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Controlled, synchronized actuation of microdroplets by gravity in a superhydrophobic, 3D-printed device

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HIGHLIGHTS

- A 3D-printed device was coated with a superhydrophobic spray and placed in a holder.
- Controlled actuation of 10–50 μL droplets was possible by gravity alone.
- The superhydrophobic coating allowed actuation of various biological fluids.
- Using an integrated microelectrode, glucose in droplets of rat serum was measured.
- The device is readily customizable for other applications.

GRAPHICAL ABSTRACT

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ABSTRACT

Droplet manipulation over open surfaces allows one to perform assays with a large degree of control and high throughput, making them appealing for applications in drug screening or (bio)analysis. However, the design, manufacturing and operation of these systems comes with high technical requirements. In this study we employ a commercial, low-friction, superhydrophobic coating, Ultra-Ever Dry[®], on a 3D-printed microfluidic device. The device features individual droplet compartments, which allow the manipulation of discrete droplets (10–50 μL) actuated by gravity alone. Simply by angling the device to normal in a 3D-printed holder and rocking in a “to and fro”-fashion, a sequence of droplets can be individually transferred to an electrochemical microelectrode detector and then to waste, while preserving the (chronological) order of samples. Multiple biological fluids (*i.e.* human saliva, urine and rat blood and serum) were successfully tested for compatibility with the device and actuation mechanism, demonstrating low slip angles and high contact angles. Biological matrix (protein) carryover was probed and effectively mitigated by incorporating aqueous rinse droplets as part of the analysis sequence. As a proof-of-concept, the enzyme-coupled, amperometric detection of glucose was carried out on individual rat serum droplets, enabling total analysis in ≈ 30 min, including calibration. The device is readily customizable, and the integration of droplet generation techniques and other sensor systems for different analytes of interest or applications can be realized in a plug and play fashion.

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1. Introduction

Precise control over (sub-)microliter-size volumes of fluids is one of the principal benefits of microfluidics. The predictability of fluid movement in complex geometries, created through micro-engineering, allows for a large degree of on-chip experimental control and versatility. Device functionality can be further increased by incorporating (microfabricated) sensors. Droplet-based platforms differ from continuous flow-based microfluidics in that the small volumes manipulated are in fact discrete droplets [1–3]. This presents a number of advantages. For example, generating higher throughput in a system is relatively facile through parallelization. Without the need to significantly increase size or complexity, multiple analyses can be run on a single device [1,2]. Droplets containing analytes or reagents can be actuated and mixed with each other with remarkable precision, either over surfaces [2,4] or through channels in multiphase systems [1,3] (based on the immiscibility of liquids or gases). Furthermore, the high surface-area-to-volume ratios of the droplets enable fast reaction times, e.g. for mass transfer or diffusion processes [4,5]. As a result, droplet microfluidics has been utilized for high-throughput applications in biochemical assays [6–8], drug development [9,10] and in point-of-care diagnostic tools [11]. The simplicity and robustness of droplet microfluidic devices combined with biosensors [12] has also been exploited to develop a droplet-based diagnostic tool for malaria [13,14]. We focus on systems where droplets are actuated over hydrophobic surfaces. In these systems parallelization for high-throughput, reproducible analysis and manipulation of samples and reagents can be performed with the greatest flexibility, since the droplets are separate, discrete containers [2,4]. This is especially interesting for the manipulation, maintenance and sequential actuation of droplet series (e.g. samples from continuous-flow based external devices).

Electrowetting on dielectric (EWOD) is a common technique for droplet actuation over open surfaces. Here, the macroscopic contact angle (CA) of a droplet on a hydrophobic surface is changed when a potential difference is applied between the droplet and a dielectric-coated electrode. This facilitates precise droplet manipulation when an electrode array is utilized [4,15,16]. However, the use of EWOD devices in diagnostic tools is limited, as contamination of the hydrophobic surface due to protein adsorption is regularly reported, necessitating employment and optimization of surfactants [17]. Another disadvantage is the high level of technical requirements for laboratories that aim to produce and operate EWOD-based devices. A different approach to droplet actuation in microfluidic devices employs magnetic fields. In this case, droplets consisting of a liquid with high magnetic susceptibility are actuated by applying a magnetic force in a desired direction. Alternatively, paramagnetic microparticles added to the droplets can be used to move these droplets with applied magnetic force. In this approach, known as magnetofluidics, external, permanent magnets [18,19] or built-in electromagnets are used [20,21]. Magnetofluidics, too, is most often performed on hydrophobic surfaces ($CA \geq 120^\circ$).

Superhydrophobic (SH) surfaces, inspired by dust- and water-repellant wax coatings seen in nature (e.g. *Lotus* leaves [22]), give rise to CAs greater than 150° for water droplets. As a consequence, an oil phase layer, which is often used to surround droplets on hydrophobic surfaces to further decrease friction and prevent evaporation of the water phase, is no longer necessary. Like their naturally occurring counterparts, synthetic SH surfaces exhibit high CAs due to micro- and nanometre scale features. This phenomenon has been thoroughly described in the Cassie-Baxter model [19,23]. The advantages of SH surfaces have also been recognized in the context of analytical devices. One example is the enhanced analysis of biological fluids using paper spray-based mass spectrometry,

facilitated by the use of SH paper substrates [24]. Patterned SH surfaces have also been applied for sample-confinement on MALDI-TOF mass spectrometry target plates [25] and as substrate for protein micro-arrays [26]. Interestingly, magnetofluidics on a commercially available SH coating has also been demonstrated [19,27,28]. In the work of Mats *et al.* [19], amorphous polytetrafluoroethylene (PTFE, or Teflon® AF, a hydrophobic coating commonly used in DMF), *Colocasia* leaves (a natural SH surface) and Ultra-Ever Dry® (UED, a SH formulation of fluorinated silica nanoparticles) were compared with respect to their suitability for actuation of droplets using superparamagnetic particles. Both the natural and commercially available SH surface enabled magnetic droplet actuation at relatively low magnetic particle concentrations, and forces as low as 900 nN were required to actuate a 10- μ L droplet. The PTFE surface exhibited higher friction, however, which prevented easy droplet actuation at the particle concentrations studied.

In fact, the dramatic decrease in surface-droplet adhesion when using SH substrates makes actuation of droplets by mere gravity possible. Gravity actuation has the potential to significantly decrease the complexity, improve the robustness and thus enhance the potential of (disposable) point-of-care diagnostic devices, especially in low-resource settings. This study presents a simple, yet elegant, compartmentalized, 3D-printed device to sequentially deliver droplets by gravity-actuation to an electrochemical detector. The device is aerosol-coated with UED to minimize droplet adhesion. It is placed in a holder that positions it at an angle relative to normal, to establish a potential gravitational energy. The device is then rocked in a “to-and-fro” fashion, to transfer droplets between offset compartments along opposite sides of the device. With each rocking motion, each droplet is brought one compartment closer to the detector compartment at the end of the device. Discrete transport of a sequence of droplets towards a detector housed within one of the compartments of the device can now be accomplished. Moreover, during the actuation of the sequence, the (chronological) order of the droplets in which droplets were sampled or added is preserved. As a proof-of-concept, an electrochemical sensor is integrated and used for glucose measurement in droplets of rat serum with free enzyme. The 3D-printed device we present is readily customizable and would be compatible with a variety of detection methodologies.

2. Materials & methods

2.1. Superhydrophobic surface coating

UED was purchased from Jeroen van Beurden Special Products (Vlijmen, The Netherlands) as a two-component coating set, an adhesive base coat and a top coat containing fluorinated silica nanoparticles. Standard glass microscope slides (Thermo Fisher Scientific, Breda, The Netherlands) were aerosol-coated with the base coat and left to cure for approximately 3 h at room temperature. The glass was then coated with the SH top layer, which was left to cure overnight, also at room temperature. Contact angle measurements and assessment of protein sorption on the surface were performed on surfaces treated in this way (see Section 1 of the Supplementary Information (SI) for detailed methods).

2.2. 3D-printed device and holder

To allow droplets to be actuated by gravity, a compartmentalized device was designed to envelop the glass substrate. Design of the device was done in SolidWorks (Waltham, MA, USA) – see Fig. S1 in SI. The device was printed in polylactic acid (PLA) (EasyFil filament, $d = 1.75$ mm, Formfutura, Amsterdam, The Netherlands)

using a fused deposition modeling 3D printer (Felix, de Meern, The Netherlands, version 3.0). Work with this printer has been described in detail previously [29]. In this case, a heated (202 °C) nozzle was used to deposit PLA on a heated (55 °C) printing bed. After the first 16 layers were extruded, the printing was paused to place a glass microscope slide on top. This functions as a permanent bottom surface in the device. Printing was then resumed. A second, removable microscope slide was added after layer 44, which can function as a lid. After the device was completed, the top surface of the device – including the bottom glass surface – was coated with UED as described above (2.1). A holder for the device was also designed and printed using the same settings – see Fig S2 through S6 in SI. Both the holder and device feature slots through which sensors can be inserted or waste droplets can be removed – see Fig. 1A.

The holder contains a threaded pin that allows adjustment of the angle at which the device is fixed in the holder with respect to the normal (see Fig. 1B–C). When the holder is rocked back and forth, droplets placed in the compartments of the device can be sequentially moved to the next lowest compartment on the opposite side of the device – see Fig. 2 and the video in the SI. This is done in a synchronized and controlled way, which maintains the order (sequence) of droplets. After interrogation, the droplets are removed from the device using a vacuum line connected to a waste collection port.

Supplementary video related to this article can be found at <http://dx.doi.org/10.1016/j.aca.2017.08.010>.

2.3. Sensor preparation

Platinum needle-type microelectrodes were used for the measurement of glucose in combination with miniaturized Ag/AgCl reference electrodes made using the method described by Wahono *et al.* [30] A description of electrode fabrication can be found in Section 2 of the SI. Both the reference and working electrode were inserted through a small piece of PEEK tubing (ID 750 μm , OD 1.5 mm, Da Vinci Laboratory Solutions, Rotterdam, The Netherlands) and fixed with a drop of epoxy glue (see Fig. 3A). The PEEK tubing with the electrodes was inserted in the sensor slot of the 3D-printed device. The tubing with fixed electrodes protruded

Fig. 2. Compartmentalized droplet actuation. (A) Top view of the device containing droplets in the compartments. Rocking the holder towards the operator causes the droplets to move down, into the next compartment. (B) Rocking the holder away from the operator moves the droplets to the previous side of the device, however, each droplet is now located one compartment below its previous position. (C) The first yellow droplet can now be interrogated by the sensor. (D) The next time the device is rocked, the measured droplet is transported to the waste outlet, vacating the measurement position. The next (blue) droplet can be moved to the sensor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

about 1 mm (see Fig. 3B) into the sensing compartment of the device, and was fixed and sealed with a drop of silicone glue (RS Components B.V., Haarlem, The Netherlands). The use of silicone glue allows easy removal and exchange of the sensor. In this way, the device can be recycled for new experiments.

2.4. Determination of glucose in rat serum using a standard-addition approach

Glucose is not electrochemically active, but it can be

Fig. 1. (A) The 3D-printed holder (black) and device (gold) printed in PLA. Both the device and holder have ports through which waste droplets can be removed with vacuum, or sensors inserted. See Figs. S1–6 for dimensions. (B) Side-view of the holder with the curved bottom part removed. The steel pin in the back can be used to set the angle that allows gravity to move the droplet sequence in the direction of the sensor and waste. By tilting the substrate $\approx 6^\circ$ and rocking the device, 30- μL droplets can be actuated. (C) As shown in this end view of the holder, the bottom of the holder is curved in such a way that rocking of the device in a “to-and-fro” fashion is easily done. (D) Schematic diagram of the needle-type working electrode used for the detection of glucose in serum. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. (A) A working electrode (WE, yellow lead) and reference electrode (RE, blue lead) inserted in a piece of PEEK tubing, and fixed with a drop of epoxy glue. (B) The PEEK tubing is inserted into the sensor port of the 3D-printed device, after which the electrodes protrude into the measurement compartment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enzymatically converted by glucose oxidase, producing H_2O_2 , which can be oxidized on an electrode surface at an appropriate potential. Equations (1) and (2) show the chemical reactions associated with both the oxidation of glucose and hydrogen peroxide to generate an electrical current.

Glucose oxidase (Sigma) was dissolved in water to prepare a stock solution with a concentration of 100 U/mL. Fifty- μL aliquots of this solution were then dispensed into cups and stored at -20°C for no longer than 3 months. D-glucose (Merck) was dissolved in 10 mM phosphate-buffered saline (PBS; from tablets, Gibco, Thermo Fisher Scientific, The Netherlands, pH = 7.5) to make a 50 mM stock solution. Rat serum (50 μL , Envigo, Horst, The Netherlands) was mixed with 10 μL of glucose oxidase (Sigma), spiked with different amounts of the glucose stock solution and filled to 100 μL with PBS to make 5, 10 and 15 mM glucose solutions. One sample did not contain glucose oxidase and was used for a background measurement. The sample from which the glucose content was to be measured contained only enzyme, serum and PBS. All tubes were incubated at 37°C for 30 min in order to convert all glucose to H_2O_2 . Thirty- μL droplets of each (spiked) sample were pipetted in sequence into the compartments of the device. By rocking the holder of the device back and forth, the sequence of droplets was moved to the sensor for interrogation. Each sample droplet was bracketed by three 30- μL washing droplets of Millipore-water to rinse the sensor and prevent carryover. Neither the reference nor working electrode did at any point protrude out of the droplets. Using a FEMTO DDP-300 current amplifier (Berlin, Germany) as a potentiostat, a potential of +700 mV vs. the Ag/AgCl reference electrode was applied to the Pt working electrode. At this potential, the H_2O_2 that was generated by the enzyme during glucose conversion was oxidized. This amperometric set-up registers the current generated from the resulting electron transfer at the electrode surface, which is directly proportional to the original concentration of glucose. Measurements were performed for 10 min per droplet. The average current of the last 30 s of each measurement was used for the analysis. After all the droplets had

been interrogated, a line was plotted through the linear portion of the calibration curve. The standard addition method was then used to calculate the concentration of glucose in the original sample. The limit of detection (LOD) of each sensor was calculated using the baseline noise (standard deviation of the background sample read-out) and sensitivity of each sensor.

3. Results & discussion

3.1. Characterization of the superhydrophobic surfaces and 3D-printed device

Contact angles of the biological fluids (*i.e.* water, human saliva and urine, rat serum and blood) were measured by pipetting appropriate volumes of the fluid on UED-coated glass slides. Photographic images (*e.g.* Fig. 4) of these droplets were analyzed in ImageJ to ascertain the contact angle. Fig. 4 also displays the results of these measurements, presented as averages $\pm$ SEM ($n = 3$).

All measured contact angles exceed 150° . In all cases, the fluids tested were easily actuated inside the 3D-printed device, as can be seen with (dyed) water in the SI video. Sequences of droplets can be transported without loss of the (chronological) order in which the droplets were placed in the compartments. The angle under which the device was placed using the long steel screw attached to the holder was $\approx 6^\circ$ for 30- μL droplets. The device enables this angle to be easily adjusted depending upon the surface-droplet adhesion. The device provided consistent actuation of 20- μL droplets when angled from 4 to 40° (*i.e.* from one compartment to another below and on the opposite side of the device 100% of the time over the course of five trials at approximately 5° increments). The device was manually rocked from an angle of 30° – 150° (*i.e.* 120° of motion) over a 1-s interval (see SI video). Below 4° the droplet would not reproducibly fall to the next compartment and above 40° the droplet would fall more than one compartment or to the bottom of the device. Generally, smaller droplets could be reproducibly actuated at higher tilt angles.

The sliding angles for 20- μL droplets of fluids tested (*i.e.* urine, saliva, serum, blood, water) were all less than 5° and could be actuated in a device angled $\approx 6^\circ$. Given the observed contact angles on the surface, and the smaller angle necessary to actuate the droplets on the device ($<10^\circ$), the substrate can indeed be characterized as superhydrophobic. Droplets of 30 μL were used for later electrochemical interrogation, although droplets as small as 10 μL and as large as 50 μL can be used without fission when colliding with the opposing walls. However, in the case of viscous biological fluids (*i.e.* human saliva, rat blood and serum), droplets smaller than 20 μL were difficult to pipette onto the surface due to the relatively high hydrophilicity of the pipette tip compared to the surface. Smaller droplets might be used when a different system is employed to dispense the droplets. Devices can be coated multiple times if the SH layer is damaged, for example by a pipette tip. After thorough rinsing with water, devices were re-coated up to at least 3 times in this study without loss of performance when actuating droplets.

Sample carryover can be a considerable problem for devices and methods, contributing to poor reproducibility. This is especially cumbersome when analytes are either flowed or actuated through or over the same device real estate. Proteins are particularly problematic and often necessitate the use of surfactants to mitigate adsorption to surfaces. We evaluated the carryover with electrochemical measurements on droplets before and after aqueous rinsing (see 3.2), and the protein sorption using a standard protein assay (Bio-Rad DC) on coated and uncoated wells of a polystyrene (PS) 96-well plate. Both methods showed that adsorption and sample carryover are mitigated with the addition of aqueous

Fig. 4. Droplets of different biological fluids on glass microscope slides coated with UED. Contact angles are displayed as averages $\pm$ SEM ($n = 3$). Volume of droplets: water and urine droplets were 10 μ L, saliva and serum 30 μ L, and blood 50 μ L.

rinsing steps between droplet analysis. Protein staining was performed to qualitatively assess the extent of protein sorption, and examine the utility of a simple droplet wash step to remove adsorbed proteins. Water (30 μ L) was deposited in uncoated and coated PS wells for 60 s, and then removed, followed by the addition of protein staining reagents. In other coated and uncoated wells 30 μ L of serum was deposited for 60 s in each well and removed, after which protein staining reagents were added. To a third group of wells 30 μ L of serum was added, but each well was rinsed three times with 30- μ L water droplets for 10 s each time. A photograph of the resulting staining of the reagents for each condition can be seen in the SI (Fig. S7). The contents of each well were then transported to a clean, transparent 96-well plate, after which the absorbance was measured at 650 nm. The average absorbance values are displayed in Fig. 5. In both coated and uncoated wells exposure to only water leads to minimal absorbance. However, when only serum was added, protein material is stained, as is reflected in the absorbance measurements. Interestingly, the samples from UED-coated wells result in significantly ($p < 1 \times 10^{-7}$) lower absorbance measurements ($75.5\% \pm 4.90$). The absorbance in wells that were washed after exposure to serum droplets was lower than without washing in both uncoated ($84.8\% \pm 1.64$ decrease) and coated ($83.3\% \pm 3.49$ decrease) wells. However, the absorbance after washing in uncoated wells was significantly higher ($p < 1 \times 10^{-7}$) than in the uncoated control wells (exposed to only water). In contrast, in the case of UED-coated wells no significant difference between the absorbance measurements in washed wells

Fig. 5. Average absorbance ($\pm$ SD; $n = 3$) as measured after the protein adhesion testing using the Bio-Rad DC Protein Assay. Uncoated and UED-coated wells were exposed to either water, only serum or serum followed by three rinsing water droplets.

and coated control wells was found. Although the protein tests were conducted in polystyrene microwells, the substrate material should not affect protein sorption since the material is completely covered by the coating. This indicates that rinsing with water droplets is effective in preventing significant carryover of protein material on surfaces coated with UED, as would be the case when performing a sequential analysis of biological fluids.

3.2. Glucose sensing

A wide range of observed glucose concentrations in serum from non-diabetic rats has been reported, with values at least as low as 4.6 mM [31] and as high as 8.5 mM [32]. Therefore, a sensor that aims to measure physiologically relevant concentrations in non-diabetic rats should have a linear dynamic range that at least spans this concentration range. Droplet-based, enzyme-coupled glucose assays have been reported recently, employing both optical [33] and electrochemical [34] detection methods. These devices employ channels to manipulate and actuate droplets to perform on-chip mixing of (immobilized) enzyme and substrate-containing droplets. They feature complex structures that make them hard to operate and, especially, fabricate in non-specialized labs. The facile approach we have developed to perform a glucose assay in the 3D-printed and SH-coated device involves first mixing a glucose calibration sample with enzyme (glucose oxidase), and then incubating the sample for 30 min to allow the glucose to be converted into the electroactive hydrogen peroxide. The assay utilizes a standard addition approach where known amounts of analyte are added to the sample at three different concentrations, and then linear regression is used to determine the unknown concentration. Standard addition is particularly useful for samples possessing a complex matrix (such as serum) where blank matching would be difficult.

Results of the glucose assay conducted on 30- μ L rat serum droplets using the integrated needle-type electrodes are depicted in Fig. 6. Considering the fact that most sensors did not yield a linear response for the droplets containing 15 mM added glucose, only the 0, 5 and 10 mM samples were used to calculate the original concentration of glucose in the rat serum. These concentrations cover the physiologically relevant concentrations.

The average measured glucose concentration lies within the range that is expected in rat serum [31,32]. Furthermore, the sensors showed good linearity up to at least 10 mM standard added to the serum sample. It follows that the linear range of these sensors is well within the physiologically relevant range. The small variations in the performance parameters of the sensors are a consequence of the manufacturing process. For example, electrode fabrication is conducted manually and thus the reproducibility of the Nafion coating and surface area of the Pt wire extending from the silica capillary (see Fig. 1D) is limited. Up to 10% variation of length of the

Fig. 6. Typical amperogram of a calibration of a sensor using the standard addition method. Each measurement is performed in a 30- μ L droplet. First a background measurement is performed (droplet containing just serum and PBS), followed by analysis of (spiked) droplets containing the sample (serum, PBS, enzyme) and increasing concentrations of glucose (5 and 10 mM). After every analyte droplet three washing droplets (30 μ L Millipore water) are moved past the sensor to prevent carryover of signal (see inset). The efficiency of these washing steps is also reflected in the linearity of the calibration lines, displayed in the lower right corner (three different sensors). The sensor parameters and measured glucose concentration in rat serum droplets are shown in the table.

working electrode alone is to be expected (unpublished results).

Fig. 6 also shows a typical amperogram for a sequenced analysis of droplets in the 3D-printed device. The inset presents a magnification of the amperogram, showing the washing of the sensor between droplets containing sample only and 5 mM added glucose. The three 30- μ L water droplets decrease the generated current substantially; after the 5 mM droplet, washing results in an average $91.3\% \pm 2.6$ ($n = 3$) decrease of the generated current to levels similar to the blank measurement. Combined with the good linearity of the sensors (see lower right corner of Fig. 6), and the results from the protein adhesion test (see Fig. 5), this shows that carryover of the analyte can be effectively prevented on the SH coating. Fig. 6 also shows the relatively long response time for the sensors: measurements of 600 s per concentration were necessary. This may at least partially be explained by the fact that the droplets are stagnant when in contact with the sensor, and diffusion is the only means of transport for H_2O_2 towards the electrode (the diffusion coefficient of H_2O_2 is $1.46 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ at 25°C [35]). However, a complete measurement including calibration of the sensor (sample, 5 and 10 mM standard addition) takes ≈ 30 min.

Reduced incubation time would be achieved by immobilizing glucose oxidase on the electrode surface, for example in a hydrogel [36], or using a cross-linker [37]. Immobilizing enzymes has the advantage that less enzyme is needed to achieve similar performance. Also, as the electroactive hydrogen peroxide will then be

generated close to the electrode surface, response time will likely decrease. However, the enzyme layer can suffer from both (bio) fouling and general loss of enzyme or enzyme activity, leading to a decrease in the sensitivity of the sensor [38,39]. Furthermore, the manufacturing process of the sensors would become more complex and time-consuming.

Although an electrochemical detector was used in the detection port for this study, it follows that other detectors (e.g. optical fiber-based detectors) can be easily integrated within the gravity-actuated droplet device. Through the use of 3D printing techniques, prototyping of new designs can now be performed with unprecedented flexibility and speed [40]. In the future, interfacing of the presented device with continuous-flow microfluidic devices and off-chip analytical instrumentation will also be explored. As was recently described, the capability to store and manipulate sequences of droplets in chronological order can be combined with fluorescence-based detection of amino acids [41], or the detection of peptides using mass spectrometry [42]. In both these studies capillaries were used to guide the sequences of droplets, but the principle could also be reproduced in open surface systems. Finally, a continuous-flow based device could be employed to generate smaller droplets [5,43] which can then be actuated using gravity in the presented device.

4. Conclusion

The combination of 3D printing with the commercially available, superhydrophobic coating, Ultra-Ever Dry, allows research labs to develop customized devices. Due to the superhydrophobicity, droplets can be actuated using gravity alone. Through the utilization of coated substrates with 3D-printed compartments, and control of the tilt angle and movement of the device with a 3D-printed holder, droplets can be translated discretely, and in sequence, to undergo detection. It was shown that the typical high contact angles ($>150^\circ$) associated with SH surfaces are also observed when biological fluids were applied to UED, and that these fluids can also be gravity-actuated in the 3D-printed device. Protein staining revealed that the UED-coated surfaces can be effectively washed with droplets of water to eliminate carryover of biological matrix when serum is used, which agrees well with our electrochemical measurements. The gravity-actuated device was equipped with a needle-type working electrode and miniaturized reference electrode. Using the standard addition approach, the glucose content of rat serum was amperometrically analyzed. The sensors exhibited a linear response in the physiologically relevant concentration range. It was also shown that incorporation of three, 30- μ L water droplets bracketing the serum samples in the analysis sequence effectively prevented carryover of the analyte. The fact that 3D printing is used in the fabrication of the devices allows for very flexible customization and sharing of (existing) designs. This also enables the facile integration of different sensors for other analytes of interest. Future work will focus on expanding the analytical palette in the device, as well as the development of diagnostics-oriented applications. Of particular interest as well is the use of this droplet-handling approach to “discretize” or digitalize the output of continuous-flow microfluidic devices for off-chip analytical instrumentation. Our future experiments will also focus on the implementation of different 3D-printed structures that will allow the controlled fission and fusion of microdroplets in superhydrophobic devices. The approach presented in this work will improve the accessibility of development of diagnostic tools, especially in resource-limited situations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.aca.2017.08.010>.

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