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User-Friendly Microfabrication Method for Complex Topological Structure and Three-Dimensional Microchannel with the Application Prospect in Polymerase Chain Reaction (PCR)

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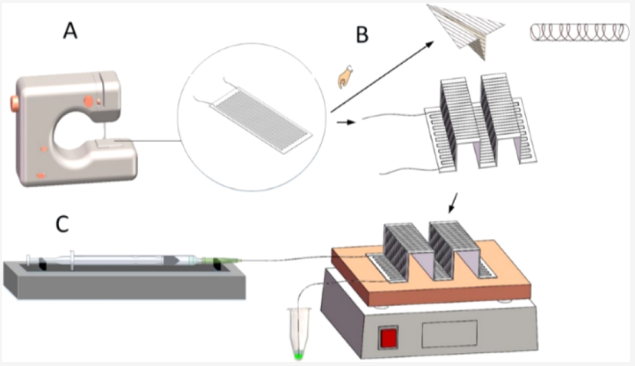
ABSTRACT: One of the most important challenges in the field of microfluidics is the rapid fabrication of microchips with complex topologies. Although the processing method of microfluidic chips has made brilliant achievements in the past 20 years, almost all traditional processing methods still face huge obstacles in the production of complex topologies and three-dimensional microchannel. Nowadays, the main methods of manufacturing microfluidic chips such as numerical control microprocessing, laser ablation, inkjet printing, photolithography, dry etching, and lithography, galvanoformung and abformung (LIGA) technology are not only inapplicable to the complex topological structure and the rapid processing of three-dimensional microfluidic chips but also rely on expensive processing equipment, complex manufacturing process, and low yield. To solve the problems of these traditional processing methods, we propose a low-cost methodology to obtain a microfluidic chip by sewing the chip pipe to the substrate with an embroidery machine as low as $6. Compared with the above-mentioned traditional microprocessing technologies, the new chip processing technology proposed by us does not involve professional microprocessing equipment and professional skills. Therefore, this new chip processing technology can significantly improve the efficiency of microprocessing.



Since our research group first put forward the concept of miniaturized total analysis system (μTAS) in 1990,[1] it has gained wide interest from international academia in the past 20 years.[2−4] This technology usually relies on controlling fluids or gases in micron-scale piping systems.[5] With microfluidic technology as the core, a chip-type micro-total analysis system could be processed and applied to the fields of biology, chemistry, medicine, fluid, electronics, materials, machinery, and the like.[2,6−8] Compared with traditional technology, the miniaturized total analysis system has a series of advantages. It can process a large number of samples in parallel with fast analysis speed, low power consumption, and little pollution.[9]

The core of the miniaturized total analysis system is the microfluidic chip.[10] Also, thus the core of the microfluidic chip is how to process it. In the past 20 years, the processing methods of microfluidic chips have developed rapidly and become more and more perfect. At present, some of the most common processing methods include photolithography, computer numerical control, CNC engraving method, laser etching, LIGA technology, embossing method, and so on.[11−15] These processing methods often rely on complex and expensive processing equipment; at the same time, in the chip processing process, a complex chip bonding process is needed, and it is difficult to realize the chip processing of complex topology and three-dimensional (3D) structure.

First, almost all the aforementioned traditional processing methods require expensive and large processing instruments.[16,17] In addition, they also rely on complex technological processes. Among them, photolithography is one most commonly used methods of chip processing.[18,19] This kind of technology usually requires a spin-coating photoresist template on a silicon wafer. Also, then by ultraviolet exposure to form the master model, which is further applied to the microstructure replication of materials such as poly(dimethylsiloxane) (PDMS). However, this method requires expensive photoresist materials, such as Su-8, NOA, etc. At the same time, toxic chemical reagents such as developers are also needed for photolithography and LIGA technology. In contrast with photolithography, toxic chemicals are not involved in the

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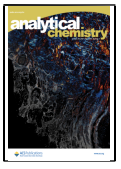

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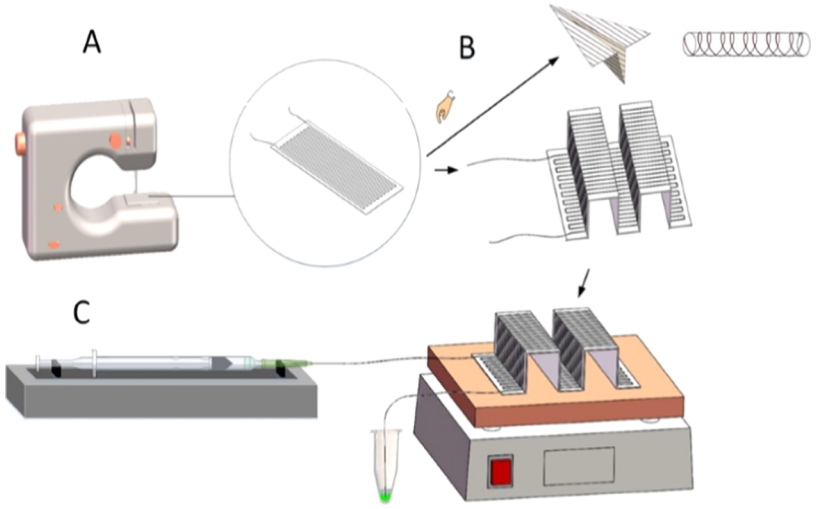

Figure 1. Novel microfabrication method and the outline of the PCR experiment. (A) Embroidery machine is applied to sew the pipe and the substrate to form a two-dimensional chip. (B) Two-dimensional chip is changed to a three-dimensional chip with various spatial structures. (C) Three-dimensional microchip is located on the heating table for the PCR test.

fabrication process of laser ablation technology, which is generally applied to process glass, quartz, and other materials. Although glass and quartz materials have good optical and electrical properties, they have high processing costs and very difficult to bond. CNC can be directly machined into a microchannel on the plastic surface, but they often have the disadvantages of rough pipe surface and cannot get rid of the reliance on expensive processing equipment.[20]

Second, almost all aforementioned traditional processing methods rely on additional bonding methods to realize the encapsulation of microchannels. Some of the most widely used bonding technologies include plasma bonding, ultrasonic bonding, embossing method, anodic bonding, and so on. Most of the bonding methods also require expensive bonding instruments and need to optimize bonding conditions to ensure better test results. At the same time, the types of materials that can be bonded by each bonding technology are limited. For example, plasma can only deal with the bonding of silicon-based materials. In addition, the embossing method is limited to plastic materials with a low melting point and the thermal quality of the chip is not stable so it is difficult to apply at higher temperatures.

In brief summary, despite a lot of traditional processing methods exist for fabricating microfluidic chips, most of them have some disadvantages, such as reliance on expensive instruments, complex processing technology, and laborious bonding conditions. In addition, most traditional fabrication methods are unable to realize the processing of three-dimensional or complex topology chips.

Therefore, the demand for a simple processing method that can easily and quickly realize the processing of complex topology channel and three-dimensional chips that do not rely on costly microfabrication machining, bonding equipment, and complex process has become more and more urgent.

This paper provides a new fabrication processing method to solve the above problem in traditional processing methods. The microtubules with the characteristics of water repellency, oil repellency, and transparency, such as poly(tetrafluoroethylene) (PTFE), poly(vinyl chloride) (PVC), and fluorinated ethylene propylene (FEP), are used. For fabricating microchips, these pipes can be purchased directly from the market with a radius easily modified by stretching. After drawing, the diameter of the microtubule is smaller, the wall of the microtubule is thinner, softer, and easy to bend, and the surface is very flat after bending. Then, the microtube can be knitted with an embroidery machine and the three-dimensional crisscross structure is made by origami art. The processing method is very simple and does not require large-scale instruments, toxic reagents, and complex materials. It has low requirements on the processing machine. A $6 embroidery machine can satisfy the fabrication purpose. Therefore, this new technology solves the problem that microfluidic chips cannot enter the general laboratory. At the same time, this method can also be applied to many fields such as electronic sensing, polymerase chain reaction (PCR) nucleic acid detection, intelligent control, and so on.

EXPERIMENTATION

To apply this new processing method to fabricate complex topology quickly, three elements are required: chip substrate, chip pipeline, and sewing equipment. First, choose a convenient and processed material as the base material, such as paper material, plastic, metal foil, metal mesh, and so on. These base materials are widely available and cheaply in the market. They can be easily found in ordinary chemical or biological laboratories without any expensive investment in laboratory consumables. The required trace map of the microfluidic pipe is drawn on the substrate. Second, the appropriate pipeline is chosen as the pipeline of the chip. Generally, PTFE, PVC, or FEP are used in the laboratory due to their good tensile properties, water repellency, oil repellency, and transparency. After stretching, the diameter of the pipe is smaller, the wall is thinner, and the plane is smoother after bending. In this paper, the original PTFE pipe (I.D. = 0.30 mm, O.D. = 0.50 mm) is used to obtain the pipe (I.D. = 0.16 mm, O.D. = 0.32 mm) after stretching. Finally, a two-dimensional (2D) microfluidic pipe chip is obtained by sewing the microchannel on the substrate with low-cost Jiarou or Fanhua embroidery machines, according to the planned microfluidic pipe trajectory. After a series of operations, such as folding and flipping, the two-dimensional microfluidic chip is finally modified to a complex topology.

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Method of Machining Microfluidic Chip by Embroidery Machine. First, the sewing thread is put on the embroidery machine successively from the winding rod, the take-up rod, the thimble screw, and the presser foot. Suitable embroidery needles are selected according to the thickness of the chip substrate. Second, the pipe is inserted into the embroidery machine and pulled out clockwise. The pipe is lifted by the needle from the middle hole of the cover plate, and the tail of the pipe is cut off to keep 50 mm on the panel. Third, the substrate is placed under the presser foot when the handwheel is turned. According to the specified pipeline trace, the pipeline is sewn to the substrate to form a 2D chip. When the embroidery machine power is turned off, the sewn 2D chip can be taken out. Fourth, the 2D chips are converted into 3D chips through origami art.

Reagents for PCR Experiment. The DNA fragment of modified pgem-3zf (+) is amplified by a microreactor. The primer sequence of pgem-3zf (+) gene fragment is: 5′-CCG GCG AAC GTG GCG AGA AAG GGG AAG AAA GC-3′ (forward) and 5′-TCG CCT TGC AGC ACA TCC CCC TTT CGC CAG C-3′ (reverse). The length of the amplified product was 137 bp. PCR reagent consists of 1× premix Taq (ex Taq version 2.0 plus dye), 0.6 μg/μL bovine serum albumin (BSA) (as25483; Ameko, China), 1 μm forward and reverse primers, 10^8 copies per μL DNA template. The DNA template sequence of the modified pgem-3zf (+) is GGA AAG CCG GCG AAC GTG GCG AGA AAG GAA GGG AAG AAA GCG AAA ATT CGC CAT TCA GGC TGC GCA ACT GTT GGG AAG GGC GAT CGG TGC GGG CCT CTT CGC TAT TAC GCC AGC TGG CGA AAG GGG GAT GTG CTG CAA GGC GAT TAA GTT GGG TAA CGC CAG GGT TTT CCC AGT CAC GAC GTT GTA AAA CGA CGG CCA GTG AAT TGT AAT ACG ACT CAC TAT AGG AGC GGG CGC TAG GGC GCT GGC AAG TGT AGC GGT CAC GCT GCG CGT AAC CAC CAC ACC CGC CGC GCT TAA TGC GCC GCT ACA GGG CGC GTC C.

Overall Flow Chart of Chip Making and PCR. Figure 1 shows the sewing-mechanism-based manufacturing process of the three-dimensional microchip and the overall flow chart of the downstream application in the nucleic acid amplification test. The whole process is divided into three parts: first, the stretched pipe and the substrate are stitched together according to a predetermined track with an embroidery machine, and the pipes about 50 mm between the boundaries are sewn into a two-dimensional chip; second, the stitched two-dimensional chip can be modified into a three-dimensional chip with a complex spatial topology using origami art such as folding and rotation, as shown in Figure 1B. Third, the folded three-dimensional chip is fixed on the heating plate with an adhesive tape, and the temperature of the hot plate and the height of the chip are adjusted so that the temperature of the reagent inside the chip can reach the temperature cycle of 95 and 60 °C, so as to achieve the purpose of nucleic acid amplification.

RESULTS AND DISCUSSION

Influence of Sewing Thread on Chip Pipe. In the process of chip manufacturing, few kinds of bad pipeline marks may occur between the sewing surface line and the chip assembly line. It is because the selection of the pressure-regulating plate between the surface line and the pressing plate affects the fixing of the pipe on the substrate, thus affecting the quality of sewing. The pressure-regulating plate adjusts the tightness of the surface line winding on the bottom line. Taking fanhuafhsm-505a embroidery machine as an example, its regulating plate could be divided into nine grades, and the first gear is the lowest pressure, resulting in the loosest winding on the pipe, while the ninth gear is the tightest. Clearly, the tight pipeline can lead to the difficulty or even blocking of the transport of the liquid inside the microchip, while too loose a pipeline makes the pipeline unstable and affects the accuracy of temperature.

Figure 2a shows the case that the pressure-regulating plate is adjusted to the first gear (starting gear), but the presser footplate is not put down. Under this condition, the pressure of the sewing thread on the pipe is small during the chip manufacturing process, not only causing the sewing thread between the pipe and the substrate to become too loose but also causing the sewing thread on both sides of the substrate to become too loose. As a result, the pipe attaches to the substrate with difficulty, causing the pipe to easily fall off the substrate. Figure 2b shows the sewing results when the pressure-regulating plate is adjusted to the first gear (starting gear) and the presser footplate is put down. Under this condition, the pressure of the sewing thread on the chip pipe is appropriate and the sewing thread is neither tight nor loose. The pipe is not squeezed by the sewing thread, and the pipe and the substrate are perfectly combined. Figure 2c shows the sewing results when the pressure-regulating plate is adjusted to the fifth gear (middle gear) and the presser footplate is put down. Under this condition, the pressure of the sewing thread on the pipe is large, and the pipe has an obvious bulge under the pressure of the sewing thread. The pipe is squeezed by the sewing thread, and the resistance increases obviously during the injection of red ink, but the liquid can continuously flow. Figure 2d shows the sewing results when the pressure-regulating plate is adjusted to the ninth gear (the highest gear) and the presser foot is put down. In this case, the pressure of the sewing thread on the chip pipe is too large, and the sewing thread completely compresses and blocks the chip pipe. Under such a condition, the chip pipe is squeezed by the sewing thread and the integrity of the pipe is damaged and the injection of red ink fails.

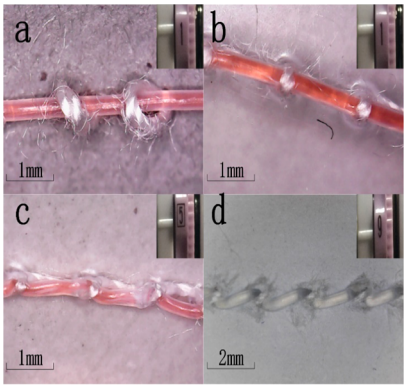

Figure 2. Effect of the tightness of the sewing thread on pipe quality. (a) Pressure-regulating plate is adjusted to the first gear (starting gear) but the presser footplate is not put down. (b) Pressure-regulating plate is adjusted to the first gear (starting gear) and the presser footplate is put down. (c) Pressure-regulating plate is adjusted to the fifth gear (middle gear) and the presser footplate is put down. (d) Pressure-regulating plate is adjusted to the ninth gear (the highest gear) and the presser footplate is put down.

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Diversified Fabrication of the Microfluidic Chip/ Diversification of Microfluidic Chip Pipeline. On the basis of the previous experiments, it is found that the perfect combination of the sewing thread to the pipe and the substrate can be realized by adjusting the working parameters of the embroidery machine. In the process of experiment, pipes with different diameters may be used as the carrier of reagents. To meet the needs of multispecification pipes for laboratory testing, chip pipes with different diameters can be used to realize the diversification of microfluidic chips.

For the sewing of pipes with different diameters on the same substrate as shown in Figure 3, we can adjust the pressure of the surface lines so as to adjust the tightness of the pipes by selecting the surface line pressure knob. Through experiments, it is found that tubes with different diameters can be perfectly stitched on the substrate to meet the requirements of tube diversity in chip manufacturing.

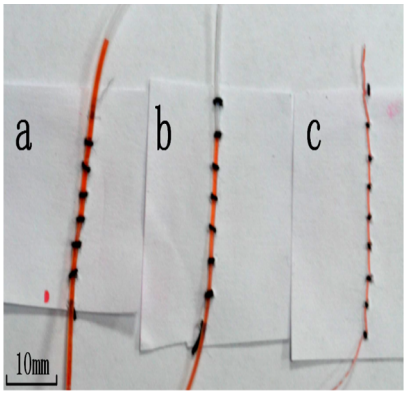

Figure 3. Sewing pipes of different diameters on the substrate. (a) Chip pipe is a PTFE pipe with an outer diameter of 700 μm and an inner diameter of 500 μm. (b) Chip pipe is a PTFE pipe with an outer diameter of 500 μm and an inner diameter of 300 μm. (c) Chip pipe is a PTFE pipe with an outer diameter of 320 μm and an inner diameter of 160 μm.

Diversity of the Microfluidic Chip Substrate. To meet the needs of laboratory experiments, it is not only necessary to select the appropriate pipeline but also to ensure the diversity of the chip substrate to meet the needs of different experiments.

In Figure 4, we show the chips sewn with different substrate materials and pipes of the same diameter. We have done experiments with polyethylene (PE), iron mesh (Fe), polypropylene (PP), paper, and copper foil (Cu), respectively. Through the comparison of the magnification image of Figure 4b, we find that PE, iron mesh, paper material, copper foil, and other chip substrates can be perfectly sewn together with pipes. When comparing PP with PE, it is found that the strength, rigidity, and hardness of PP are stronger than those of PE. When the pipe is sewn on PP, the substrate is damaged greatly. Also, thus the pipe has an obvious extrusion phenomenon.

Diversity of the Spatial Structure of Microfluidic Chip. In the process of microfabrication, the diversification of the microchip can be realized by changing the pipeline and the substrate. The topology diversity of the microchips also can be realized by changing the spatial structure (Figure 5).

The two-dimensional planar microfluidic chip is fabricated by sewing and the three-dimensional complex topological microfluidic chip is realized by bending and twisting the spatial

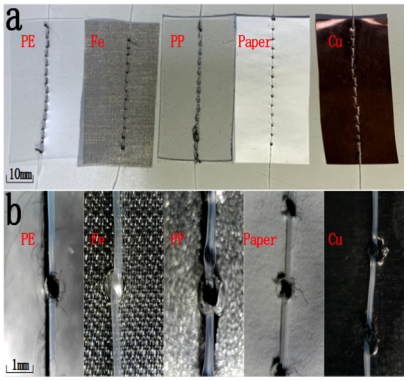

Figure 4. Different materials can be used as substrates for the fabrication process. (a) Different substrates from left to right are polyethylene (PE), iron mesh (Fe), polypropylene (PP), paper material, and copper foil (Cu). (b) Ten times enlarged image after the pipe is sewn to the substrate.

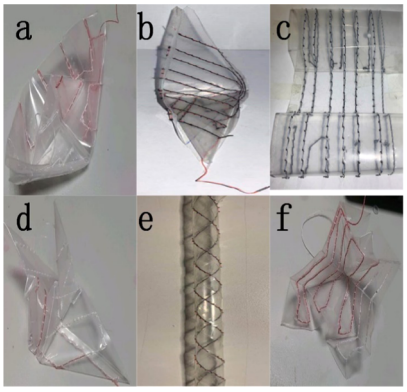

Figure 5. Microfluidic chips with different spatial structures. (a) Ship-structured chip; (b) shuttle-structured chip; (c) concave-structured chip; (d) paper crane-structured chip; (e) double-helix-structured chip; and (f) flower-structured chip.

structure. This kind of microfluidic chip, which can be bent and folded at will, can perfectly meet the needs of laboratory experiments. It is found that the pipeline of the microfluidic chip is not affected by the change in space structure, and the pipeline is damaged and squeezed. The ink can flow perfectly and normally in the pipeline.

Figure 6a shows a ship-structured chip floating on the water surface; Figure 6b–f shows the flow of the excited fluorescent

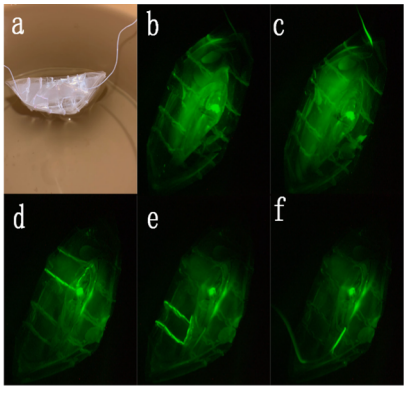

Figure 6. Microfludic boat floating on water.

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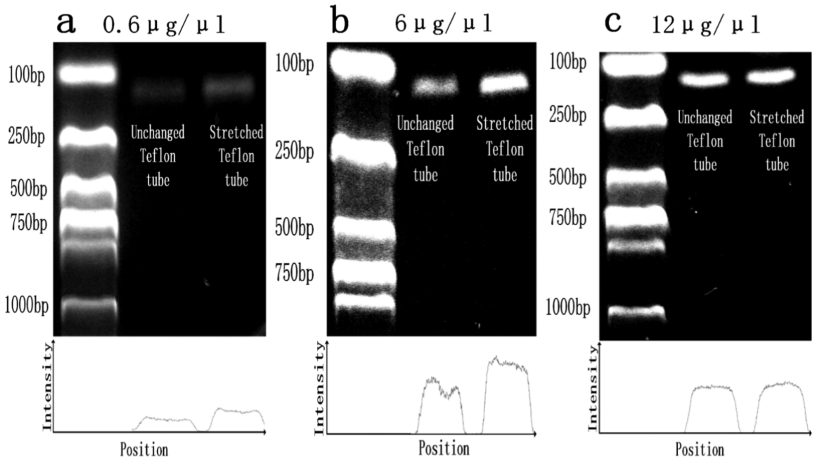

Figure 7. Effect of different concentrations of BSA passivation in microfluidic chips with different PTFE pipes.

liquid in the ship-type pipe. This new chip can be used not only in a normal environment but also in water.

Passivation Effect of BSA in Different Thicknesses of PTFE Microfluidic Chips. We use a heater to create a thermal cycle for the PCR reactions inside different PTFE pipes. The untwisted PTFE tube and the stretched PTFE tube are wrapped in two PDMS blocks of the same height, shape, and size respectively, and then placed on a single heater for about 30 min, so as to reach the required temperature before sample injection. The denaturing temperature is adjusted to about 95 °C and the annealing temperature to about 60 °C to analyze the PCR amplification efficiency of the microdevice. At the same time, the flow rate of the reagent around the PTFE tube and the stretched PTFE tube is controlled to be 90 s per cycle.

The concentration of BSA in the reaction reagent is 0.6, 6, and 12 $\mu g/\mu L$ in turn. Figure 6a–c shows that the increase of the BSA concentration from 0.6 to 6 $\mu g/\mu L$ would enhance the reaction efficiency of cyclic amplification. However, when the concentration of BSA increases from 6 to 12 $\mu g/\mu L$, it is found that the difference in the brightness of the product is not obvious, which indicates that the BSA concentration could enhance the reaction efficiency of the reagent in a certain range.

Moreover, a comparison of Figure 7a and Figure 7b shows that the reaction efficiency of the product of the stretched pipe is better than that of unstressed pipe at the same concentration of BSA.

The thickness of the unstressed pipe is much thinner than that of the stretched pipe, which leads to better temperature control, while passivation could heighten the amplification efficiency for both cases. It is found from Figure 7c that when the concentration of BSA is large enough, the reaction efficiency of the stretched pipe is similar to that of the unstressed pipe.

Figure 8 shows that under the same concentration of BSA, the reaction efficiency of the PTFE pipe after stretching is better than that of the PTFE pipe. Especially, when the concentration of BSA is low, the difference in the product efficiency of the stretched pipe is more obvious than that of the nonstretched pipe.

PCR Results of a Continuous Flow Three-Dimensional Paper Chip. According to the analysis of the previous experimental results, a continuous flow microfluidic chip is fabricated using the stretched PTFE tube as the chip pipe. The PDMS block can be inserted into the hollow tank to ensure the

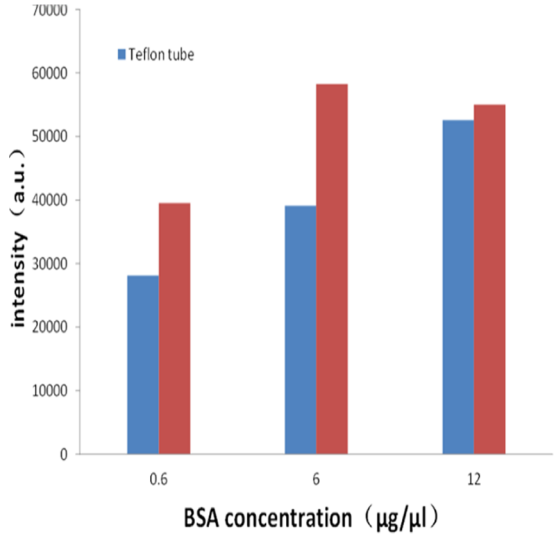

Figure 8. Comparison of the brightness of the products between the nonstretched and stretched pipes under different BSA concentrations.

thermal insulation effect. However, it is found that when the concentration of BSA is too high, the reaction reagent easily blocks the stretched pipe, resulting in difficulty of liquid flow. The reaction reagent with the BSA concentration of 0.6 $\mu g/\mu L$ is used here.

As shown in Figure 9, the electrophoretic results from left to right are paper-based continuous flow chip, ladder, traditional

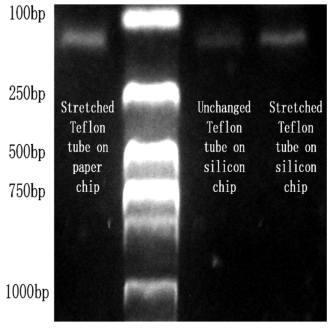

Figure 9. Comparison of the results of the continuous flow paper chip and the traditional continuous flow chips.

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continuous-flow microchip with an unchanged pipe, and a traditional continuous-flow microchip with a stretched pipe. It can be clearly seen from the electrophoretic graph that the reaction efficiency of the paper-based microfluidic chip is close to the amplification efficiency of the traditional continuous flow microchip with a stretched pipe, which is superior to that of the traditional continuous-flow microfluidic chip with an unchanged stretched pipe. This means that the new microfluidic chip can be used in the PCR reaction.

CONCLUSIONS

This paper introduces the fabrication of a simple, fast, and cheap microfluidic chip with a complex topology. Without the use of expensive processing equipment and reagents, we can use the low-cost embroidery machine to process the chips without difficult operating conditions. We can design and change the spatial structure of the chip and choose the appropriate substrate according to the specific needs of the experiment. The processed chips can perfectly transport the liquid segment. At the same time, the new microfluidic chip can be used perfectly in the PCR field.

In view of the above advantages, we believe that the embroidery sewing technology can significantly reduce the manufacturing cost of 3D microfluidic chips, thus reducing the difficulty of processing microfluidic chips in the laboratory, and can easily produce microfluidic chips with complex topology to meet the needs of various laboratories.

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Notes

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