpaths and presented flocking of fibers in a laboratory environment.[5] Here, we work with flocked devices that were produced by industrial methods, present design features for flow control and reagent integration, and demonstrate the biocompatibility of flocked devices in the case of an enzyme-amplified colorimetric assay.

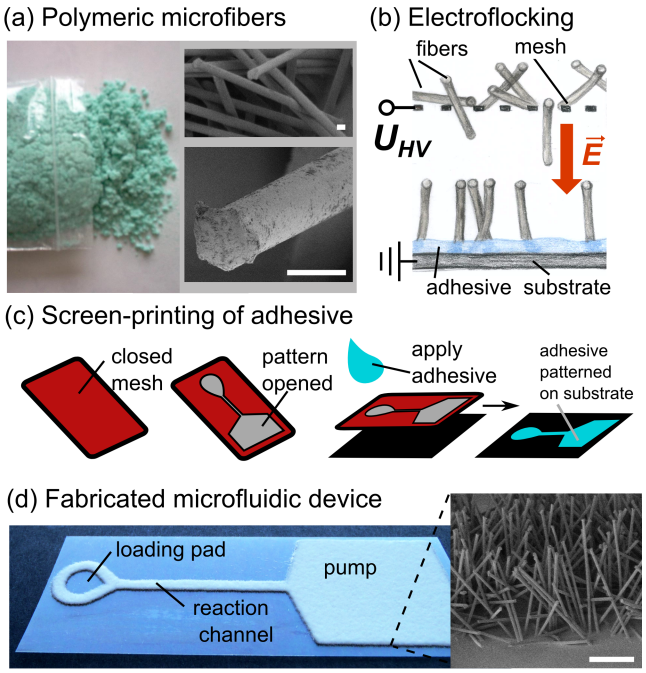

Fig. 1: Flock-based microfluidics. (a) Polymeric microfibers (scale bar: 20 µm). (b) Fibers are deposited onto an adhesive-coated substrate using a high voltage electric field. (c) Patterning of adhesive onto a substrate using a partly closed mesh. (d) Flocking of microfibers forms the wicking layer of a microfluidic chip, here comprising a loading pad, a reaction channel and a pump. Inset: SEM image of flock showing the orientation of fibers and their packing density (scale bar: 100 µm).

EXPERIMENTAL

Microfluidic flowpaths having loading pads, pumps and additional features were designed with a minimum linewidth of 1 mm and using a graphic design program. These flowpaths were flocked onto polymer substrates by a print shop (Flock+Print, Bretnig-Hauswalde, Germany) using screen-printing to pattern a layer of adhesive onto the substrate (Fig.1c) and electrostatic flocking of white nylon fibers. After drying, the devices were cut, assembled, and decorated with reagents.

To demonstrate the release of reagents 2 µL of green dye in a saturated solution of NaCl were pipetted onto the reagent zone and dried. Then, 200 µL of yellow colored water were pipetted onto the loading pad to monitor the filling and dissolution. For stop-and-go flow devices the surface area around the network was made hydrophobic by wiping it with a cotton swab dipped in a solution of 1% methacryloxytrietoxysilane in EtOH. Two substrates with flocked patterns were then placed on top of each other, flocked sides facing the center, in a way that the flocked rectangle on the top layer was aligned with the discontinuity of the channel in the bottom layer. A frame around the pattern, on both top and bottom side, served as the alignment mark and as a vertical spacer. For glucose detection experiments, 10 µL of 600 mM KI, 2 µL of 150 U/mL horseradish peroxidase (HRP) with 750 U/mL glucose oxidase (GOx) in phosphate buffered saline (PBS), and 10 µL of 50 mM glucose were spotted into the reagent zones, as indicated in Figure 3b. After drying, artificial urine, spiked with 50 mM glucose, was pipetted on the loading pad and the color change was evaluated after 10 min.

RESULTS AND DISCUSSION

Microfluidic devices, as received from the print shop, comprise flowpaths of mostly upright standing microfibers, forming an anisotropic, porous structure with hydrophilic surfaces (Fig.1d). These devices spontaneously wick liquids, pipetted onto the loading pad, and transport these through the reaction channel towards the pump. Design features in the network add functionality to microfluidic devices. Splitting and merging channels can be used to release integrated reagents in a controlled manner, similar to reagent integrators for rectangular microchannels.[6] Figure 2a shows how an incoming sample splits into a channel holding reagents and two diluter channels, and then rejoins. The contribution of each channel to the total flow is defined by their hydraulic resistances ($R_{reagent}$ and $R_{diluter}$). Their ratio sets the dilution of reagents dissolved in the reagent channel after the point, where the channels merge. A stop-and-go flow, using a normally closed valve, is shown in Figure 2b. The valve is based on a discontinuous flowpath that stops the filling of liquid at a certain position. Pushing down the cover of the flocked device closes the discontinuity by inserting fibers from the top layer into it. As a consequence thereof, the liquid can pass through the valve, similar to capillary soft valves in microchannels.[7] Furthermore, flocked devices can be used to perform biological assays, such as the detection of glucose in urine. An enzymatic oxidation of glucose by glucose oxidase (GOx) forms hydrogen peroxide, which is then visualized by the oxidation of iodide to brown iodine in the presence of horseradish peroxidase (HRP), see Figure 3a.[8] Splitting of flowpaths allows a user to perform multiple reactions at the same time. In this case, two additional channels provide glucose or omit GOx for positive and negative internal controls, respectively (Fig.3b). Figure 3c presents experimental results for the detection of 50 mM glucose in artificial urine after an incubation time of 10 min. The presence of brown-colored iodine in the signal detection zone of the glucose detection channel can be attributed to glucose in the sample. The positive and negative controls result in a more intense color and no change in color, respectively. This suggests that the signal is specific to the glucose in the sample. The inhomogeneity of color in the signal detection zone is most likely related to drying effects and evaporation-based transport of iodine molecules to the borders of the detection zone. Furthermore, it should be noted that the enzymatic activity in this experiment is maintained and we do not see a much evidence of chemical substances leaking out of the adhesive or being washed off the fibers.

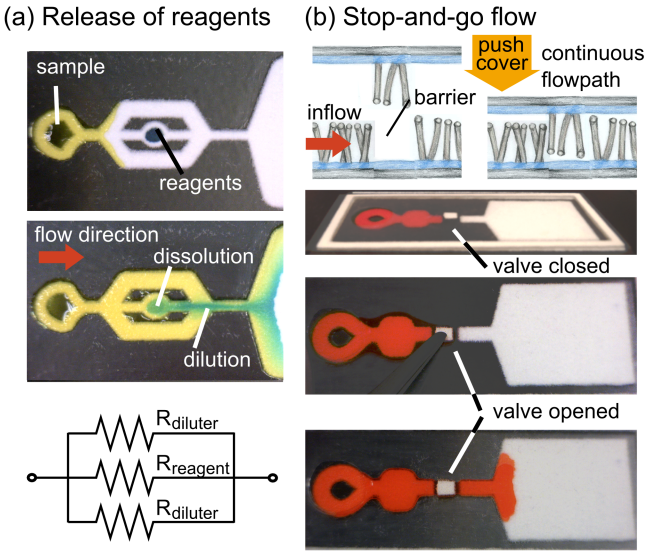

Fig. 2: Design features for flock-based microfluidics. (a) Green, dry colorant is dissolved in a stream of sample and diluted in streams that bypassed the reagents. Bottom: electrical equivalent circuit representing the contribution of each channel to the total network. (b) Discontinuous flock patterns stop an advancing filling front of a red-colored liquid. Pushing down a flocked cover continues the flowpath.

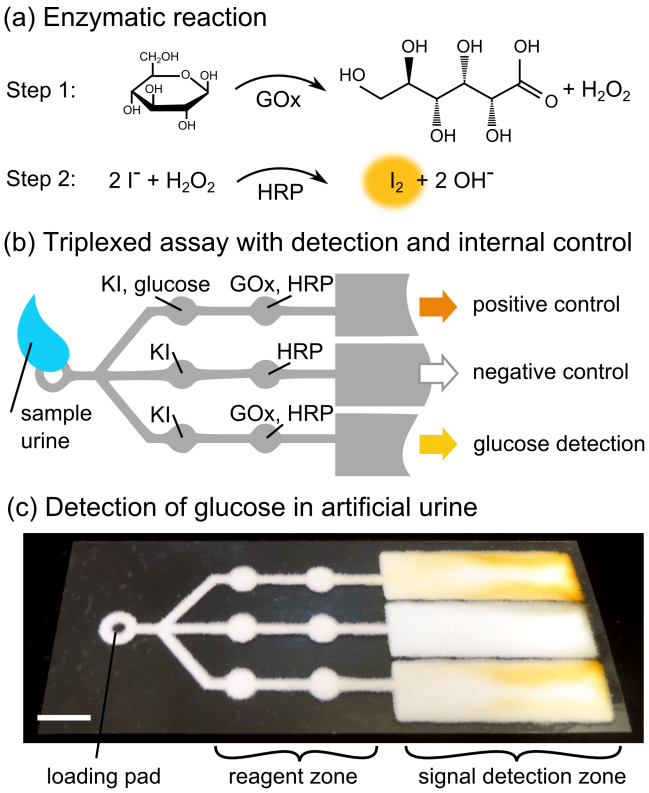

Fig. 3: Detection of glucose in urine. (a) The assay involves an oxidation of glucose to gluconic acid under formation of H_2O_2, which is then used to convert KI into brown I_2 by HRP. (b) Parallel flowpaths simultaneously enable a positive/negative control and a detection of glucose in the sample. (c) Experimental results for 50 mM glucose in artificial urine, 10 min after the sample was pipetted onto the loading pad. (Scale bar: 8 mm)

CONCLUSION

Flock-based microfluidics provide an exciting concept for the development of diagnostic devices. We foresee the functionalization of microfibers with reagents before flocking as a convenient method for integrating dissolvable and surface-immobilized reagents into microfluidic devices. In contrast to the fabrication of a liquid-handling platform, followed by loading it with reagents using pipetting robots and pick-and-place tools, electroflocking has the potential to build a microfluidic flowpath and integrate reagents at the same time. As a result, the flexibility to manufacture devices for different applications is increased and quality control of single components can be done before their assembly, which is beneficial for production yields.

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