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Surface Hydrophilic Modification for Chip of Centrifugal Microfluidic Immunoassay System

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Abstract: The surface of a centrifugal microfluidic immunoassay system chip such as polymethyl methacrylate (PMMA) is often hydrophobic, which leads to problems such as poor liquid transfer efficiency and easy-to-block siphon channels, leading to bad fluid control. Therefore, surface hydrophilic modification for such chips is necessary to improve the rapidity and sensitivity of the system. Chemical modification is commonly used, but there is little research on the hydrophilic effect of different concentrations of hydrophilic reagents. According to function requirements for different microchannels of the chip (some only need to ensure the liquid can flow into the next chamber, and some also need to ensure the function of “closing the door” during immunoassay incubation), we explored the best combination of hydrophilic reagent and concentration through experiments. Firstly, three hydrophilic reagents were used for modification. Secondly, the hydrophilic effects of different reagents and concentrations were explored by contact angle test, the influence of different modification methods on liquid transfer efficiency was characterized by residual liquid calculation in the chamber. Finally, the effect of different hydrophilic reagents on absorbance was also tested. By experimental results and comprehensively considering the stability of the modification effect and the function requirements, Tween-20 (2.0% v/v) was chosen as the modifying reagents of the first siphon valve and the second siphon valve, and TritonX-100 (2.0% v/v) was chosen for the third siphon valve, which effectively reduces the contact angle and improves the liquid transfer efficiency, leading to further improvement of the rapidity and sensitivity of the centrifugal microfluidic immunoassay system by efficient siphoning and high plasma separation efficiency (99%).

Keywords: hydrophilic modification; contact angle; liquid transfer efficiency; centrifugal microfluidic immunoassay system

1. Introduction

Centrifugal microfluidics is an important branch of microfluidic technology, which is widely used in immunoassay, biochemical analysis and molecular diagnosis [1–5]. Among them, the centrifugal microfluidic immunoassay system plays a great role in disease prevention and diagnosis. However, different from the traditional laboratory, the centrifugal microfluidic immunoassay system is based on the microchannel network, which integrates all steps, including fluid loading, reaction and detection onto a “lab-on-a-chip” where precise step-by-step liquid transfer is required [4,5]. Then, the performance of the liquid flow on the chip greatly affects their rapidity and sensitivity. On the one hand, the roughness and geometry of the microchannel have a certain impact on the liquid flow, for example, Kasiterpoulou et al. [6] used numerical methods to deeply study the impact of wall roughness on the fluid flow and found that near the rough wall, the fluid particles tend to be trapped in the wall cavity, and the flow rate will decrease with the narrowing of the channel.
Arash et al. [7] also used numerical methods to study how a water droplet interacts with micrometer-sized patterns of a hybrid hydrophobic/-philic surface and revealed the details of the droplet–surface interaction. On the other hand, the hydrophilicity of microchannels also affects the flow of liquid, especially when capillary force is dominant. For example, Kitsara et al. [8] increased the surface free energy by adding hydrophilic reagents on the surface of microchannels; in this way, they can enhance the surface hydrophilicity and promote the sequential loading of liquid in series siphon valves.

The surface of the commonly used microfluidic chip (PMMA) is often hydrophobic, which may bring problems of poor liquid transfer efficiency and easy channel blockage, leading to bad fluid control [9]. Therefore, surface hydrophilic modification is necessary. Different modification methods can be divided into physical modification and chemical modification methods [10,11]. Physical modification methods generally introduce hydrophilic groups to the material surface through oxygen plasma. The chemical modification method is to graft the reagents containing hydrophilic groups onto the surface of the chip. Compared with physical modification, chemical modification has the advantages of simple operation and good durability.

The commonly used hydrophilic modification reagents include Tween-20 [11], Triton X-100 [12] and polyethylene glycol (PEG) [13]. However, there are few studies on the hydrophilic effect under different concentrations. When the concentration is too low, chemicals are difficult to graft on the channel surface, leading to a poor hydrophilic effect [14]. When the concentration is too high, chemicals can easily pollute the liquid in the channel [15], and may rupture the cell membrane, affecting the accuracy of the results [16]. Moreover, for the PMMA chip we designed for immunoassay system, the functional requirements of different microchannels are different. The purpose of the first and the second siphon valves is only to ensure the liquid can flow into the next chamber. So, these channels only need to adopt the modification method with strong hydrophilicity and good stability. The third siphon valve should not only realize the transfer of liquid, but also ensure it plays the role of “closing the door” during immunoassay incubation. Therefore, it is necessary to explore the best combination of hydrophilic reagents and concentration to meet the hydrophilic treatment requirements of the chip for centrifugal microfluidic immunoassay systems.

In this work, firstly, we used the three reagents above to carry out hydrophilic modification. Secondly, the hydrophilic effects of different reagents and concentrations were explored by contact angle test, and the influence of different modification schemes on liquid transfer efficiency was characterized by residual liquid calculation in the chamber. Finally, the effect of reagents on absorbance was also tested. The experimental results show that: 1. The higher the surface tension, the better the control of the liquid, but the efficiency of liquid transfer also decreases. 2. With the increase in reagent concentration, the contact angle becomes smaller and the hydrophilicity is better. 3. There is no significant difference in absorbance among the three different treatment methods. Considering the stability of the hydrophilic effect and the functional requirement of each siphon valve, Tween-20 (2.0% v/v) was chosen as the surface modification reagents of the first siphon valve and the second siphon valve, and TritonX-100 (2.0% v/v) was chosen as the surface modification reagents of the third siphon valve. This scheme effectively reduces the contact angle and improves the liquid transfer efficiency, which meets the hydrophilic treatment requirements of step-by-step liquid transfer on centrifugal microfluidic chip, and is expected to further improve the rapidity and sensitivity of the centrifugal microfluidic immunoassay system by the realization of efficient siphoning and high plasma separation efficiency (99%).

2. Materials and Methods

2.1. Materials and Instruments

Instruments: ELX800 microplate reader (Winooski, VT, USA), PDC-MG plasma cleaning machine (Chengdu, China), OCA 25LHT contact angle measuring instrument (Shanghai, China), XGQ-2000 blast drying oven (Yuyao, China).
Materials and reagents: Tween-20, TritonX-100 (Hefei, China), PEG400 (Shanghai, China), Deionized water (Chengdu, China).

2.2. Hydrophilic Treatment Mechanism of PMMA

Hydrophilic treatment methods include physical modification (plasma treatment) and chemical modification. Plasma is known as the fourth state of substances. When enough energy is added to the gas to ionize it, it will become a plasma state. The active components of plasma include ions, electrons, atoms, activated groups, excited nuclides and photons. By introducing reactive gases such as oxygen, nitrogen and hydrogen into the plasma cleaning machine, complex chemical changes can occur on the material surface and new functional groups can be introduced. In this experiment, oxygen plasma treatment is adopted. When oxygen enters the plasma cleaning machine and contacts with air, it will produce -COOH, -OH and other groups on the surface of the material and increase their hydrophilicity [17].

For chemical modification, the three reagents used in the experiments are TritonX-100, Tween-20 and PEG400. Their chemical structure formula is shown in Figure 1.

![Chemical structural formulas and related interaction mechanism](image)

Figure 1. Chemical structural formulas and related interaction mechanism: (A) TritonX-100; (B) Tween-20; (C) PEG400; (D) arrangement mechanism of Tween-20, TritonX-100 in water; (E) interaction mechanism between Tween-20, TritonX-100 and the PMMA surface.

The molecules of the first two reagents have amphiphilic structure, PEG chain segment is a hydrophilic group, while long alkyl chain is a hydrophobic group. When these molecules are in contact with water, the hydrophilic groups and water molecules have strong hydration, resulting in the molecules dissolving in water. While the hydrophobic groups and water molecules have no affinity and escape from the water. As shown in Figure 1D, the two opposite forces make the molecules enriched at the water/air interface, and the hydrophilic group is in the aqueous phase and the hydrophobic group is in the oil phase (or air). The hydrophilic group in the aqueous phase produces positive adsorption, which reduces the surface tension [18]. It should be noted that the amphoteric groups must be basically matched before they can have significant surface activity. Any groups too strong or too weak will significantly weaken the surface activity of amphiphilic molecules.

2.3. Hydrophilic Treatment and Contact Angle Test

The microfluidic chip we designed consists of a chip lid and a chip substrate (Figure 2). The chip substrate is a disk with a radius of 33 mm (Figure 2B), which is composed of different microchannels and micro chambers (the cross-sections of the channels are rectangular). There are three different siphon valves with the same depth of 0.1 mm on the chip substrate. The first siphon valve is 20.29 mm long and has the minimum width of 0.2 mm. The second siphon valve is 18.68 mm and the third siphon valve is 19.04 mm; they both have the maximum width of 0.25 mm. It is worth noting that the process of hydrophilic modification needs to be carried out on the chip substrate and be completed before bonding process.
2.3. Hydrophilic Treatment and Contact Angle Test

The microfluidic chip we designed consists of a chip lid and a chip substrate (Figure 2), substrate made up of different microchannels and microchambers in which 1, 2, 3 refer to the first, second and third siphon valve, respectively.

Tween-20 and TritonX-100 were diluted with 95% ethanol to four concentrations with volume fraction of 0.5% $v/v$, 1.0% $v/v$, 1.5% $v/v$ and 2.0% $v/v$, and PEG400 was diluted with deionized water to four concentrations with mass fraction of 5 wt%, 10 wt%, 15 wt% and 20 wt%.

After finishing the preparation of reagents, we placed the PMMA chip we designed in 95% ethanol solution for ultrasonic cleaning for 15 min, and then placed it in distilled water for ultrasonic cleaning for 15 min. After cleaning, we dried the surface with nitrogen and dried it in an oven for 30 min at 40 °C. PMMA with clean surface and no moisture were divided into two groups. The physical modification group was treated with PDC-MG oxygen plasma cleaner at 150 W for 60 s. In chemical modification groups, PMMA were directly immersed in different concentrations of Tween-20, Triton X-100 and PEG400 for 24 h, and then were taken out and placed in a dry and matte environment.

In order to compare the hydrophilicity effect between untreated PMMA and PMMA treated by different methods, the contact angle of PMMA surface was measured by dynamic contact angle measuring instrument (Figure 3A).

A total of 2 µL of deionized water was added to the PMMA surface vertically with a pipette gun. After the droplet was stabilized on the surface, the distance from the light source to the loading platform was adjusted until a clear droplet profile was displayed on the screen to measure the contact angle. Three to five points at different positions of PMMA surface were selected, the above steps were repeated, and the contact angle was measured.
and averaged. In order to study the stability of various treatment methods, the contact angle was tested on the fifth, tenth, fifteenth and twentieth day after treatment.

2.4. Liquid Transfer on Chip

Since the centrifugal microfluidic immunoassay system needs to realize step-by-step liquid transfer on the chip, the efficiency of liquid transfer in the channel after hydrophilic reagent treatment is very important. Since the third siphon valve should not only realize the transfer of liquid, but also ensure that it plays the role of “closing the door” during incubation, this channel needs to be studied separately. The purpose of the first and the second siphon valves is only to ensure the liquid can flow into the next chamber, so only a method with strong hydrophilicity and good stability is needed.

The experimental steps are as follows: 1. Built the experimental platform (Figure 3B). 2. Dropped all kinds of hydrophilic reagents (6 μL) into the third siphon valve of the chip, respectively, made the whole channel covered with hydrophilic reagents, evaporated in the air for 15 min, removed the solvent and bonded the chip (Figure 4A). The chip treated with plasma was immediately bonded after treatment (150 W, 1 min). 3. Diluted the red ink to 1:20 with deionized water. Injected 200 μL diluted red ink into the mixing chamber (Figure 4B). 4. Placed the chip on the platform, and set the speed to 3000 rpm (maximum) for 30 s to study which hydrophilic treatment method has the higher liquid transfer efficiency under maximum centrifugal force (indicating this hydrophilic treatment method plays a leading role in liquid transfer below the maximum speed). 5. After centrifugation, most of the liquid had been transferred to the next chamber (Figure 4C), and the remaining liquid in the mixing chamber was extracted with a needle for volume calculation (Figure 4D). 6. Repeated each method three times and averaged it.

Figure 4. Schematic diagram of on-chip liquid transfer: (A) add hydrophilic reagent; (B) inject red ink; (C) centrifugal process; (D) extract and calculate.

2.5. Absorbance Test

In order to explore whether the hydrophilic reagent in the siphon channel will interfere with the subsequent immunoassay detection, the absorbance of red ink (with different hydrophilic reagents added) was tested by enzyme labeling instrument. The operation process is as follows: 1. Took 6 μL of Tween-20 and TritonX-100 with volume fraction of 0.5% v/v, 1.0% v/v, 1.5% v/v and 2.0% v/v and PEG400 with mass fraction of 5 wt%, 10 wt%, 15 wt% and 20 wt% in centrifuge tubes, respectively. 2. Took 6 μL deionized water as the blank control group, added 200 μL red ink diluent into the centrifuge tube and mixed well. 3. Sucked 150 μL solution with a pipette gun, added it to the enzyme labeling plate, and tested the absorbance with ELX800 automatic enzyme labeling instrument. Repeated the measurement three times in each group.
3. Results and Discussion

3.1. Contact Angle Test

When the hydrophilic modification was completed, the contact angle was tested, and the test results are shown in Figure 5.

![Figure 5](image)

Figure 5. Contact angle results: (A) oxygen plasma; (B) Tween-20; (C) PEG400; (D) TritonX-100.

The contact angles of the four hydrophilic methods are small in a short time after treatment, and the average contact angle on the fifth day of oxygen plasma treatment group is 42.64° (Figure 5A). The average contact angles of Tween-20 treatment group (0.5% v/v, 1.0% v/v, 1.5% v/v and 2.0% v/v) on the fifth day are 42.34°, 35.79°, 6.45° and 4.46°, respectively (Figure 5B). The average contact angles on the fifth day after PEG400 (5 wt%, 10 wt%, 15 wt%, 20 wt%) treatment are 54.46°, 35.79°, 47.83° and 55.68°, respectively (Figure 5C). The average contact angles of TritonX-100 (0.5% v/v, 1.0% v/v, 1.5% v/v and 2.0% v/v) on the fifth day after treatment are 12.99°, 11.25°, 13.18° and 13.39°, respectively (Figure 5D). The contact angle of the untreated surface is 85.99°, which means that the four methods can significantly change the hydrophilicity of the PMMA surface. The surface properties of PMMA treated with oxygen plasma are prone to aging over time. Theoretically, the hydrophilic surface will gradually return to the original hydrophobicity, and the aging process will be affected by temperature, humidity and atmosphere [14]. In the experiments, all treated PMMA were placed in a dark environment with a room temperature of 25 ± 3 °C, an air humidity of 35 ± 5% and a relatively stable atmosphere, so the process of restoring hydrophobicity of PMMA was delayed.

For the PMMA treated with different reagents, since the PEG chain segment in TritonX-100 and Tween-20 is the hydrophilic group and the long alkyl chain is the hydrophobic group, they are amphiphilic. When these amphiphilic surfactant molecules are in contact with the surface of hydrophobic material (PMMA), their hydrophobic end and hydrophobic surface are closely combined through hydrophobic interaction, while the hydrophilic end and hydrophobic surface repel each other to form the structure shown in Figure 1E, so as to realize the functionalization of the hydrophobic surface and improve its affinity with water. Different from TritonX-100 and Tween-20, PEG is adsorbed to the hydrophobic surface through physical adsorption, and its high hydrophilicity is used to form a hydrophilic protective layer on the hydrophobic surface, which significantly improves the affinity between the material surface and water [19].
For Tween-20, the hydrophilic stability is the best at the treatment concentration of 2.0% v/v, and the contact angles on the 10th, 15th and 20th days are 12.23°, 22.92° and 9.88°, respectively (Figure 5B). Although the effect of other lower concentration groups is better in a short time, the hydrophilicity of the PMMA surface gradually becomes worse over time, for example, on the 20th day, there is no significant difference between the surface contact angle of PMMA treated with 0.5% v/v and the untreated one. On the premise of the same treatment time, the overall trend of hydrophilicity and hydrophilic reagent concentration shows a positive correlation.

For TritonX-100, the hydrophilic stability is the best at the highest concentration (2.0% v/v, Figure 5D), and the contact angle on the 20th day is 14.80°. The surface contact angle of low-concentration treatments changes greatly with poor stability (compared with the treatment methods of the other two reagents). It can be seen from the molecular structure (Figure 1A) that TritonX-100 contains more methyl than the other two structures, and methyl is a hydrophobic group, so the hydrophilicity is not stable enough. Based on this conjecture, we dropped these three reagents directly on the surface of blank PMMA and observed the contact angle between them and PMMA. Each group was measured three times and averaged it. As shown in Figure 6, at the same concentration, the contact angle of TritonX-100 is higher than that of Tween-20, and the standard deviation is also large, indicating that the methyl group in the molecular structure of TritonX-100 has a certain impact on its hydrophilicity.

For PEG400, the group with the highest concentration (20 wt%) still has the best hydrophilic stability. The contact angle on the 20th day is 55.60° (Figure 5C). However, the results show that the hydrophilic modification ability of PEG400 is weaker compared with the other two treatment methods. This is because given the condition of 25 °C and a standard atmospheric pressure, the surface tension of four concentrations of PEG400 is between 45–50 mN·m⁻¹, the surface tension of four concentrations of TritonX-100 is 40 ± 1 mN·m⁻¹, the surface tension of Tween-20 (0.5–1.0% v/v) is 20-30 and the surface tension of Tween-20 (1.5–2.0% v/v) is 5–10 mN·m⁻¹ [20]. The reason for this phenomenon
is that TritonX-100 and Tween-20 are amphiphilic compounds, and their hydrophobic ends will be arranged on the water surface, which destroys the interaction between water molecules on the liquid surface and reduces the surface tension of the liquid, while PEG400 is a high hydrophilic compound, which has no hydrophobic chain segment to destroy the interaction between water molecules on the liquid surface, resulting in high surface tension of the solution. The surface tension reflects the ability of liquid surface shrinkage; the smaller the shrinkage force, the easier the solution is to spread on the surface, which explains the poor hydrophilic effect of PEG400. However, because the surface tension is large and the cohesion of aqueous solution on the PMMA surface is strong, the stability of contact angle of PMMA surface treated by PEG400 is the best and will not fluctuate greatly as time goes by.

The experiments above show that hydrophilicity is positively correlated with the concentration of hydrophilic reagent and negatively correlated with the treatment time. The best effect can be obtained by selecting the reagent with a high concentration and doing the experiment as soon as possible after treatment. However, when the reagent with a too high concentration is used in immunoassay, it may destroy the cell membrane and dissociate the nuclear protein, which will affect the experimental results. Although the target of immunoassay is protein, considering the universality of hydrophilic reagent, the reagent with the same concentration above will still be used for research.

3.2. Liquid Transfer Efficiency on Chip

The methods above were applied to the third siphon valve of the centrifugal microfluidic chip. When the chip injected with liquid was fixed on the centrifugal motor, we used the maximum rotational speed to ensure the maximum centrifugal force on the chip. Under the combined action (Equation (1)) of centrifugal force $P_c$ and capillary force $P_{cap}$ acting on the liquid in the mixing chamber [21], it entered the next chamber after passing through the third siphon valve.

$$P_{total} = P_c + P_{cap} \quad (1)$$

For the same kind of liquid, the centrifugal force is independent of the hydrophilicity of the siphon channel. However, for the siphon channel with the rectangular cross-section (width is $W$ and length is $L$), as shown in Equation (2), the capillary force is closely related to the surface tension ($\gamma$) of the liquid and the contact angle between the liquid and each surface of the siphon channel.

$$P_{cap} = \gamma \left( \frac{2 \cos \theta_s}{W} + \frac{\cos \theta_t + \cos \theta_b}{L} \right) \quad (2)$$

where $\theta_s$ is the contact angle between the liquid and the side of the channel, $\theta_t$ is the contact angle between the liquid and the top of the channel, and $\theta_b$ is the contact angle between the liquid and the bottom of the channel. Obviously, under the action of different hydrophilic reagents, the hydrophilicity of the siphon channel is also different (specifically, the contact angle decreases and the surface free energy increases) [8], which makes the capillary force of the liquid greatly different. The capillary force is the main force during the siphon prime process (initial liquid transfer) under a low frequency; therefore, under the condition of maximum centrifugal force, if the liquid transfer efficiency of the siphon channel after a hydrophilic treatment method is higher, the siphon channel after the hydrophilic treatment method has a stronger liquid driving ability at low rotational speed or even stop. The liquid transfer efficiency of various methods was characterized by calculating the residual liquid in the mixing chamber (Figure 7).
The liquid transfer efficiency of oxygen plasma treatment and the Triton X-100 (2.0% Triton X-100 treatment group (0.5% Triton X-100 treatment group) is more than 85%). The liquid transfer efficiency of liquid transfer is the Tween-20 treatment group (2.0% Tween-20 treatment group) is more than 85%). The statistics of residual liquid under various methods are shown in Table 1. The best efficiency of liquid transfer is the Tween-20 treatment group (2.0% Tween-20). Followed by the Triton X-100 treatment group (0.5% Triton X-100), the liquid transfer efficiency is more than 90%. The liquid transfer efficiency of oxygen plasma treatment and the Triton X-100 (2.0% Triton X-100 and 1.5% Triton X-100) treatment group is more than 85%.

**Table 1. Statistics of liquid transfer efficiency.**

| Methods       | Concentration | Residual (µL) | Transfer Efficiency |
|---------------|---------------|---------------|---------------------|
| TWEEN-20      | 0.5% v/v      | 43.7          | 78.15%              |
|               | 1.0% v/v      | 41.5          | 79.25%              |
|               | 1.5% v/v      | 33.3          | 83.35%              |
|               | 2.0% v/v      | 4.3           | 97.85%              |
| TritonX-100   | 0.5% v/v      | 14.9          | 92.55%              |
|               | 1.0% v/v      | 37.1          | 81.45%              |
|               | 1.5% v/v      | 25.4          | 87.30%              |
|               | 2.0% v/v      | 25.0          | 87.50%              |
| PEG400        | 5 wt%         | 184.3         | 7.90%               |
|               | 10 wt%        | 160.2         | 19.90%              |
|               | 15 wt%        | 51.6          | 74.20%              |
|               | 20 wt%        | 31.2          | 84.40%              |
| Oxygen plasma |               | 20.7          | 89.65%              |

However, the difference of liquid transfer efficiency far exceeds the difference of contact angle when using different reagents. Although the chips were injection molded with the same parameters, the abrasion degree of each chip is different, so it is difficult to ensure the unity of surface roughness, which partly explains the large gap in liquid transfer efficiency. In addition, we speculate that the liquid absorption of the modified siphon channel also has a certain influence on the liquid transfer. In order to verify this speculation, the surface of blank PMMA was hydrophilized by the above treatment methods, 100 µL red ink was added to the surface with a pipette gun, the red ink was sucked out after 30 s, and the change of the thickness of the residual liquid layer on the surface with time was observed. After the ink was removed, pictures of the residual solution on the PMMA surface were taken (once every 20 s for 2 min).

The results show that compared with the blank group, almost all the modified PMMA surfaces have the phenomenon of water absorption. The liquid layer thickness of the surface treated with PEG400 is the highest, which is due to the smaller hydrophobic group of PEG compared with the other two reagents. It can be seen from the above analysis that when the hydrophobic group is too weak, the hydrophilicity is affected, and the aqueous solution has strong cohesion on its surface; therefore, the macro performance is that the adsorbed liquid layer is thicker (PEG group). This conclusion is consistent with the results of liquid transfer experiments. The difference of the thickness of the liquid

![Figure 7. Liquid transfer on chip: (A) liquid loading; (B) liquid transfer; (C) residual liquid extraction.](image-url)
layer between PMMA treated by plasma, TritonX-100 and Tween-20 and the blank group is relatively small. Taking the average liquid layer thickness as an example, the average liquid layer thickness of PMMA treated by PEG400 (10 wt%) is 2.8 times that of the blank group. When using other methods, the thickest liquid layer was found in the TritonX-100 group (1.0% v/v), which is 1.8 times that of the blank group. In conclusion, it can be considered that the main factors of the difference in liquid transfer efficiency are the uneven roughness of the chip surface together with the different adsorption ability for aqueous solution using various treatment methods.

The analysis above shows that under the treatment with low surface tension, the channel surface will hardly absorb water and all the liquid flows successfully to the next chamber, but the good hydrophilicity also means that it is difficult to “close the door” in the incubation step during immunoassay. For the treatment method with the high surface tension, the surface of the siphon channel will have a liquid layer, resulting in the reduction in the efficiency of the liquid flowing through the next chamber, which plays a role in controlling the liquid in the incubation step. However, the liquid layer that is too thick will cause a blockage in the channel, which is not conducive to the flow of the liquid. To sum up, TritonX-100 of 2.0% v/v is selected as the modification scheme for the third siphon valve, it is because its liquid transfer efficiency is high enough for liquid transfer with its thickness of the adsorbed liquid layer on the surface between those of Tween-20 and PEG400 (just fit the functional requirement of closing up), which is an ideal compromise scheme.

3.3. Absorbance Test

The main purpose of this paper is to use different hydrophilic treatment methods to realize the better “valve control” function of liquid on centrifugal microfluidic chip. Therefore, in Section 3.1, the contact angle was used to characterize the differences of different hydrophilic treatment methods, while in Section 3.2, the residual liquid volume in the mixing chamber was used to characterize the liquid transfer efficiency on the chip. After realizing the efficient transfer of liquid on chip, it is also necessary to consider whether the hydrophilic reagent will affect the absorbance detection of the solution containing organic compounds. For this, we further explored with red ink solution.

It can be seen from Table 2 that there is no significant difference in absorbance among the three different treatment methods. For the same kind of hydrophilic reagent, there are differences in absorption under different concentrations. It can be found that, in the groups treated with Tween-20 and TritonX-100, the absorbance of red ink generally decreases with the increase in hydrophilic reagent concentration. The possible reason for this phenomenon is that micelles were formed after hydrophilic reagent enters the red ink solution, and some dyes have an aggregation quenching effect, which makes the absorbance show a slight decrease.

3.4. Plasma Separation Test

The modified chip was used on the centrifugal microfluidic immunoassay prototype (Figure 3C) we designed for plasma separation to further verify the effect of hydrophilic modification. Firstly, human whole blood was added to the chip. Secondly, blood was successfully separated into pure plasma and red blood cells (RBCs) quickly (in 60 s, 10 s less than before modification) and was successfully extracted by the siphon channel after a series of rotational speed switches. Figure 8A shows the RBCs in the blood before separation, and Figure 8B shows the RBCs in the pure plasma extracted by siphon after separation with a separation efficiency of 99%. The shortening of the siphon and extraction time indicates that the hydrophilicity of the first siphon valve is improved. Finally, the diluted plasma also successfully entered the next chamber with no cross-contamination observed during the incubation process at a low frequency, which indicates the improvement for function of the third siphon valve. The experimental results above verify the effectiveness of hydrophilic modification again.
principles of different treatment methods were analyzed theoretically. Secondly, experi-
ments were designed to explore the effects of different treatment methods, including the con-
tact angle test, liquid transfer efficiency test and the absorbance test. Finally, the follow-
ing conclusions are drawn: 1. In a short time after surface modification, the hydrophilic ef-
effect is the best, and the effect decreases gradually with the increase in time. For the same reagent, the higher the concentration, the better the stability of the hydrophilic effect.

2. The treatment method with low surface tension has high liquid transfer efficiency. Using the modification method with poor liquid transfer efficiency means that it is easy to block the siphon channel, which is not conducive to the control of fluid. However, high liquid transfer efficiency also means that the liquid is transferred too quickly, which leads to difficulty playing the role of “closing the door” in the incubation step of the immunoassay.

3. There is no significant difference in absorbance among the three different treatment methods. According to the experimental results, considering the stability of the hydrophilic effect and the functional requirements of each siphon valve, Tween-20 (2.0% v/v) was chosen as the surface modification reagents of the first siphon valve and the second siphon valve, and TritonX-100 (2.0% v/v) was chosen as the surface modification reagents of the third siphon valve, which effectively reduces the contact angle between the liquid and the chip, improves the liquid transfer efficiency, ensures the “closing up” function of the third siphon valve and has little interference on the absorbance of solution. This method meets the hydrophilic treatment requirements of step-by-step liquid transfer on the chip and realizes fast and efficient blood separation (99%) and siphoning, and it is expected to

| Methods    | Concentration | Average Absorbance | Standard Deviation |
|------------|---------------|--------------------|--------------------|
| Tween-20   | 0.5% v/v      | 3.76               | 1.6%               |
|            | 1.0% v/v      | 3.706              | 3.5%               |
|            | 1.5% v/v      | 3.633              | 3%                 |
|            | 2.0% v/v      | 3.621              | 1%                 |
| TritonX-100| 0.5% v/v      | 4.087              | 2.37%              |
|            | 1.0% v/v      | 3.837              | 3.15%              |
|            | 1.5% v/v      | 3.818              | 4.54%              |
|            | 2.0% v/v      | 3.71               | 2.15%              |
| PEG400     | 5 wt%         | 3.81               | 12.9%              |
|            | 10 wt%        | 3.875              | 17.3%              |
|            | 15 wt%        | 3.884              | 9.3%               |
|            | 20 wt%        | 3.824              | 10.3%              |
| Blank group|               | 3.747              |                    |

Figure 8. Number of RBCs calculated by flow cytometry: (A) before separation; (B) after separation.

4. Conclusions

In this paper, the surface hydrophilic modification technology of a PMMA chip of a centrifugal microfluidic immunoassay system was comprehensively explored. Firstly, the principles of different treatment methods were analyzed theoretically. Secondly, experiments were designed to explore the effects of different treatment methods, including the contact angle test, liquid transfer efficiency test and the absorbance test. Finally, the following conclusions are drawn: 1. In a short time after surface modification, the hydrophilic effect is the best, and the effect decreases gradually with the increase in time. For the same reagent, the higher the concentration, the better the stability of the hydrophilic effect.
improve the rapidity and sensitivity of the centrifugal microfluidic immunoassay system in the future.

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