



A suspending-droplet mode paper-based microfluidic platform for low-cost, rapid, and convenient detection of lead(II) ions in liquid solution

Han Sun^a, Wanbo Li^a, Zhen-Zhen Dong^a, Chong Hu^a, Chung-Hang Leung^{b,*},
Dik-Lung Ma^{a,c,**}, Kangning Ren^{a,c,d,**}

^a Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

^b State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China

^c HKBU Institute of Research and Continuing Education, Shenzhen, China

^d State Key Laboratory of Environmental and Biological Analysis, The Hong Kong Baptist University, Waterloo Rd, Kowloon, Hong Kong, China

ARTICLE INFO

Keywords:

Paper-based microfluidic device

Superhydrophobic

Lead(II) ion

Iridium(III) complex

Smart phone

ABSTRACT

A paper-based microfluidic device based on unconventional principle was developed and used to detect lead ions through a two-step process including heated incubation and subsequent mixing. The device was made by generating a superhydrophobic pattern, which defines channel and reservoir barriers, on a water-impermeable paper substrate, followed by loading and drying the reagents in the defined reservoirs. Different from the conventional paper-based devices that are made of water-permeable paper, the as-prepared device holds water drops in discrete reservoirs, and the water drops will not move unless the device is tilted along the direction of the predefined channels. In this way, the liquid samples applied onto the device are handled as individual drops and could be stored, transported, and mixed on demand. Different from the conventional paper-based devices that use capillary force to drive liquid, our new device uses wetting and gravity as driving force. We name this operation principle suspending-droplet mode paper-based device (SD-μPAD). The use of a Teflon contact-printing stamp makes the production of such devices rapid, cost efficient, and mass productive. Utilizing a G-quadruplex-based luminescence switch-on assay, we demonstrated rapid, convenient, highly sensitive, and low cost detection of lead(II) ions in water samples, using a custom made battery-powered portable device, and a smart phone as the detector.

1. Introduction

Since the industrial revolution, heavy metal pollution has become a major and still growing threat to human health. Lead(II) ions are one of the major sources of water contamination, especially in drinking water, which causes serious physical defects in human body, like nephropathy, hepatopathy and encephalopathy (Gumpu et al., 2015; Needleman, 2004; Riess and Halm, 2007; Shahat et al., 2015; Verma and Gupta, 2015). Recently, reports of excessive levels of lead(II) ions (> 50 nM, Drinking Water Quality Standards of World Health Organization (WHO)) in the tap water of housing estates or schools have increased in number. In addition, high levels of lead(II) ions in the bloodstream of children have also been reported (Patrick, 2006). According to the report from WHO, 143 thousand people died and 600 thousand children suffered from intellectual disabilities caused by lead poisoning in the year of 2013 (WHO, 2013). Therefore, domestic water (like open waterbody and drinking water) and industrial water should be routi-

nely monitored for lead(II) ions. There is a significant demand of a rapid, sensitive and cost-efficient method for the on-site detection of lead(II) ions.

However, current methods of lead(II) ion detection in water are far from meeting this need. Atomic absorption spectrometry (AAS) (Palmer et al., 2006; Willis and Sturman, 2004; Xu et al., 2013) and ICP-MS (Willis and Sturman, 2004), which are widely used in research institutions, are impractical to be applied for point-of-care (POC) testing, because of the bulky and expensive instruments and complicated sample preparation processes. New methods based on fluorescence (Huang and Ding, 2011; Li et al., 2010; Zhang et al., 2011), colorimetry (Guo et al., 2011; Kuo et al., 2015), electrochemistry (Shen et al., 2008; Xiao et al., 2007) and oligonucleotide (Leung et al., 2012; Ma et al., 2011a, 2011b) have been developed for detecting lead(II) ions, which are more convenient and more cost-effective than AAS and ICP-MS. Among all these methods, the label-free oligonucleotide-based luminescence switch-on assay is outstanding, benefited from its advantages of low

* Corresponding author.

** Corresponding authors at: Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China.

E-mail addresses: duncanleung@umac.mo (C.-H. Leung), edmondma@hkbu.edu.hk (D.-L. Ma), kangningren@hkbu.edu.hk (K. Ren).

cost, high sensitivity, and high selectivity. However, its routine utilization was still hampered by the multi-step operation and sophisticated detection equipment. Moreover, the requirement of professionals or well-trained staffs for performing the aforementioned tests might be a major obstruction to the effective implementation of the methods. In general, there is a significant demand of practical method for POC analysis of lead ions in water, especially in the less-developed areas where water contamination could be more severe and less noticed.

Recently, microfluidic paper-based analytical devices (μ PAD) (Martinez et al., 2008b; Mu et al., 2015; Pollock et al., 2012; Ren et al., 2013; Zhang et al., 2013) have demonstrated promising potential in the field of POC analysis, with implementations such as environmental monitoring, glucose level metering, urinalysis, blood gas tests, pregnancy tests, nucleic-acid amplification tests, and high-throughput immunoassays. Compared with conventional microfluidic chips, the μ PADs are more cost-effective, easier to fabricate and use, and with less requirement of external apparatus (Martinez et al., 2008b; Mu et al., 2015; Pollock et al., 2012). Hence, μ PADs have attracted increasing attention as a new platform for POC testing and other rapid tests (Martinez et al., 2009; Ren et al., 2014; Yetisen et al., 2013). The most widely used paper substrates for μ PADs were chromatography paper and filter paper, which are hydrophilic and can absorb liquids instantly. These paper substrates can be chemically modified and treated by photolithography, printing (Bruzewicz et al., 2008; Carrilho et al., 2009; Cheng et al., 2010; Nie et al., 2012), or soft-lithography, in order to form patterns of hydrophobic barriers that restrict the penetration of liquid in the channels defined by the barrier pattern. This design brings the advantage of automatic liquid pumping into the channels, largely simplified the system and reduced the cost. The hydrophobic patterns, however, do not offer any control of water penetration in the uncoated (channel) regions (Miljkovic et al., 2012; Sousa and Mano, 2013); thus, water can penetrate the entire uncoated region upon contact, leaving no room for storage or on-demand manipulation of different liquid aliquots on such chip. On the other hand, in many real assays, different reagents need to be held in discrete reservoirs and mixed at specific time points. This is difficult to achieve using current water-penetrable paper devices, even though some complicated designs of valves have been demonstrated (Li et al., 2013; Renault et al., 2014). This major limitation has restricted the applications of paper-based devices in POC assays.

Here we present a self-contained paper-based system for lead(II) ion detection based on G-quadruplex-based luminescence switch-on assay (He et al., 2013; Lin et al., 2011), comprising a novel type of paper-based chip and a matching portable device. Different from the reported paper-based devices, the paper substrate we chose was art paper, which is used for printing magazines. This type of paper could prevent the absorption of liquid into the paper matrix and hold the liquid in place for a period of time; and it could also be used for temporary liquid containing like a plastic substrate (such as polypropylene (PP) and polystyrene (PS)), but the surface of the paper is inherently hydrophilic. In such a design, liquid drops are suspended on the surface of the device in designed reservoirs, rather than absorbed into the paper; when the chip is tilted, the liquid drops will move to other reservoirs according to the guidance of channels defined on the surface. To differentiate it from reported μ PAD devices that are fabricated with water-permeable paper, we name this new type of paper-based devices suspending-droplet mode paper-based microfluidic devices (SD- μ PAD). Different from the conventional μ PADs that use capillary force to drive liquid, our SD- μ PADs uses wetting and gravity as driving force. To fabricate the superhydrophobic pattern on the paper device, we developed a new microcontact printing-based method to produce inexpensive and precisely patterned superhydrophobic coating on paper. The coating material is poly(dimethylsiloxane) (PDMS), a hydrophobic and transparent silicone that has long been used for fabricating microfluidic devices. Importantly, the negative-relief stamp we used is made of Teflon, a non-stick polymer, so that the PDMS-coated paper could be peeled from the stamp flawlessly.

After such fabrication process, the stamped area of the paper is coated with a textured PDMS layer that is decorated with arrays of micro-pillars, which could provide superhydrophobic effect and most effectively hold the droplets in place; the remaining area of the paper is still hydrophilic. As a demonstration of this new design, we developed a method using the reaction characteristics of iridium(III) complex for rapid, onsite detection of lead(II) ions in liquid samples. As the reagents have already been loaded onto the paper device during fabrication, the only reagent the users need to add is water. Because of the large Stokes shift of the iridium(III) complex probe, inexpensive optical filters can be employed, and we were able to make an inexpensive, battery-powered compact device for routine portable detection using a smartphone as a detector (Coskun et al., 2013; Martinez et al., 2008a; Mu et al., 2014; Oncescu et al., 2013; Roda et al., 2014a, 2014b; Sumriddetchkajorn et al., 2014), allowing the rapid analysis and interpretation of results on site as well as the automatic dissemination of data to professional institutes, including tests even in poor rural areas in developing countries.

2. Materials and methods

All materials and chemicals used in this study are listed in the [Supplementary information](#).

2.1. Fabrication of paper-based chip

The negative-relief Teflon stamp was designed using AutoCAD 2015 and fabricated using a thermo-molding method we published before (Ren et al., 2011). The superhydrophobic coating on the art-paper substrate was fabricated using a new microcontact printing-based method (Fig. 1A). As a result, the region coated with textured PDMS on the art paper turned superhydrophobic, while the remaining regions were hydrophilic, which served as wetting-controlled microchannels (Fig. 1B). Details of the fabrication process are described in [Supplementary information](#).

2.2. Fabrication of the portable detection device

The custom-made portable detection device (Fig. 3) was made of aluminum and transparent polymethyl methacrylate (PMMA). A smartphone was used to take images of the optical signal through a filter window. Details of the device configuration are described in [Supplementary information](#).

2.3. Detection of the lead(II) ion

The principle of detection is that lead ions can assist the designed single-stranded DNAs to form a G-quadruplex conformation, which can greatly enhance the luminescence emission of the iridium(III) probe (Hu et al., 2016). The selectivity of this method for Pb^{2+} ions was evaluated by investigating the response of the system to other common metal ions, including K^+ , Na^+ , Li^+ , Zn^{2+} , Cu^{2+} , Mg^{2+} , Ca^{2+} , Hg^{2+} , Al^{3+} , and Fe^{3+} ions.

2.3.1. Preparation of the paper-based chip

The reagents were loaded and dried on the chip, and then the chip was stored at 4 °C or -20 °C before use. Details are shown in [Supplementary information](#).

2.3.2. Detection procedure

Standard samples, three types of real samples, including tap water, bottled water, and river water (collected from the Shing Mun River of Hong Kong, and filtrated using a hydrophilic PTFE membrane filter with 0.2- μm pore size), and corresponding spiked samples (100 nM Pb^{2+} ions) were tested. Details of the testing procedure and analysis of results are described in [Supplementary information](#). Also, two control

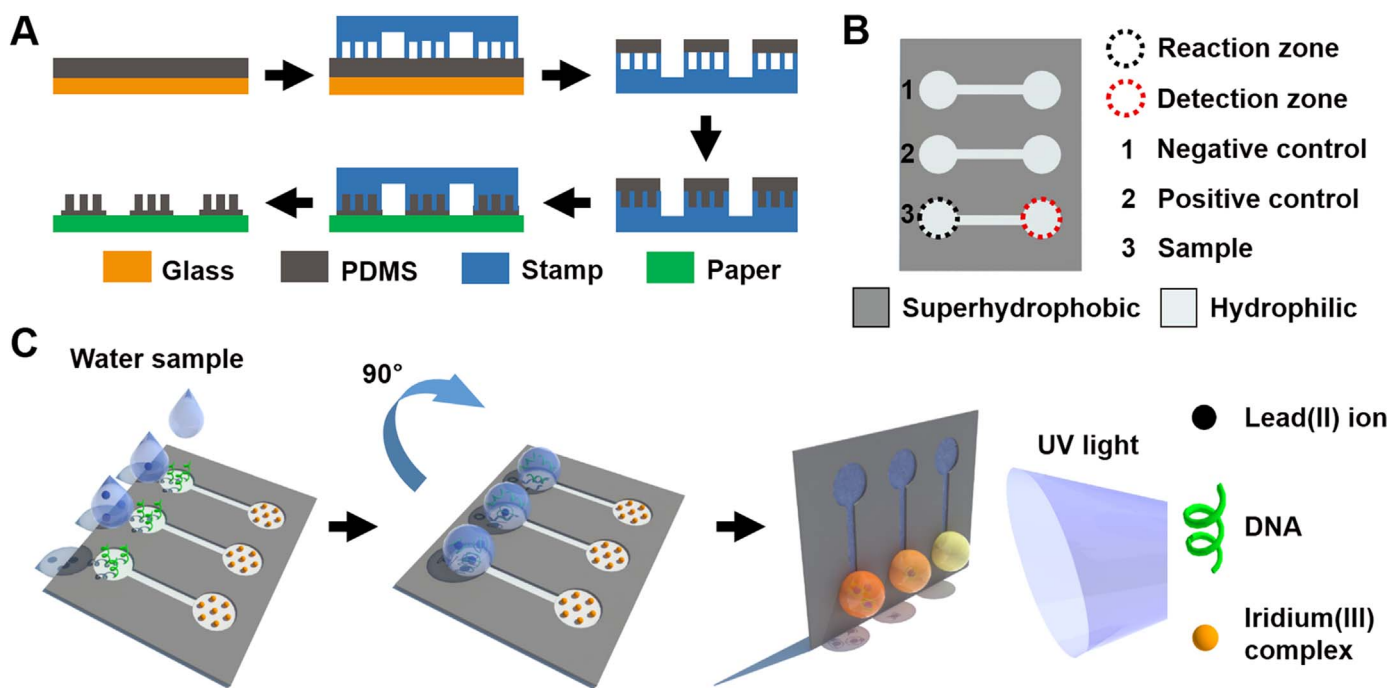


Fig. 1. Schematics of the fabrication process of SD- μ PADs. (A) The fabrication process of superhydrophobic coating on the art-paper substrate. A negative-relief Teflon stamp was attached onto a liquid-PDMS-coated glass slide and then peeled off to obtain a thin liquid PDMS coating on its surface. The stamp was kept in a vacuum chamber until the liquid PDMS completely filled the gaps in the micro-well arrays in the pattern. The stamp was then pressed onto a piece of art paper and heated at 90 °C for 15 min. The paper chip coated with superhydrophobic patterns was made, when the Teflon stamp was peeled off. (B) The configuration of the paper-based chip. The zones and channels are hydrophilic, while the rest regions are superhydrophobic. (C) Schematics of lead(II) ion detection on chip. The water samples were added to the reaction zone. After the reaction that took 3 min, the chip was tilted by 90°, upon which the droplet rolled to the detection zone and reacted with the iridium(III) complex 1.

experiments, based on AAS and fluorescence spectrophotometry, respectively, were performed for lead (II) ions detection (see [Supplementary material](#)).

3. Results and discussion

3.1. Principle of the SD- μ PAD

Considering that the G-quadruplex-based luminescence method for the detection of Pb^{2+} ions is a two-step process ([He et al., 2013](#)), the reagents need to be stored in discrete reservoirs and mixed at specified time points, which is hard to achieve with current μ PADs. Therefore, we propose here the utilization of SD- μ PAD, made of water-impermeable hydrophilic paper substrates patterned with superhydrophobic regions. The principle of our SD- μ PAD is that liquids of different contents can be loaded onto different reservoirs designed on the same device, and subject to reactions for a short period of time, e.g., heated for 10 min, after which the drops could be transferred to other reservoirs, through the guidance of predefined channels, simply by tilting the device. Note the liquid drops do not migrate when the device is put flat, although there are hydrophilic channels connecting the reservoirs. We were inspired by some recent reports on controlling liquid motion using printed patterns on superhydrophobic surface, but the fluorinated superhydrophobic substrates are costly to prepare, and the printed inks are thermally unstable, easy to cause fouling, and sometimes cause interference to optical detection ([Elsharkawy et al., 2014](#)). We therefore decided to create a platform based on bare paper substrate. To realize this idea, the candidate paper substrate should possess a hydrophilic surface but not be permeable to water throughout the testing process, so that the liquid droplets could be restricted in the hydrophilic regions due to the large difference in surface tension ([Balu et al., 2009](#)), and move along the defined channels only when the device is tilted along the direction of the channels. For this purpose, we carried out a study of the hydrophilicity of different kinds of commonly used water-impermeable commercial papers by measuring the water contact angles (WCA). As

shown in [Fig. S1](#), three types of paper (art paper, photographic paper, and weighing paper) were tested to be hydrophilic (WCA < 90°). However, the weighing paper deformed seriously upon contact with water, which could affect the testing results. Also, the photographic paper consists of multiple components, such as barium oxide, resin, sensitive emulsion, which may have negative effects on the test. Finally, art paper was chosen, because it is cleaner and more cost-effective than photographic paper. Art paper is a coated paper containing cotton and some adhesives (e.g., polyvinyl alcohol and casein), so that it could prevent the absorption of liquid, while keeping the surface hydrophilic.

3.2. Fabrication of the SD- μ PAD

The key feature of the SD- μ PADs is the capability in holding the liquid droplets in discrete reservoirs on a flat substrate, and moving the droplets on demand using gravity as driving force. This feature requires a water impermeable surface with patterns of different hydrophobicities, to retain the droplets in designed reservoirs and guide the movement of the droplets using predefined channels. Some recent studies reported that printing some hydrophilic inks/paintings on a hydrophobic surface could be used to control the movement of droplets, but this strategy has some limitations in real application: (1) the use of fluorinated coating to generate the superhydrophobic substrate raises the manufacturing cost; (2) the attachment of inks/paintings on fluorinated surfaces are not strong enough, especially when the device is heated; (3) the ink/painting may cause fouling of sample and interference with optical detection. Our SD- μ PADs, in contrast, are fabricated using a different strategy. We use art paper, an inherently hydrophilic substrate to serve as the channel/reservoir surface, and create a superhydrophobic pattern on the paper through a rapid and cost-efficient microcontact printing strategy. The created device is thermally stable and mechanically stable, and is less prone to cause channel fouling or interfere with optical detection. The superhydrophobic pattern was made of micropillar arrays, which was inspired by the superhydrophobic surface topology of lotus leaf.

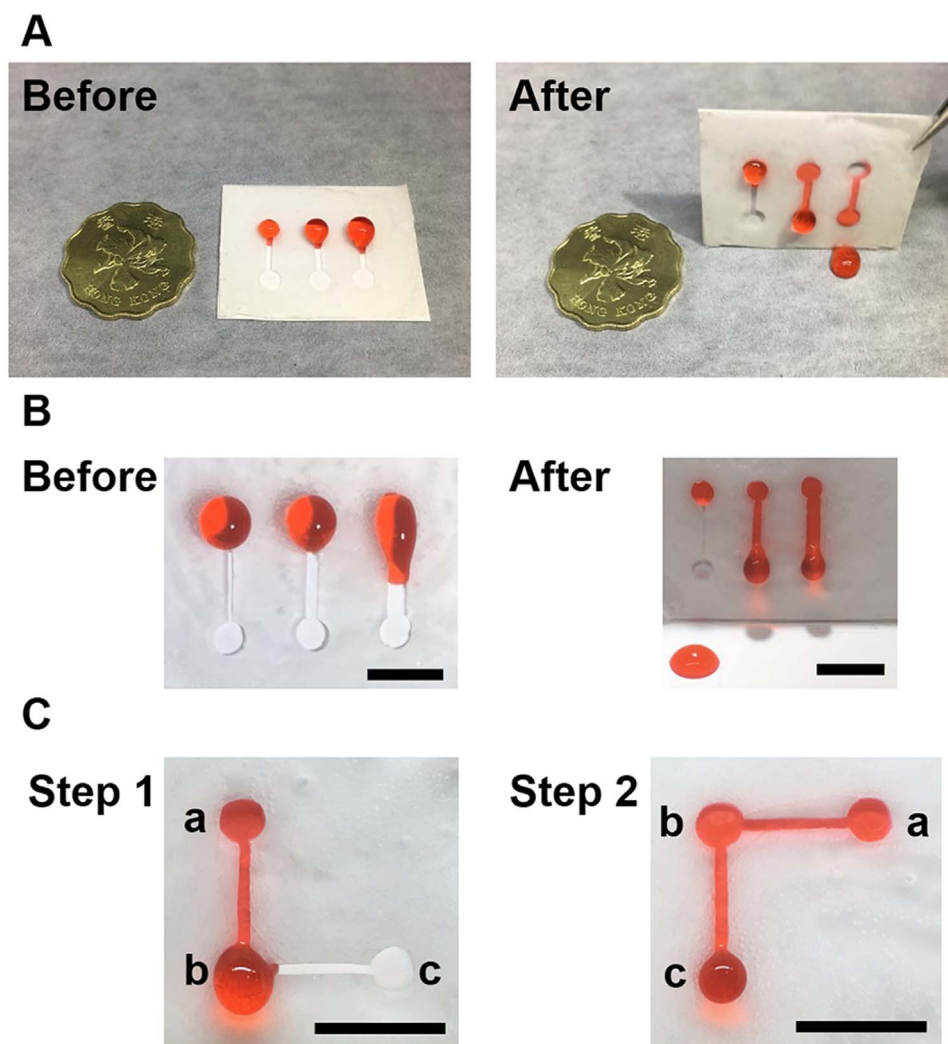


Fig. 2. The tests of droplet rolling on SD- μ PAD. (A) From left to right, the drop volumes were 10, 20, and 30 μ L, respectively. (B) From left to right, the widths of the channel were 0.3, 1.0, and 1.5 mm, respectively. (C) Multi-step transfer of the droplet on the SD- μ PAD. The scale bars represent 1 cm.

However, there was still a major challenge to address. Different from the paper substrates used conventional μ PADs, which possess rough surfaces, the paper substrate used in our work is smooth, making the adherent strength of a coating on it less than those on a rough paper surface. Owing to the high density of the pillar microarray in the superhydrophobic coating, it was difficult to completely peel off the coating from the stamp, when different stamp materials were tried, including polymethyl methacrylate (PMMA), polyethylene (PE), PP, and PS; the coating turned to stay on the stamp rather than on the paper, which might be the reason that we did not find previous report of similar device design. To address this challenge in fabrication, we made a negative-relief Teflon stamp using a thermo-molding method developed recently by our group (Fig. S2)(Ren et al., 2011). Teflon was chosen as the material of the stamp, because it is one of the best non-stick polymers, which will make the adhesion strength between the coating and the stamp weaker than that between the coating and the paper. As shown in Fig. 1A, after the PDMS was fully cured, the PDMS-coated paper could be easily peeled off from the Teflon stamp. The region on the paper coated with the textured PDMS became superhydrophobic, while the uncoated region remained hydrophilic.

3.3. Investigation of the droplet manipulation on the SD- μ PAD

Different from the conventional μ PADs that use capillary force to drive liquid, our SD- μ PADs use wetting and gravity as driving force. In

order to transfer the droplets between two reservoirs, a narrow, hydrophilic channel was designed so that the droplets would move when the device is tilted along the direction of the channels. We investigated the transfer of water droplets of different volumes between the two zones and measured the appropriate volume range of the droplets that can be reliably transferred between them. As Fig. 2A suggests, the appropriate droplet volume for transfer was around 20 μ L when the diameter of the reservoir was 3 mm, and the droplet slipped into the reservoir and stayed still if the device was tilted by 90 degrees. The sliding angle of the droplet was also tested and the result was about 85° (Fig. S3). For the given dimension of the reservoirs and the channels, a droplet too small (10 μ L) couldn't move because of the surface tension between the droplet and the paper surface; when the droplet volume was too large (30 μ L), it slipped off due to the large inertia. In addition, we investigated the transfer of droplets between two reservoirs with different widths of the channels in between. The tests were conducted using reservoirs 3-mm in diameter, and droplets 20- μ L in volume that were tested appropriate in size for the reservoirs; the tested widths of channels were 0.3, 1.0, and 1.5 mm, respectively. The plot in Fig. 2B shows the results of the tests: it was difficult to transfer the droplets with a narrow channel, and it was hard to hold the droplet in the reservoir when the channel is too wide. Our observation was in accordance with previous reports suggesting that the appropriate combination of the droplet volume and the width of channel can be predicted using modified Young-Dupré equation (Balu et al., 2009).

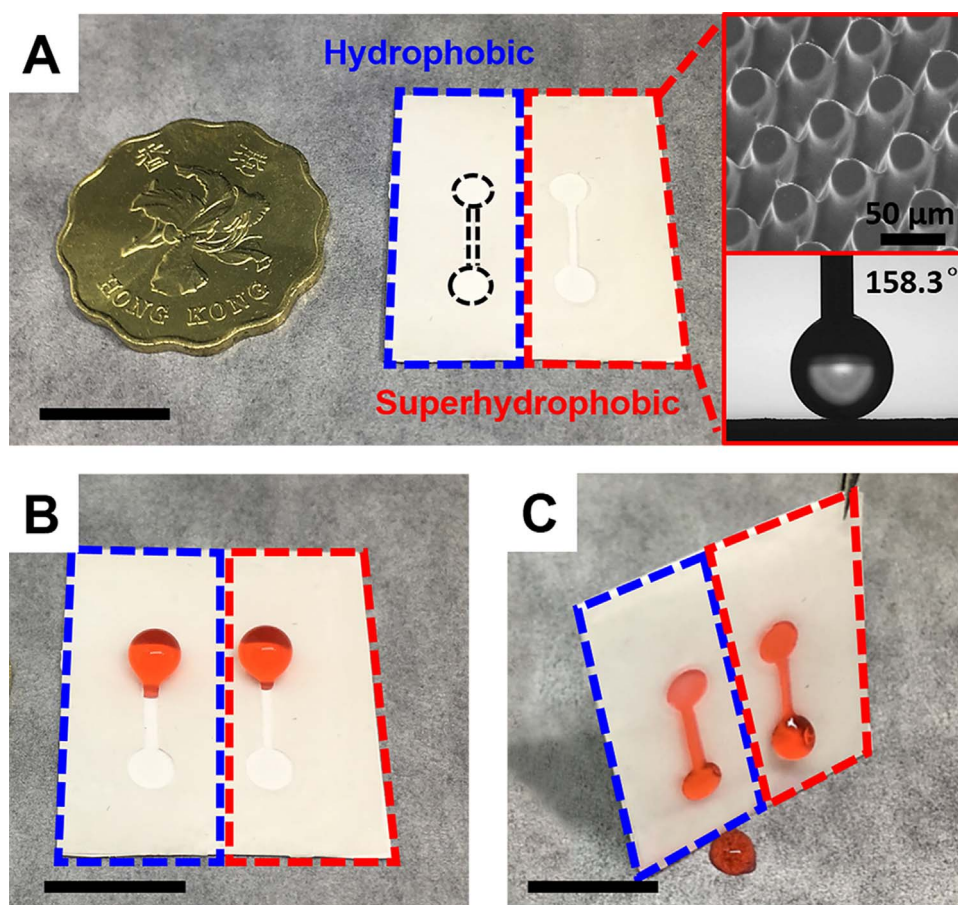


Fig. 3. The comparison between hydrophobic coating and superhydrophobic coating in the functionality of the paper-based chip. (A) Half of the coating was superhydrophobic (right), while the rest was hydrophobic (left). Inset: SEM characterization of the superhydrophobic coating and advancing water contact angle test. (B) Water droplets dyed in red were added to the reaction zones. (C) The droplets flowed to the detection zones when the chip was turned by 90° along the direction of the channel. The scale bars represent 1 cm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Moreover, we performed a test of multi-step transfer of the droplets, which proved that our device could be used for multi-step reaction (Fig. 2C). The principle of the multi-step transfer of the droplet is that channels can be placed in different directions on the device and the droplets only moves when the chip is tilted along the direction of the channel. We found that when the chip was laid flat, a small amount of liquid reflowed to the channel, which may influence the testing results of the SD- μ PAD. Therefore, we designed the detection method with the chip laid vertically, to ensure the accuracy for the detection.

Also, we made a comparison between hydrophobic coating and superhydrophobic coating in the performance of holding droplets in place. As shown in Fig. 3A, half of the tested chip had superhydrophobic pattern, and the other half had hydrophobic pattern. The SEM image shows the microstructures on the superhydrophobic region, which contains micropillar arrays; the advancing contact angle of water on the textured PDMS surface was 158.3°. A solution of a red dye was used as the sample in the test; after the chip was turned to 90° along the direction of the channel, part of the droplet flowed out of hydrophobic barrier of the detection zone, while the whole droplet stayed inside the detection zone with the superhydrophobic barrier (Fig. 3C). Therefore, the high contrast of surface tension did have a positive effect on the restriction of the liquid, which proved that the superhydrophobic coating is essential in this work.

3.4. Design of the SD- μ PAD device for lead ion detection

The method of G-quadruplex-based luminescence detection includes two steps: the formation of G-quadruplex under heat; the

subsequent mixing of G-quadruplex and iridium(III) complex (Fig. S4). We performed analysis about the method's selectivity to Pb^{2+} ions against ten other types of common metal ions. The results show that only Pb^{2+} ions could significantly enhance the photoluminescence emission of the complex 1/PS2.M system (Fig. S5). In this work, we set two zones on the paper-based chip, namely reaction zone and detection zone, to perform the two-step process of the G-quadruplex luminescent based detection of Pb^{2+} ion. On each SD- μ PAD device, three pairs of channels were set up for positive control, negative control, and sample, respectively, which could increase the reliability of detection. Deionized water was used as negative control, while lead(II) ion standard solution with the concentration equal to Drinking Water Quality Standards of WHO (50 nM) was used as positive control. Hence, the results of the samples could be easily judged by comparing with the results of the controls, and disqualification could be readily identified when the former is greater than that of the positive control. To make a user-friendly μ PAD for Pb^{2+} ion detection, we added the DNA solution and iridium(III) complex 1 to the reaction zone and detection zone, respectively, during the preparation of the device. These components attached to the paper surface firmly when they dried out at 4 °C. During storage, a thin cling wrap was used to protect the components on the chip. Such device was found to be stable for at least 30 days when kept at 4 °C (Fig. S6). In this way, users of the device will only need to add water to perform the assay, which is expected to be convenient for users, especially those non-expert users. In real implementation, the deionized water could be simply replaced with bottle water of trusted brands.

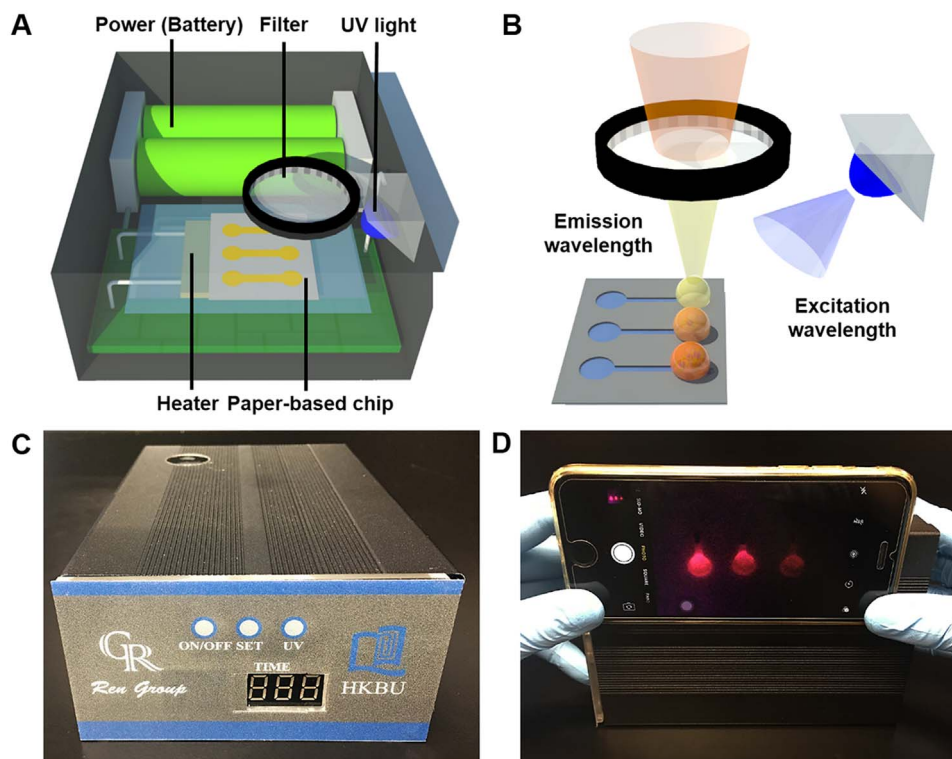


Fig. 4. Schematics of the detection principle and photos of the detection device. (A) Schematic of the layout structure of the portable detection device. (B) Schematic of the detection principle. (C) Photo of the detection device. (D) Image of the lead(II) ion detection using a smartphone.

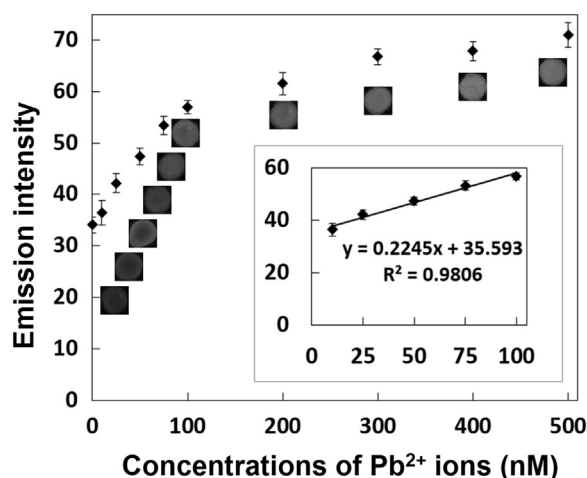


Fig. 5. The emission intensity obtained in the tests with different concentrations of Pb²⁺ ions (nM). The emission intensity showed a good linearity when the concentrations of the Pb²⁺ ions were between 10 nM and 100 nM.

3.5. Design of the portable device

The main reason for designing a portable detection device was the inconvenience of using conventional equipment on site, as well as the need of trained person to run the conventional tests. The main detection principle is shown in Fig. 4; the whole system is less than one pound and driven by battery, which allows it to be conveniently used in POC tests, especially for those remote and less developed areas. In our design, we utilized a self-adjusted heater to help form G-quadruplex structure in the reaction zone. After the heating process, the whole device was tilted by 90° to let the liquid sample flow to the detection zone and react with the iridium(III) complex. The iridium(III) complex has a large stoke shift: the excitation wavelength at around 365 nm and the maximum emission wavelength at about

650 nm, which allows inexpensive filters to be used for optical filtration. To make the whole system portable, a battery-powered LED light source was used for the excitation. The optical signal can be easily captured using a low-cost portable device, such as a cellphone with an integrated camera. In this way, the whole cost of the device was below 20 US dollars, which allows our device to be widely used in low-income areas, where water contamination is a greater and more common issue. The detection results with this device could be captured and analyzed in two ways: one is fast qualitative analysis by naked-eye observation for the POC testing, when the concentration of Pb²⁺ is high (> 100 nM); the other is quantitative analysis by a smartphone, of which the result could either be analyzed using a small program for cellphone or be uploaded immediately to the database of professional institutes.

3.6. Lead(II) ion assay on the fully portable system

We performed assays of different concentrations of lead(II) ion samples on the SD-μPAD, and captured the results using a cell phone and analyzed the images using a free software ImageJ 1.4. As shown in Fig. 5, the results indicate a good linearity in the correlation between the emission intensity and the Pb²⁺ ion concentration (10–100 nM), which is comparable to the results in our previous work (He et al., 2013). Moreover, the results of the two control experiments based on AAS and fluorescence spectrophotometry, respectively, were consistent with the results obtained using the SD-μPAD, which proved that our system is accurate enough (Fig. S7). In addition, we tested three types of real samples and the corresponding spiked samples (100 nM Pb²⁺ ions). The results indicate that the spiked Pb²⁺ ions could be accurately measured in these samples (Tab. S1). However, for some samples, such as sewage and sea water, pretreatment should be done; otherwise the detection results would be unconvincing. Aside of the positive and negative control zones, current design includes one sample zone on each SD-μPAD, but the number of sample zones could be easily increased for repetitive or high-throughput parallel detection.

4. Conclusion

We developed a SD- μ PAD and a corresponding portable instrument for rapid, sensitive, and low-cost detection of Pb^{2+} ions in liquid samples. Different from conventional μ PADs, the SD- μ PADs hold liquid droplets on top rather than absorb the liquid. Art paper, a water-impermeable paper substrate, was used to fabricate the SD- μ PAD, which is a new kind of μ PAD material. To some extent, quite a few materials often used for disposable tests, such as the glass slides for blood type test, and some polymers used for rapid detection of ions, could be substituted by the hydrophilic water-impermeable μ PADs for a much lower price. Different from the conventional μ PADs that use capillary force to drive liquid, our SD- μ PADs uses wetting and gravity as driving force. To fabricate the SD- μ PADs, we developed a new strategy of microcontact printing method using a Teflon stamp to achieve precisely patterned robust superhydrophobic coating on paper surface, which is cost-efficient and well scalable. The high contrast of surface tension allows storage, transportation, and mixing of liquid drops on demand on the SD- μ PADs, which allow stepwise manipulation and thereby more flexibility in on-chip assays, such as the implementation of G-quadruplex luminescent based detection of Pb^{2+} ions demonstrated here. In addition, the extreme simplicity and convenience of using an inexpensive portable device could widen its applicability. Benefitted from the rapid development of smartphones, e.g., the powerful image process programs, we expect that the smartphone-based SD- μ PAD systems will be greatly expanded to several research fields, such as environmental monitoring, health monitoring, diagnosis, and food safety tests.

Acknowledgements

This work was supported by Hong Kong Baptist University (FRG2/15-16/002, FRG2/16-17/007, SDF 03-17-096), Hong Kong RGC (22200515), the National Natural Science Foundation of China (21505110, 21575121, 21628502), Innovation and Technology Fund (ITF/16-17/08-CHEM), Matching Proof-of-Concept Fund (MPCF-002-2016/2017), the Hong Kong Baptist University Century Club Sponsorship Scheme 2016, the Science and Technology Development Fund, Macao SAR (077/2016/A2).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2017.07.073.

References

Balu, B., Berry, A.D., Hess, D.W., Breedveld, V., 2009. *Lab Chip* 9 (21), 3066–3075.
 Bruzewicz, D.A., Reches, M., Whitesides, G.M., 2008. *Anal. Chem.* 80 (9), 3387–3392.
 Carrilho, E., Martinez, A.W., Whitesides, G.M., 2009. *Anal. Chem.* 81 (16), 7091–7095.
 Cheng, C.-M., Mazzeo, A.D., Gong, J., Martinez, A.W., Phillips, S.T., Jain, N., Whitesides, G.M., 2010. *Lab Chip* 10 (23), 3201–3205.
 Coskun, A.F., Wong, J., Khodadadi, D., Nagi, R., Tey, A., Ozcan, A., 2013. *Lab Chip* 13 (4), 636–640.
 Elsharkawy, M., Schutzius, T.M., Megaridis, C.M., 2014. *Lab Chip* 14 (6), 1168–1175.

Gumpu, M.B., Sethuraman, S., Krishnan, U.M., Rayappan, J.B.B., 2015. *Sens. Actuators B* 213, 515–533.
 Guo, Y., Wang, Z., Qu, W., Shao, H., Jiang, X., 2011. *Biosens. Bioelectron.* 26 (10), 4064–4069.
 He, H.-Z., Leung, K.-H., Yang, H., Chan, D.S.-H., Leung, C.-H., Zhou, J., Bourdoncle, A., Mergny, J.-L., Ma, D.-L., 2013. *Biosens. Bioelectron.* 41, 871–874.
 Hu, C., Lin, S., Li, W., Sun, H., Chen, Y., Chan, C.-W., Leung, C.-H., Ma, D.-L., Wu, H., Ren, K., 2016. *Lab Chip* 16 (20), 3909–3918.
 Huang, C., Ding, C., 2011. *Anal. Chim. Acta* 699 (2), 198–205.
 Kuo, S.-Y., Li, H.-H., Wu, P.-J., Chen, C.-P., Huang, Y.-C., Chan, Y.-H., 2015. *Anal. Chem.* 87 (9), 4765–4771.
 Leung, C.-H., Chan, D.S.-H., He, H.-Z., Cheng, Z., Yang, H., Ma, D.-L., 2012. *Nucleic Acids Res.* 40 (3), 941–955.
 Li, C.-L., Liu, K.-T., Lin, Y.-W., Chang, H.-T., 2010. *Anal. Chem.* 83 (1), 225–230.
 Li, X., Zwanenburg, P., Liu, X., 2013. *Lab Chip* 13 (13), 2609–2614.
 Lin, Z., Chen, Y., Li, X., Fang, W., 2011. *Analyst* 136 (11), 2367–2372.
 Ma, D.L., Chan, D.S.H., Man, B.Y.W., Leung, C.H., 2011b. *Chem.-Asian J.* 6 (4), 986–1003.
 Ma, D.-L., Xu, T., Chan, D.S.-H., Man, B.Y.-W., Fong, W.-F., Leung, C.-H., 2011a. *Nucleic Acids Res.* 39 (10), (e67–e67).
 Martinez, A.W., Phillips, S.T., Carrilho, E., Thomas, S.W., III, Sindi, H., Whitesides, G.M., 2008a. *Anal. Chem.* 80 (10), 3699–3707.
 Martinez, A.W., Phillips, S.T., Whitesides, G.M., 2008b. *Proc. Natl. Acad. Sci.* 105 (50), 19606–19611.
 Martinez, A.W., Phillips, S.T., Whitesides, G.M., Carrilho, E., 2009. *Anal. Chem.* 82 (1), 3–10.
 Miljkovic, N., Enright, R., Wang, E.N., 2012. *ACS Nano* 6 (2), 1776–1785.
 Mu, X., Zhang, L., Chang, S., Cui, W., Zheng, Z., 2014. *Anal. Chem.* 86 (11), 5338–5344.
 Mu, X., Xin, X., Fan, C., Li, X., Tian, X., Xu, K.-F., Zheng, Z., 2015. *Chem. Commun.* 51 (29), 6365–6368.
 Needleman, H., 2004. *Annu. Rev. Med.* 55, 209–222.
 Nie, J., Zhang, Y., Lin, L., Zhou, C., Li, S., Zhang, L., Li, J., 2012. *Anal. Chem.* 84 (15), 6331–6335.
 Oncescu, V., O'Dell, D., Erickson, D., 2013. *Lab Chip* 13 (16), 3232–3238.
 Palmer, C.D., Lewis, M.E., Geraghty, C.M., Barbosa, F., Parsons, P.J., 2006. *Spectrochim. Acta Part B* 61 (8), 980–990.
 Patrick, L., 2006. *Altern. Med. Rev.* 11 (1), 2–23.
 Pollock, N.R., Rolland, J.P., Kumar, S., Beattie, P.D., Jain, S., Noubary, F., Wong, V.L., Pohlmann, R.A., Ryan, U.S., Whitesides, G.M., 2012. *Sci. Transl. Med.* 4 (152), (152ra129–152ra129).
 Ren, K., Dai, W., Zhou, J., Su, J., Wu, H., 2011. *Proc. Natl. Acad. Sci.* 108 (20), 8162–8166.
 Ren, K., Zhou, J., Wu, H., 2013. *Acc. Chem. Res.* 46 (11), 2396–2406.
 Ren, K., Chen, Y., Wu, H., 2014. *Curr. Opin. Biotechnol.* 25, 78–85.
 Renault, C., Anderson, M.J., Crooks, R.M., 2014. *J. Am. Chem. Soc.* 136 (12), 4616–4623.
 Riess, M.L., Halm, J.K., 2007. *J. Gen. Intern. Med.* 22 (8), 1212–1215.
 Roda, A., Guardigli, M., Calabria, D., Calabretta, M.M., Cevenini, L., Michelini, E., 2014a. *Analyst* 139 (24), 6494–6501.
 Roda, A., Michelini, E., Cevenini, L., Calabria, D., Calabretta, M.M., Simoni, P., 2014b. *Anal. Chem.* 86 (15), 7299–7304.
 Shahat, A., Awual, M.R., Khaleque, M.A., Alam, M.Z., Naushad, M., Chowdhury, A.S., 2015. *Chem. Eng. J.* 273, 286–295.
 Shen, L., Chen, Z., Li, Y., He, S., Xie, S., Xu, X., Liang, Z., Meng, X., Li, Q., Zhu, Z., 2008. *Anal. Chem.* 80 (16), 6323–6328.
 Sousa, M.P., Mano, J.F., 2013. *Cellulose* 20 (5), 2185–2190.
 Sumriddetchkajorn, S., Chaitavon, K., Intaravanne, Y., 2014. *Sens. Actuators, B* 191, 561–566.
 Verma, R., Gupta, B.D., 2015. *Food Chem.* 166, 568–575.
 WHO, 2013. (<http://www.who.int/mediacentre/news/notes/2013/lead-20131018/en/>).
 Willis, J., Sturman, B., 2004. *J. Anal. At. Spectrom.* 19 (5), 706–708.
 Xiao, Y., Rowe, A.A., Plaxco, K.W., 2007. *J. Am. Chem. Soc.* 129 (2), 262–263.
 Xu, H., Xu, P., Gao, S., Zhang, S., Zhao, X., Fan, C., Zuo, X., 2013. *Biosens. Bioelectron.* 47, 520–523.
 Yetisen, A.K., Akram, M.S., Lowe, C.R., 2013. *Lab Chip* 13 (12), 2210–2251.
 Zhang, L., Han, B., Li, T., Wang, E., 2011. *Chem. Commun.* 47 (11), 3099–3101.
 Zhang, M., Ge, L., Ge, S., Yan, M., Yu, J., Huang, J., Liu, S., 2013. *Biosens. Bioelectron.* 41, 544–550.