

Hydrophilic coating film used to drive flow in a microfluidic point-of-care testing (POCT) device

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The liquid is usually driven by capillary force to flow forward in a channel network of a point-of-care testing device. However, it is not enough to drive the liquid flow forward that only relies on the surface tension of the chip material itself. While the liquid drops into the chip, the unstable liquid front shape may produce bubbles or the liquid flowing will even stop which will resist the signal detection. Here, a novel method, hydrophilic coating, is demonstrated. This method used an automatic device to dip the chips from the self-prepared TiO₂ sol forming the gel film on the surface of chips' channel. Results have shown that the coating film will enhance the liquid flow performance and improve the detection precision and accuracy.

1. Introduction: Point-of-care testing (POCT) is a rapid detection and analysis technique near the patient's bedside and its core technology is the microfluidic chip. In recent years, polymer microfluidic chips because of their low fabrication cost, high analytical performance, solvent consumption, and the advantages of good biological compatibility have been applied in many fields [1–3]. A typical microfluidic POCT device may usually involve a sample injection inlet, a mixing/reaction chamber, a detection channel network, a waste collection unit, and some valves. The size of the channel is always tens of microns to several millimetres [4, 5]. In a microfluidic device, liquid sample traverses the channel network to achieve testing results. Also, the liquid is usually driven by capillary force to flow forward in a channel network. However, in case, it is not enough to drive the liquid flow forward that only relies on the surface tension of the chip material itself [6, 7]. While the liquid drops into the chip, the unstable liquid front shape may produce bubbles, or because the surface tension is too little, the liquid flowing will even stop. As the liquid sample flows through the device, the target ligand in the sample reacts with the test reagent which has been deposited on the surface of the mixing/reaction chamber or the channel to generate the response signals. The antigens contained in the liquid sample must react with the antibodies to generate response signals. The unsmooth flow will prevent this reaction, which makes the quantitative analysis of the antigen concentration to be inaccurate [8]. Also, for mass production, small differences between chips are required. The batch surface modification of microfluidic devices especially the POCT devices is facing great challenges.

To enhance the surface tension of polymer microfluidic chips, the existing technology mainly includes plasma modification and nano-coating modification. For example, polymethylmethacrylate (PMMA) substrates were treated by oxygen plasma etching by some researchers [9]. The surface roughness of the substrate was changed by changing the etching time and temperature of the plasma. Although the method has a good effect, it may damage the chip's surface microstructure and obtain small differences between chips. In addition, the process requires expensive equipment, high energy consumption, high cost, is not conducive to the realisation of mass production [10–13]. Besides, about the nano-coating modification, some researchers prepared the self-cleaning

hydrophilic film on the PC substrate surface. First, they prepared the SiO₂ sol, then, TiO₂ powder added into the SiO₂ sol to get TiO₂-SiO₂ sol. Also, the sol was coated on the substrate by spin coating. In addition, the process of the method is complex for mass production, and they do not use it in a polymer POCT device, and the spin method is not suitable for rectangular structure chips [14]. In addition, to solve the question, some methods such as ultraviolet (UV) surface modification and some physical methods were studied. Also, we did not get high-quality experimental results from a lot of trying. Thus, it is necessary to develop a novel method for the modifying of the channel applicable to the mass production in a POCT device [15, 16].

In this Letter, a method used to enhance the surface tension in the POCT device channels is demonstrated. This method uses a semi-auto dipping apparatus with a special fixture to fix chips. An R&D developed TiO₂ sol is used for the dip-coating. The hydrophilic coating will enhance the local channel surface tension and generate stable liquid velocity and stable liquid front. Also, it can obtain small differences between chips. The parameters of the dip-coating process and the composition of the dip-coating sol are investigated and optimised.

2. Experimental section

2.1. Materials and solutions: The experimental devices are fabricated on PMMA (Asahi Kasei Corporation) plates. The PMMA plates have a glass transition temperature of ~105°C. The main reagents including tetrabutyl titanate (TBOT, AR grade, Tianjin Kermel Chemical Reagent Co. Ltd), absolute ethanol (EtOH, GR grade, Tianjin Fuyu Fine Chemical Co. Ltd), nitric acid (HNO₃, AR grade, Tianjin Damao Chemical Reagent Co. Ltd), alkanolamide [diethanolamine (DEA), CP grade, China Sinopharm Chemical Reagent Co. Ltd], acetylacetone (AcAc, AR grade, Tianjin Kermel Chemical Reagent Co. Ltd), and deionised water (water purification equipment is from Beijing Jiayun Water Purification Equipment Manufacturing Co. Ltd). The PMMA microfluidic chip was used as the substrate, and a thin film was coated on the substrate. Before coating, the substrates are cleaned in deionised water containing DZ-1 cleaning agent in the ultrasonic cleaning machine, and a cleaning agent and deionised water according to 1:20 proportion, then cleaned two times in deionised water after ultrasonic cleaning and dry it. The human

serum used for the experiments is provided by the School of Life Science and Biotechnology, Dalian University of Technology.

2.2. Device design: The structure of the experimental device is shown in Fig. 1a. It is composed of a cover plate and a substrate. The size of the device is about 100 mm × 20 mm × 2 mm. The microstructures are fabricated on the substrate. A fan-shaped reservoir is designed for liquid sample injection and reserving. The trapezoidal mixing chamber and fan-shaped sample introduction reservoir structures are designed for sample introduction. The channel is 2 mm wide, 23 mm long and 70 μm deep.

The special fixture for the experimental chips is mounted onto a dip-coating machine whose moving accuracy is 1 μm. The machine can automatically pull the chips up and down to finish dip-coating (as shown in Fig. 1c). The thickness of the film can be adjusted by changing the velocity of dipping. The sol used in dip-coating is put into a solution cylinder which is placed below the chips. The sol will form a gel film on the substrate during the dip-coating.

2.3. Device fabrication: The microstructures are in a silicon mould which is fabricated by using UV-Lithographie, Galvanoformung and Abformung (LIGA) technique. The microstructures are transferred to a PMMA substrate by hot embossing. The enclosing of the microstructures is achieved by using the ultrasonic welding

process. The hot embossing processes of the substrate are as follows. First, the PMMA plate is cut into the chip outline shape using a laser cutting machine, and the cleaned PMMA substrate is placed onto the hot embossing plate of an embossing machine (developed by our group), the machine is shown in Fig. 1b. The temperature is increased to 90°C at a rate of 0.5°C/s and kept for 2 min. Second, the temperature is increased to 120°C at a rate of 0.2°C/s. Meanwhile, the embossing pressure is put on the substrate through the mould at a rate of 0.02 MPa/s. Third, as the temperature rises to 120°C and the pressure increases to 2.0 MPa, a remaining process is conducted. The temperature of 120°C and the pressure of 2.0 MPa will be retained for 5 min. Four, the substrate will be cooled to 40°C and the pressure will be reduced to 0.5 MPa after the remaining process. Finally, the temperature will be increased to 70°C, and the de-moulding process is performed. The fabricated microstructures are shown in Fig. 1a. The ultrasonic welding processes are easier than the hot embossing, and the ultrasonic welding machine is shown in Fig. 1d. First, the substrate and cover plate are put into the fixture after aligning. Second, the welding pressure is set to 0.2 MPa, and the welding time and holding time are set to 0.01 and 5 s, respectively. Third, the chip is pressed by the welding horn with the high-vibration and the welding process is finished.

2.4. Sol preparation and flow visualisation: In this Letter, tetrabutyl titanate (TBOT) was used as the precursor, absolute ethanol as the solvent, nitric acid as the catalyst, DEA and acetylacetone (AcAc) as complexing agents. TiO₂ sol was prepared by hydrolysis of TBOT. The preparation flow chart of the sol is shown in Fig. 1. Firstly, 20 ml TBOT was dissolved in 80 ml absolute ethanol and was stirred by a magnetic stirrer (Shanghai Biaohe Instrument Co. Ltd, ZNCL-BS230*230). Meanwhile, DEA and AcAc were each taken 6 ml and dropped into the solution, continuously stirred for 30 min, then formed solution A. Then 40 ml absolute ethanol was dissolved in 4 ml deionised water, and 2 ml nitric acid was added to it, and then formed solution B. The solution B was slowly added to solution A, and after 2 hours of continuous mixing, a uniform clear yellow sol was formed. After 5 days of sealed light aging, the sol can be used as the dip coating solution. In addition, the volume fraction of each component in the sol system was TBOT: absolute ethanol: DEA: AcAc: nitric acid: deionised water = 1:6:0.3:0.3:0.1:0.2.

The flow visualisation is done by recording the liquid front through a measuring microscope (developed in our lab). The human serum is dropped into the sample introduction reservoir. The liquid will be pulled by the drag force to passively migrate within the device. Without the hydrophilic coating, the liquid front may usually stop or produce bubbles. After dip-coating, the liquid will flow smoothly and uniformly in the channel.

3. Results and discussion

3.1. Theoretical analysis of the effect of hydrophilic coating: It is driven by the capillarity that fluid flows in the channel in an automatic capillary system. The liquid is dropped into the testing channel of a POCT chip, and it is driven by the capillarity to flow to the testing area. Martin *et al.* studied the flow characteristics of the capillary system in a Polydimethylsiloxane (PDMS) microfluidic chip, and (1) pointed out that the changing of the microfluid velocity in a capillary system [17]

$$Q = \frac{1}{\eta} \frac{\Delta P}{R_F}, \quad (1)$$

where Q is the fluid velocity, η is the viscosity of the liquid, ΔP is the gas–liquid pressure difference in the capillary, R_F is the flow resistance in the capillary. So the larger ΔP is the larger Q is, and the smaller R_F is the larger Q is.

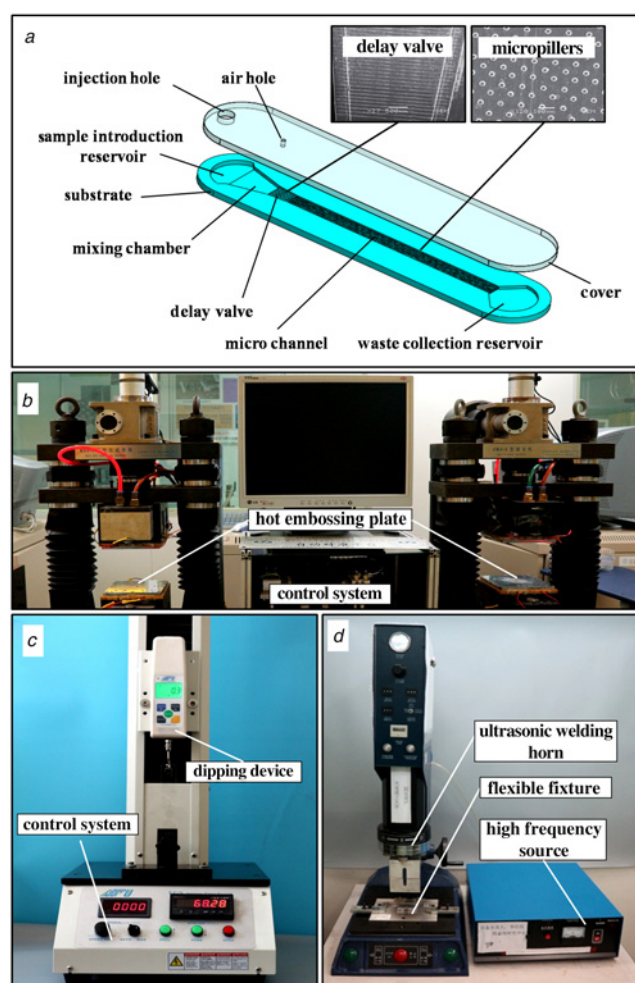


Fig. 1 Experimental microfluidic chip and experimental apparatus
a Microfluidic chip, which is composed of sample introduction reservoir, mixing chamber, delay valve, microchannel and waste collection reservoir
b Hot embossing machine which is used for device fabrication
c Dip-coating machine which is used for film coating
d Ultrasonic welding machine which is used for chip sealing

The cross sections of the channel of experimental chips are rectangular, and in the rectangular capillary system, the gas-liquid pressure conforms to the following theory:

$$P_c = -\gamma \left(\frac{\cos\alpha_b + \cos\alpha_t}{a} + \frac{\cos\alpha_l + \cos\alpha_r}{b} \right), \quad (2)$$

where P_c is the difference of gas-liquid meniscus pressure in the capillary and γ is the surface tension of a liquid α_b , α_t , α_l , and α_r , are the contact angles of the top, bottom, left and right inner surface the capillary channel. Besides, a and b are the length and width of the rectangular capillary section. The formula shows that the smaller the contact angles of the inner channel are, the greater the absolute value of the capillary pressure is, and the larger the velocity of the fluid is in the microfluidic channel.

So we can make the smaller contact angles of the inner surfaces of the channel to accelerate the liquid flow. In the preparation of TiO_2 sol and the formation of the gel film, the reactions in the process are shown in Fig. 2a. First of all, the precursor, TBOT, is hydrolysed and condensed in an organic solvent to form a sol. Once the hydrolysis reaction occurs, the condensation reaction occurs in the sol system, the reaction between the hydrolysis products. As reaction proceeds, the condensation will continue to occur. After the reaction, the sol film can be formed on the surface of the substrate, and then the polycondensation reaction in the sol film will continue through the drying process. After the above reaction process, the substrate surface has realised the conversion of the sol film to the gel film, and the gelation process of the film has been basically completed. At the same time, a uniform and stable coating film will be formed on the surface of the substrate. The surface of the sol film formed by the reaction contains some hydroxyl groups, which we think is the reason why the sol film enhances the hydrophilicity of the PMMA surface. This

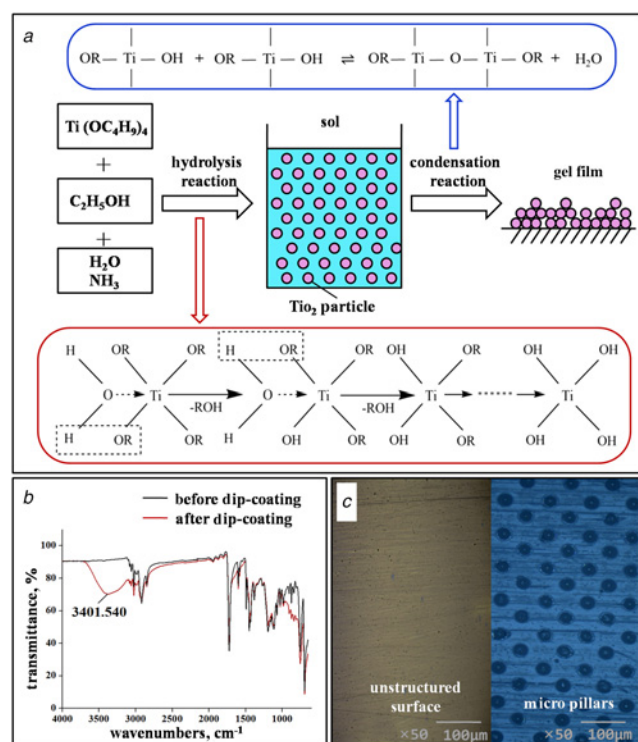


Fig. 2 Forming principle, surface group analysing and micrograph of the hydrophilic film

a Forming principle of hydrophilic film and reaction principle formula
b Contrast analysis of the infrared spectrum of the substrate surface before and after the coating
c Micrograph of the no-structure area and the pillars in the channel after hydrophilic coating

conclusion has also been verified by experimental data, as shown in Fig. 2b, which is the contrast analysis of the infrared spectrum of the PMMA surface before and after the coating. The hydroxyl peak after the coating has been greatly improved, so the increase of the surface hydroxyl groups increases the surface hydrophilicity, that is, the contact angle between the surface and water becomes smaller. In addition, the micrograph of the no-structure area and the pillars in the channel after hydrophilic coating are shown in Fig. 2c.

As a result, the sol-gel coating can increase the driving force of the capillary and can be applied to the POCT chip channel to drive the liquid stream to flow smoothly.

3.2. Effect of different solution components: To study the influence of the different proportion of the complexing agent on the hydrophilicity of the film, we set up three groups of different sol proportion experiments. To equal volume (6 ml) of DEA and AcAc, two kinds of complexing agents were added in the sol, and only added the same amount of DEA sol as the complexing agent, and only AcAc as a complexing agent. In addition, other processes of the three different sols were the same. The substrates (to facilitate the measurement of experimental data, we used the PMMA boards without any structure) were coated in the three kinds of sol by using the same process parameters (the velocity of dipping was 2 mm/s, and the temperature is 20°C). The contact angles were measured by the contact angle measuring instrument (PA3220-MK2 type optical contact angle measuring instrument, Germany) based on Young's measuring method and the drip volume is 1 μl . We measured five points from each substrate at random for the average value, and we set up a control experiment. The results of the measurements are shown in Figs. 3a-d. The experimental results show that the contact angle

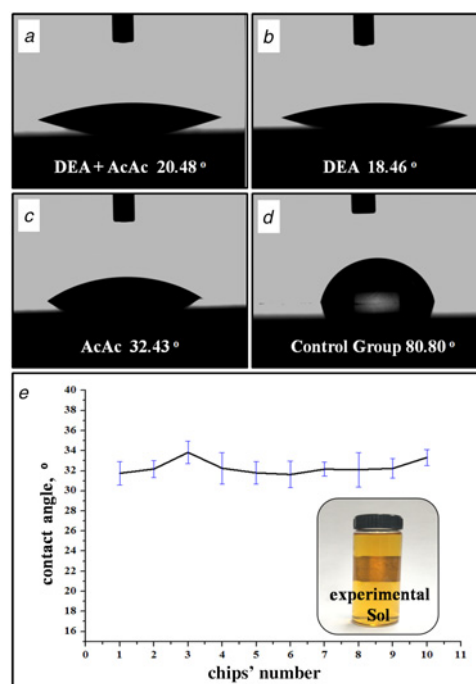


Fig. 3 Contact angles' analysis of different coating sol composition

a Contact angle of the coating surface while DEA and AcAc both as the complexing agents of sol
b Contact angle of the coating surface while DEA as the complexing agents of sol
c Contact angle of the coating surface while AcAc as the complexing agents of sol
d Contact angle of the untreated surface
e Differences of contact angles among chips while the AcAc as the complexing agents of sol

of only DEA sol is the minimum, and the average value is 18.46°. The average contact angles of DEA-AcAc sol and the untreated PMMA plate are 20.48° and 80.80°, respectively. The only AcAc sol is 32.43°, which is the closest value to the requirement. So the sol of only DEA as the complexing agent is chosen to the coating sol of the follow-up experiment.

Besides, in order to study the difference of dip-coating hydrophilic films between substrates' surfaces, we measured ten groups of substrates using only AcAc as the dip-coating solution and with the same process parameters (the velocity of dipping was 2 mm/s, and the temperature is 20°C). The contact angles were also measured by the contact angle measuring instrument based on Young's measuring method and the drip volume is 1 µl as the same. We measured five points from each substrate at random. As shown in Fig. 3e, the differences of contact angles among chips and different points of on chip are both not more than 10%.

As a result, experiments show that while DEA, AcAc, and DEA & AcAc as the complexing agents of sol the coating film both have good hydrophilicity and its average contact angles are 18.46°, 32.43°, and 20.48°, respectively. For the requirements of testing, the only AcAc sol is chosen for the coating sol. The differences of contact angles among chips and different points of one chip are both not more than 10%.

3.3. About the qualities and properties of the coating film: The ZYGO surface profiler was used to detect the surface topography of the coated substrate. 50 times the objective lens was used, and ZOOM value was measured at 1. Besides, the detected surface of the hydrophilic coating solution used AcAc as a complexing agent, and we set up a control group. After cleaning the substrate under the same conditions with hydrophilic coating under the same experimental environment (20°) of detection, the detection results are shown in Fig. 4b, and it is the hydrophilic surface

contour map and Fig. 4a is untreated surface. It is shown that the surface roughness of the untreated substrate is 0.015 µm, and the hydrophilic coating has a surface roughness of 0.013 µm. The difference between the roughness of coating surface and the untreated surface is little. So the reason for the increase of the substrate surface hydrophilicity is the presence of surface hydroxyl groups rather than the changing roughness of the substrate surface after dip coating. In addition, we used the step apparatus (ET4000M, Kosaka, Japan) to measure the thickness of the coating film while the dip velocity was 2 mm/s and the temperature is 20°C. Half of the substrate was coated with a sol film and the other half was not. The probe of the step apparatus scanned from the coating part to the blank part, and by measuring the height changing of the probe to estimate the thickness of the coating film. As shown in Fig. 4c, it is the height changing of the probe on the substrate surface. A baseline was the average height of the probe in the coating film. Two points were chosen as the measuring points from the untreated part. The measurement shows that the distance between the measuring point 1 and the baseline is 245 nm and point 2 is 223 nm, so the thickness of the coating film is about 234 nm. Besides, according to the method of DIN EN ISO 2409:2007 standard coated mesh cutting test, we measured the adhesion between the coating and the substrate and the result is shown in Fig. 4d. After the grid cutting experiment, the shedding of the surface coating film is about 8%, which shows that the TiO₂ hydrophilic coating has good adhesion.

In conclusion, the average thickness is 234 nm while the dipping speed is 2 mm/s and AcAc is used as the complexing agent, and it is far less than the channel size of the structure, so the dip coating will not affect the original channel microstructure. The ZYGO surface morphology measuring also confirms the above conclusion. It is also proved that the increase of surface hydrophilic is the presence of surface hydroxyl groups rather than the changing roughness of the substrate surface after dip coating. Besides, the surface adhesion

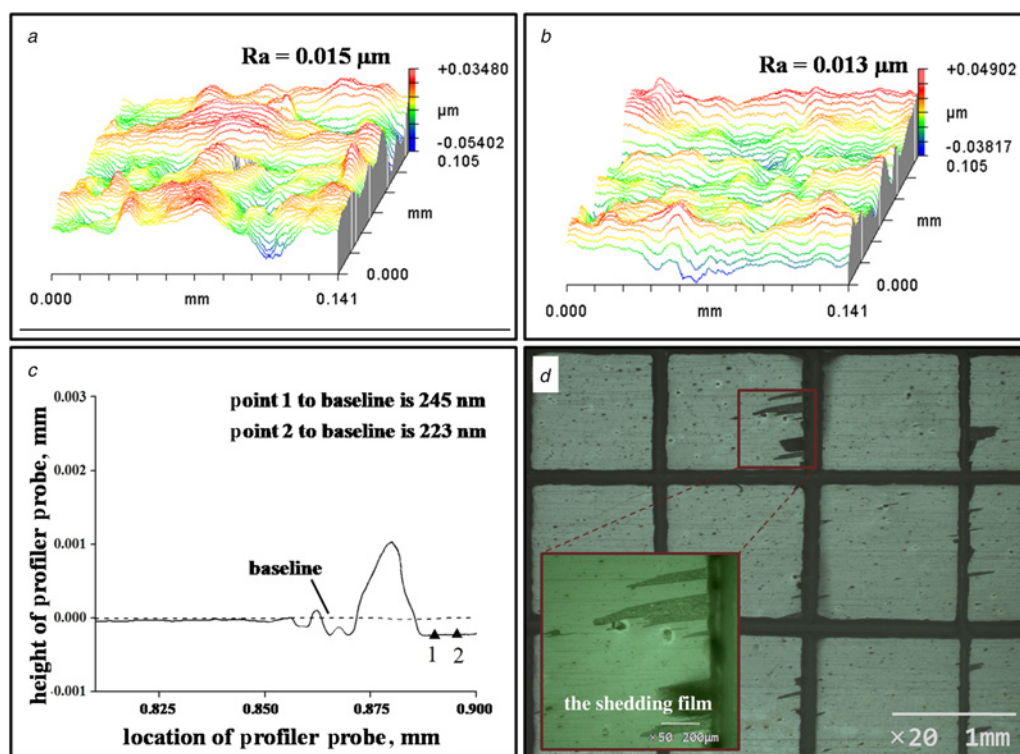


Fig. 4 Qualities and properties of the coating film

a Surface contour map by ZYGO surface profiler before coating

b Surface contour map by ZYGO surface profiler after coating

c Height changing of the probe of the step apparatus on the substrate surface

d Micrograph of the coated surface after adhesion testing by tool microscope

testing shows that the hydrophilic film had good adhesion on the PMMA surface.

3.4. Performance of liquid in the chips: The flow of serum in the microchannel of the microfluidic chip was observed with a stereo microscope, and the flow stability of the microchannel was investigated by comparing the flow time of the serum in groups of different microchannels. We set two groups of chips and each group contains ten chips. One group of chips was coated with hydrophilic films and another did not. First, the surface of a substrate coated with a hydrophilic film and the hydrophilic sol used AcAc as the complexing agent. The control group was untreated. Second, the two groups of microchannel sidewalls were marked with hydrophobic lines to avoid the generation of bubbles while the liquid flow in the channel. Third, conductive filter papers were inserted into each substrate to drive liquid into the microchannel. Finally, after cleaning, we welded the cover plates on the substrate by ultrasonic welding machine.

Then, 150 μl of serum was injected into the chip and the liquid flow in the microchannel. Also, record the flow time and the changing of the liquid front by screening under a stereo microscope. The result is shown in Fig. 5. In the control group, six chips flow stopped, and the remaining flow times were obviously too long or irregular flow occurred. The flow rate of the chip coated with the hydrophilic film is stable, and the flow velocity fluctuates within acceptable limits (as shown in Fig. 5a), which is required for the single testing. In addition, the average flow velocity is stable, and the deviation value is not more than 15%. Meanwhile, as shown in Fig. 5b, the channels were segmented into seven parts, and the average velocity in each segment was taken. The difference of liquid velocity among each segment after the hydrophilic coating is small. So the liquid velocity is stable in the same location from different chips. Besides, in the chip without hydrophilic coating, the shape of the liquid front is unstable (shown in

Fig. 5c), and it is easy to pack bubbles in the channel to influence the flow of the liquid affecting the detection. The liquid front in the channel after the hydrophilic coating is stable and the irregular liquid flow does not occur, as shown in Fig. 5d.

According to the research of the liquid flow in the channel before and after dip coating, the results show that after hydrophilic coating the flow velocity becomes faster and the liquid front became more stable. So the chip surface modified by hydrophilic coating can effectively improve the performance of liquid flow in the chip.

3.5. Detection of antigens by using the POCT device: The experimental POCT device is used to detect the cTnI, which is a biomarker of myocardial infarction. Before the fluorescence detection, the mixing chamber of the substrate was pipetted with water-soluble fluorescence substances, and the testing area was pipetted with antibodies by using a biological sample spotting machine. While the serum flowed to the channel, the samples will mix with water-soluble fluorescence substances in the mixing chamber, and then the cTnI in the serum will bind with fluorescence materials. Subsequently, the serum will flow into the detection channel and react with the antibodies within the channel to generate a testing fluorescence peak, and the testing machine is shown in Fig. 6a.

We compared the different performance of a hydrophilic coating to UV-light modification which is a popular method to enhance the surface tension of PMMA at present. The contact angle of chips is about 32° after 183 s UV-light modification and the contact angle of coated chips is about 32° as well. Fig. 6b shows the peak of the cTnI in the serum sample. It can be seen that the baseline mean value is about 4000 and the peak value is about 23,000–27,000 while the microchannel is modified by UV-light. In contrast, the single peak values are all about 35,000 by coating modification. The peak heights have increased to about 10,000. Also, the multi-peaks occurred by the UV-light modification, that

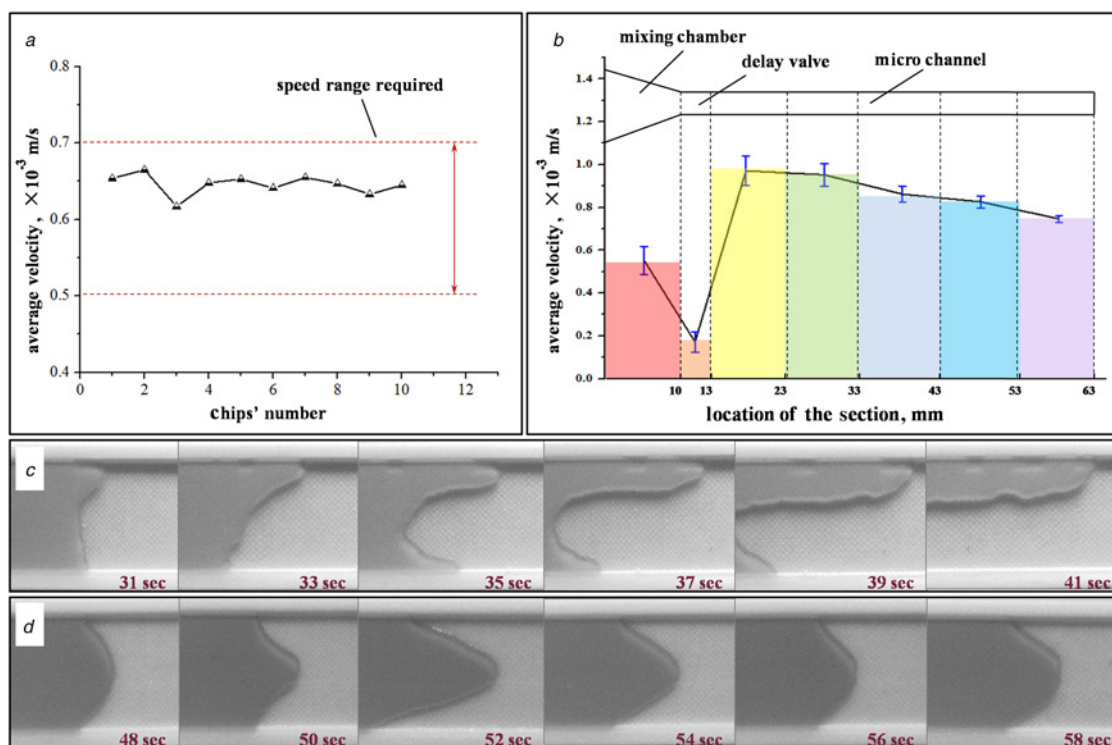


Fig. 5 Performance of liquid in the chips before and after coating
a Differences of average flow velocity among different chips after coating
b Differences of liquid velocity among each segment of ten chips after hydrophilic coating
c Shape changing of the liquid front before coating
d Shape changing of the liquid front after coating

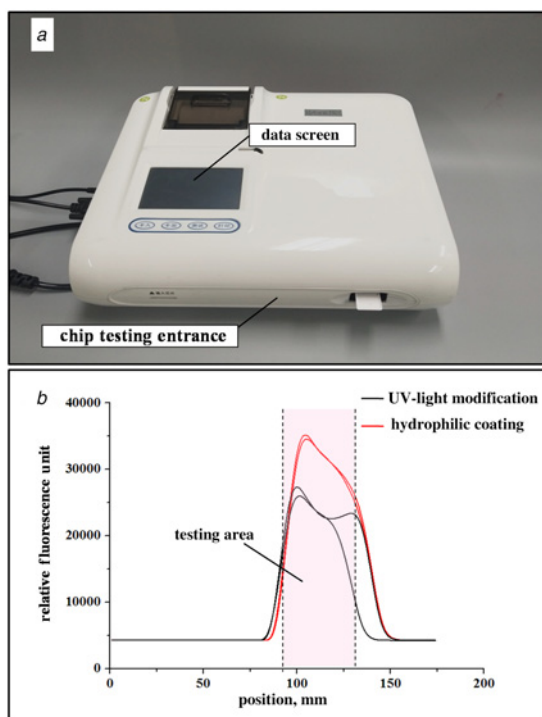


Fig. 6 Fluorescence testing equipment and testing results
a Fluorescence intensity tester
b Contrast fluorescence value testing figure of UV-light modification and hydrophilic coating

is because the uniformity of UV-light is difficult to control. Also, UV-light treatment varies considerably between batches.

In addition, results have shown that the peak heights for cTnI have been increased obviously and no multi-peaks occurred by using a hydrophilic coating. The hydrophilic coating has exhibited its benefit in improving the detection precision and accuracy compared with the popular UV-light.

4. Conclusions: This Letter demonstrates that the sol–gel coating can increase the driving force of the capillary and can be applied to the POCT chip channel to drive the liquid stream to flow smoothly. The DEA, AcAc, and DEA & AcAc are used as the complexing agents of sol the coating film, and we chose only AcAc sol for the coating sol to match the requirements of testing. In addition, the average thickness is 234 nm, and it is far less than the channel size of the structure, so the dip coating will not affect the original channel microstructure and have a good quality. After hydrophilic coating, the flow velocity becomes faster and the liquid front became more stable. So the chip surface modified by hydrophilic coating can effectively improve the performance of liquid flow in the chip. Also, the peak heights for cTnI have been increased obviously and no multi-peaks occurred by using a hydrophilic coating. The hydrophilic coating has exhibited its benefit in improving the detection precision and accuracy compared with the popular UV-light.

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