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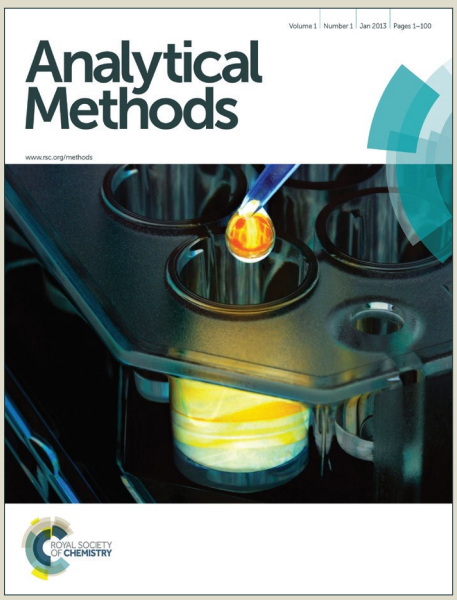

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Title

BSA Coated NOA81 PCR Chip for Gene Amplification

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Abstract

Norland Optical Adhesive 81 (NOA81) is an excellent material of microfluidic chip, but this chip has been rarely used in the field of gene amplification due to the existence of inhibition on the polymerase chain reaction (PCR). In this study, a NOA81 PCR chip (NP-chip) was developed with a simple, fast fabrication method. To overcome this inhibition effect, a simple bovine serum albumin (BSA) coated method was introduced and the BSA coated pH and concentration was optimized for a better gene amplification effect. The PCR results of HLA-DRB1 indicated that the BSA coated method could greatly improve the PCR efficiency on the NP-chip. Moreover, two kinds of PCR were performed on the NP-chip to validate the effectiveness of the BSA coated method. i) gene mutation of anti EBV variable region gene (AEB-HC6-6) was observed under the condition of cobalt radiation. ii) Short Tandem Repeats (STRs) PCR was performed on the NP-chip as the multiple-primers PCR. The results showed that the NP-chip with BSA coated was able to successfully realize single and multiple primers gene amplification. With further improvement of the precise temperature controlling, this kind of NP-chip would be widely applied in gene amplification and promote the development of the miniature gene amplification device in point of care testing (PCOT) and forensic detection, etc.

Keywords: NOA81 PCR chip, BSA coated, gene amplification, STRs PCR

1.Introduction

The invention of polymerase chain reaction (PCR) by Kary Mullis in 1986 brought a revolutionary impact on molecular biology and clinical diagnosis[1]. PCR was mainly used in the field of biological genotyping, genetic disease diagnosis, cancer diagnosis, and forensic medicine and so on.

With the appearance of microfluidic chip techniques, PCR chip based on this techniques caught researchers' eyes at the very first it comes up. To date, various kinds of quantitative PCR technique, such as real-time PCR [2-4], digital PCR[5-6], and droplets PCR[7-10] have been realized on the microfluidic chip over the years. In addition, other gene amplification techniques such as Short Tandem Repeats (STRs) PCR[11-15] and Loop-mediated isothermal amplification (LAMP)[16-18] was also applied more and more widely in microfluidic chip.

These microfluidic chips for PCR were mainly fabricated by the materials such as glass, polymethyl methacrylate (PMMA), and polydimethylsiloxane (PDMS)[19-22] due to their good light transmittance, heat tolerance and well biocompatibility. However, three major disadvantages limited the wide application for gene amplification, that is, poor thermal conductivity, complicated fabricated process, and difficulty in making 3D structure chamber and channel.

A new microfluidic chip material named Norland Optical Adhesive 81 (NOA81) was firstly reported and used by Denis Bartolo[23] for fabrication of microfluidic chip. NOA81 could solidify in a few seconds once it was exposed to the UV light. Moreover, this material possesses the ability of greater solvent and pressure resistance, low auto-fluorescence, impermeable to air and water vapor, especially fast and easy fabrication and good biocompatibility. Bertrand Levache´[24] used different UV exposure time to change the surface properties of NOA81 for successful cell culture. NOA81 sticker for cell- and tissue-based assays in micro-channels was also reported by Morel. Mathieu[25].

Due to favorable ability of NOA81 and its advantages mentioned above, NOA81 was used as the material of the microfluidic chip for PCR in our present study[26]. In the

experiment, we found that the PCR efficient was low and unstable. The possible reason was that the PCR was inhibited by NOA81sticker. Previous studies[27-30] also demonstrated that low amplification efficiency was the major limitation of chip materials because the PCR was inhibited by the unmodified surface of NP-chip. Therefore, it would be particularly important for the modification of NP-chip in this study. Previously extensive research efforts have been made in surface passivation of microfluidic chip, common surface coated reagent were BSA, PEG, Tween20, silane agents[31-36], etc. C. A. Kreader[37] reported that the optimum concentration of BSA was 200 to 400 ng/μL to relieve the inhibition of PCR. J. Besecker[38] adopted dynamic passivation with BSA to overcome the LTCC mediated inhibition of PCR and S. Petralia[39] reported the stability evaluation of BSA coating on the Lab-on-chip device. However, there was no relevant report about BSA coated method on NP-chip to improve the PCR efficiency. We firstly choose BSA solution as the surface passivation reagent in consideration of its compatibility with PCR.

In this study, we simplified the fabricated process of NP-chip. In order to make PCR more stability and efficiently, we employed the BSA solution to coat the NOA81 surface and optimized the concentration and pH of BSA solution. Moreover, two typical PCR were achieved on NP-chip and got well PCR efficiency. With miniaturization of the NP-chip and the peripheral control unit, the POCT devices for gene amplification diagnosis based on NP-chip would be realized in the future.

2. Materials and methods

2.1 Materials

The NOA81 was purchased from Norland Products Inc. (USA) and used as received. The Peltier (TEC1-07102T125(23 mm × 23 mm)) was purchased from Beijing Huimao Cooling Equipment Co., Ltd. (China). Chrome plate (SG2506) was purchased from Changsha Shaoguang Chrome Blank Co., Ltd. (China). The PT1000-senor was purchased from HAYASHI DENKO Co., Ltd. (Japan). The center-control circuit board was designed and made in author's lab.

BSA (A4737-5g) was purchased from Sigma-Aldrich Co. LLC (USA) and prepared with PBS for the experimental needs. The PCR kit (Bioteke), agarose (Promega), DNA 500 marker (Sangon) were used without any further purification. The AmpFlSTR ®Identifiler PCR Kit and standard female DNA was purchased from Applied Biosystems Co., Ltd. (USA). The DNA sample was extracted from human blood with the Human blood DNA Extraction Kit purchased from Bioteke (China). The primers of HLA-DRB1 and AEB-HC6-6 shown in Table.1 were all synthesized by Sangon Co. Ltd., Shanghai, China.

<Table 1 near here>

2.2 Fabrication of NP-chip

The NP-chip was made of NOA81 with three plastic film mask A, B, C, shown in Fig.1. The main structure of NP-chip was five PCR chambers, a PT1000-senor fixed region and several alignment holes, shown in Fig.1.B. The detail size of PCR chamber was showed in Fig.1.D with two sample loading holes, two standby sampling holes and a PCR chamber with a capacity about 20μL.

<Figure 1 near here>

The NP-chip has three NOA81 layers and each layer was made by UV lithography with

the corresponding plastic film mask covered. Three NOA81 layers were piled up together to 3D PCR chamber structure depending on their curing ability. This fabricated method without any PDMS or stamp mold was fast and simple. Firstly, we dropped 0.6 mL of NOA81 to the Peltier or chromium plate with a rectangular cofferdam which was cut from the film mask and was fixed in Peltier or chromium plate by using NOA81, Fig. 2A. Secondly, covering the film mask A, B, and C shown in Fig.1 carefully and exposing to the UV light for 30s, Fig.2B. Thirdly, peeling off the film mask A, B, and C carefully and the NOA81 layer would rest on different attachment, Fig.2C. The adhesive force to cured NOA81 was NOA81 > Peltier > Film mask > chromium plate. Then, washed the uncured NOA81 by using alcohol and acetone mixture (1:1, volume). Finally, bonding the NOA81 layer B3 and C3 to layer A3 successively and each bonding step needed UV re-curing for 30s, Fig.2.D1. Then fixed the PT1000-sensor in the middle of the NP-chip and aged the NP-chip in the dry oven at 50 °C for 2 h. The finished NP-chip with PCR sample was shown in Fig.2.D3.

<Figure 2 near here>

2.3 Optimization of the O2 plasma treatment time on the NP-chip

The hydrophilic and hydrophobic property of the NP-chip played an important role in surface modification. To a large extent, the water contact reflected the hydrophilic and hydrophobic property of the NP-chip. So, the water contact angle in different time of O2 plasma treatment was tested for optimizing the O2 plasma treatment time. In the experiment, the NP-chip was treated by O2 plasma treatment for 1, 2, 3, 4, 5, 6, 7, 8min. Then, the water contact was tested through dropping a drop of water on the surface of the NP-chips. The NP-chip was cleaned by YZD08-5C O2 Plasma cleaning machine (Tangshan yanzhao institute of technology, China) with a power of 40 Watt. The water contact angle was measured by XG-CAMB1 standard contact measuring device (Shanghai Xuanyichuangxi Industrial Equipment Co.,Ltd., China).

2.4 Optimization of the BSA coated time

The concentration of BSA adsorbed on the NP-chip was measured for optimizing the BSA coated time by Bradford method. BSA was prepared to 5 mg/mL BSA solution with PBS (pH=4). NP-chip was cut into small pieces and was putted into a 1.5 mL centrifuge with 1mL BSA solution. Each time 5μL of the BSA solution was sampled from the centrifuge tube after being coated for 5, 10, 20, 40, 60, and 120 min. Then, the corresponding BSA concentration in different time and the concentration of BSA adsorbed on the NP-chip were measured by Bradford method. Moreover, the NP-chip was cut to 5x5x2mm pieces and coated with 5mg/ml BSA solution for 10, 60, 120min to characterize the BSA adsorption effect on the NP-chip by SEM Quanta FEG250 (FEI Company, USA).

2.5 Optimization of the BSA coated pH and concentration

PCR method was employed to optimize the BSA coated concentration and pH. Firstly, several groups of NP-chip were firstly fabricated as same as Fig.2D.3 and the NP-chip was cleaned by O2 plasma for 3min. Secondly, sampled 5 mg/ml BSA solution into each PCR chamber, sealed the inlet and outlet with heat resistant adhesive tape, then coated at 50℃ for 1 hours. Then cleared the BSA solution from the PCR chambers and dried the NP-chip. Thirdly, the PCR reagents (10μl 2xPCR mix, 1μl DNA sample, 1μl forward HLA-DRB1 primer, 1μl reverse HLA-DRB1 primer and 7μl sterile water, total volume 20μl) were premixed in the PCR tube and sampled into the PCR chambers. Finally, the NP-chip was sealed by NOA81 to avoid sample leakage in the PCR process. After PCR, all the PCR products were detected by agarose-gel electrophoresis and the electrophoretic bands were scanned to fluorescence intensity value (FI value) by using ChemiDox XRS electrophoresis analyzer (BioRAD, USA) for convenience in display. By using the PCR method, both pH and concentration of BSA solution was optimized with a positive control amplified in a conventional thermocycler. In the process of optimizing pH and concentration, we firstly treated the NP-chip with two kinds of 5 mg/ml BSA solution prepared by pH4 and pH7 PBS buffer solution. Then, we treated NP-chips with 1, 3, 5, 10mg/ml BSA solution prepared by pH4 PBS buffer solution. The optimal BSA coated pH and concentration could be obtained by comparing the

HLA-DRB1 PCR efficiency on the NP-chip with different treatment method.

2.6 The influence of cobalt radiation on anti EBV gene

We observed the influence of anti EBV heavy chain variable region gene under cobalt radiation on the NP-chip. Two groups were set in the experiment: on-chip PCR method and in-tube PCR method. The main difference between two methods was that the former was operated on the NP-chip and the latter was operated on the Techne primer5 PCR amplifier (TECHNE, England). We took 200 gray radiation doses on the Puc57 gene and used them as templates to amplify the AEB-HC6-6 with two PCR methods. The primers and program showed in Table.1 and Table.2 respectively. The 20μl PCR system (10μl 2x PCR reaction Mix, 0.5μl forward, 0.5μl reverse primer, 1μl Puc57 plasma DNA and 8μl sterile water) was premixed in the PCR tube for using in two PCR methods. All the PCR products were detected by agarose-gel electrophoresis and analyzed by using ChemiDox XRS electrophoresis analyzer (BioRAD, USA).

< Table 2 near here>

2.7 STR-PCR on the NP-chip

With the BSA coated NP-chip, the STRs-PCR was targeted for 17 STR gene site simultaneously amplification using AmpFlSTR Identifiler PCR Amplification Kit. In the same way, we employed the on-chip STRs-PCR method and in-tube STRs-PCR method to compare the efficiency of STR amplification. 20μl STR amplification reagents (8μl AmpFlSTR PCR reaction Mix, 4μl AmpFlSTR identifiler primer set, 1μl human blood DNA and 7μl sterile water) was premixed in the PCR tube for amplifying. The on-chip and in-tube STRs-PCR were implemented simultaneously. All of the STRs-PCR products were analyzed in the 3130XL genetic analyzer (ABI, USA).

3. Results and discussion

3.1 Water contact angle testing on NP-chip

In order to improve the BSA coated efficiency, we first observed the relationship between the water contact angle and O2 plasma treatment time on the surface of NP-chip. With the O2 plasma treatment time increasing, the water contact angle was decreased at first then increased, Fig.3A. The surface of NP-chip showed strong hydrophobicity at first with a maximum water contact angle of 66.7°, Fig.3B. With the 3min O2 plasma treatment, the water contact angle became the minimum of 9.7°, Fig.3C. In the process of BSA coated, high surface hydrophilicity was more beneficial to BSA coated on the surface of NP-chip because of the similar hydrophilic principle. Then the BSA would be coated on the surface of NP-chip depending on the intermolecular forces between BSA and the surface molecule of NP-chip. So, the optimal O2 plasma treatment time we optimized was 3min because that the surface of NP-chip showed strongest hydrophilicity in this time.

< Figure 3 near here>

3.2 The optimal BSA coated time

In order to get maximal BSA adsorption capacity on the NP-chip, we measured the relationship between BSA adsorption value and BSA coated time by Bradford method. With this method, the corresponding BSA adsorption value in different coated time could be calculated by the standard concentration curve of BSA, Fig.4A. With the BSA coated time increased, the BSA adsorption value, which was 0.15 mg at first 5min, was increased slowly and then reached the maximum of 0.21mg at 60min, Fig.4B. The BSA adsorption capacity was no longer rising in 60-120min because it had reached saturated adsorption capacity on the NP-chip. Therefore the optimal BSA coated time we optimized was 60min when the BSA adsorption value tended to be the maximum.

< Figure 4 near here>

3.3 SEM characterization of BSA coating on the NP-chip

To observe the effect of BSA coating directly, the NP-chip with BSA coated for 10min,

60min and 120min were examined by the scanning electron microscopy (SEM). The surface of NP-chip was flat at first (Fig. 5A). After BSA coated for 10min, the BSA gathered and sparsely absorbed on the surface of NP-chip (Fig. 5B) . The density of BSA was obviously increased after 60min and not significant increase was observed for 120min coating (Fig.5C and D). All the results shown in SEM pictures indicated that the NP-chip could be coated sufficiently in 60min by BSA which was consistent with the Bradford method in the optimal BSA coating time. According to the results, BSA could be well coated on the surface of NP-chip with the mentioned method above and the 60min was chosen for the optimal BSA coating time in subsequent experiments.

< Figure 5 near here>

3.4 The Optimal BSA coated pH and concentration

The pH of BSA solution would influence the efficiency of BSA coated on the NP-chip. The PCR results showed that the pH4 BSA coated NP-chip had a better PCR efficiency than the pH7 BSA coated NP-chip. The FI value of pH4 was 12813 almost three times higher than the pH7 which the FI value was 4956, Fig.6. The main reason caused this gap probably was that the pH of buffer solutions would change the charge of BSA, thus affecting the adsorption characteristic of BSA on the NP-chip. The BSA solution was positively charged in the pH4 buffer solutions while the surface of NP-chip was negatively charged by the O_2 plasma treated. Therefore, the optimal BSA coated pH was pH4 which was more favorable to BSA coated on the NP-chip by comparing with the FI value of pH7.

< Figure 6 near here>

The concentration of BSA solution would also influence the efficiency of BSA coated on the NP-chip. The PCR results showed that different concentrations of BSA coated had different PCR efficiency. With the concentration increasing, the FI value was increased and reached the maximum value, then decreased, shown in Fig.7. The

maximum FI value was 10500 at the concentration of 5 mg/ml BSA coated. The lower BSA coating concentration may lead to an insufficient coating effect. The higher concentration may lead to a decreasing on the PCR efficiency caused by the higher concentration of dissolving BSA in the PCR system which was higher than 400 ng/μl[41,42]. Through the experiment contrasts, 5 mg/ml of BSA solution was chosen as the optimal BSA coated concentration.

< Figure 7 near here>

In addition, an effective comparison experiment was developed for verifying the PCR efficiency before and after the BSA coated on the NP-chip. With and without BSA coated, two groups were set for comparing the PCR results on the NP-chip. The FI value of two groups were 10154 and 1248 respectively, Fig.8. The results showed that the PCR efficiency of NP-chip with BSA coated was much higher than the NP-chip without BSA coated which means the PCR efficiency was significantly improved by the BSA coated method. The main reason was that the DNA polymerase in the PCR system was seriously adsorbed on the bare surface of the NP-chip. Once the Taq enzyme was adsorbed on the NP-chip, the PCR efficiency would drop dramatically . With the BSA coated, the surface of NP-chip would be coated by BSA and would not absorb too much Taq enzyme any more. Therefore, it very necessary to implement BSA coated on the NP-chip for the NP-chip widely used in the area of gene amplification.

< Figure 8 near here>

3.5 NP-chip for amplification of AEB-HV gene with and without cobalt radiation

To evaluate the reliability of PCR on NP-chip, the PCR of AEB-HV gene, which has been undertaken by many researchers in the early diagnosis of nasopharyngeal carcinoma (NPC), with and without cobalt radiation was performed. A specific gene AEB-HC6-6 (416bp) was amplified by the on-chip PCR method and in-tube PCR method. The PCR products with two methods were measured by agarose

electrophoresis was showed in Fig.9. It can be found from the results of two methods that the electrophoretic bands of AEB-HV gene without cobalt radiation were clear and bright, and there was a dramatic decrease in brightness with the cobalt radiation. Moreover, all the bands of on-chip PCR method were corresponding dimmer than the in-tube PCR method but the decreasing tendency of the two PCR methods was consistent. Obviously, the cobalt radiation could cause the target gene mutations and rupture, then lead to a decrease of the PCR efficiency.

< Figure 9 near here>

3.6 NP-chip for STR Amplification

Short Tandem Repeats (STRs) PCR, which were short repeat units usually with 2-6 bp in length surrounding the chromosomal centromere, was performed to explore the possibility of multiple-primers PCR on the NP-chip. STRs-PCR with highly genetic polymorphism had been usually used for human identification which was the primary task in forensic test. STRs-PCR was a challenge for gene amplification on chip because of 17 pair primers in the same system. Fig.10 shows that the 17 pairs of STRs gene were well amplified on the conventional thermocycler with an approximately average peak intensity of 3734. 17 pairs of STRs gene were also completely amplified on the NP-chip with an approximately average peak intensity of 2565, Fig.11. By comparing the STRs-PCR results amplified by the NP-chip and conventional approach, all the STR gene sites were amplified successfully and the peaks were clear and strong enough. Although the average peak intensity of NP-chip was slightly lower than the conventional thermocycler but the intensity were strong enough for human identification. Therefore, the NP-chip is a new strategy in STRs-PCR and has potential applications in forensic test.

< Figure 10 near here>

< Figure 11 near here>

Conclusions

We developed a simple and fast fabricated PCR chip with the seldom-reported material of NOA81. For a better PCR efficiency, we first introduced an effective BSA coated method on the NP-chip to reduce the inhibition induced by the surface of NOA81. With 3min O_2 plasma treated and 60min the pH4, concentration (5mg/ml) BSA coated, the PCR efficiency of NP-chip was greatly improved. The fact that the BSA would prevent the Taq enzyme absorbing on the surface of NP-chip too much in a competitive way and the dissociative BSA has an effect on the stability and activity of Taq enzyme in the process of PCR. Additionally, the successful amplification of AEB-HV gene and STRs gene indicated that the NP-chip was able to realize the single-primer and multiple-primers PCR. In this study, we emphasize the effect of BSA coating on NP-chip that the PCR efficiency of BSA coated NP-chip is 284.56% higher than the uncoated NP-chip. It is undeniable that the PCR efficiency of the BSA coated NP-chip is 32.2% lower than the standard tube/thermacycler PCR. The main reason was that the temperature on the NP-chip didn't match the real PCR temperature. This temperature difference causes by the heat loss when the heat transferred from Peltier via the bottom layer of NP-chip to the PCR chamber. If further develop the temperature correction and the miniature automatic control system, the proposed NP-chip has great potential applications in the area of point of care test (POCT) and human identification such as paternity test, forensic test and so on.

Acknowledgements

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Figure captions

Fig.1. The design schematic of NP-chip (A) the film mask of bottom layer (B) the film mask of middle layer (C) the film mask of top layer (D) the dimension of PCR chamber in detail.

Fig.2. The fabricating process of the NP-chip (A) fabricating the bottom NOA81 layer on a Peltier (Step1：fixing cofferdam on a Peltier; Step2: dropping the NOA81 and covered the film mask; Step3: UV curing and peeling up the film mask of bottom layer) (B) fabricating the middle layer on a chromium plate (Step1: fixing cofferdam on a chromium plate; Step2: dropping the NOA81 and covered the film mask; Step3: UV curing and peeling up film mask of the middle layer) (C) fabricating the top layer on chromium plate (Step1: fixing cofferdam on a chromium plate; Step2: dropping the NOA81 and covered the film mask; Step3: UV curing and peeling up the film mask of top layer) (D) the finished NP-chip and the fabricating color legend (D1: the structural schematic diagram of the NP-chip; D2: the color legend; D3: the photo of the finished NP-chip).

Fig.3. The relationship between water contact angle and O_2 plasma treatment time on the surface of NP-chip (A) the water contact angle curve (B) the maximum of the water contact angle (C) the minimum of water contact angle.

Fig.4. The relationship between BSA adsorption value and BSA treatment time on the NP-chip (A) the standard concentration curve of BSA (B) the adsorption value curve.

Fig.5. SEM pictures of the NP-chip with different BSA coating time. (A) NP-chip without BSA coated (B) NP-chip with BSA coated for 10min (C) NP-chip with BSA coated for 60min (C) NP-chip with BSA coated for 120min.

Fig.6.The FI value of HLA-DRB1 PCR products with different BSA coated pH.

Fig.7. The FI value of HLA-DRB1 PCR products with different BSA coated concentration.

Fig.8. The FI value of HLA-DRB1 PCR products with different BSA coated method (A) in-tube PCR method (B) on-chip PCR method without BSA coated (C) on-chip PCR method with pH4, 5mg/ml BSA coated.

Fig.9. The agarose-gel electrophoresis results of AEB-HC6-6. 1 was the gene marker.

2-4 was the PCR results with in-tube PCR method and 5-7 was the PCR result with on-chip method. 1 and 4 was the negative control; 2 and 5 was the contrast group without cobalt radiation; 3 and 6 was experiment group with 200 gray cobalt radiation doses.

Fig.10. The STR results with the in-tube STRs PCR method.

Fig.11. The STR results with the on-chip STRs PCR method.

Table captions

Tab.1. The primers of HLA-DRB1 gene and AEB-HC6-6 gene in detail

Tab.2. The PCR programs of three different PCR in this paper.

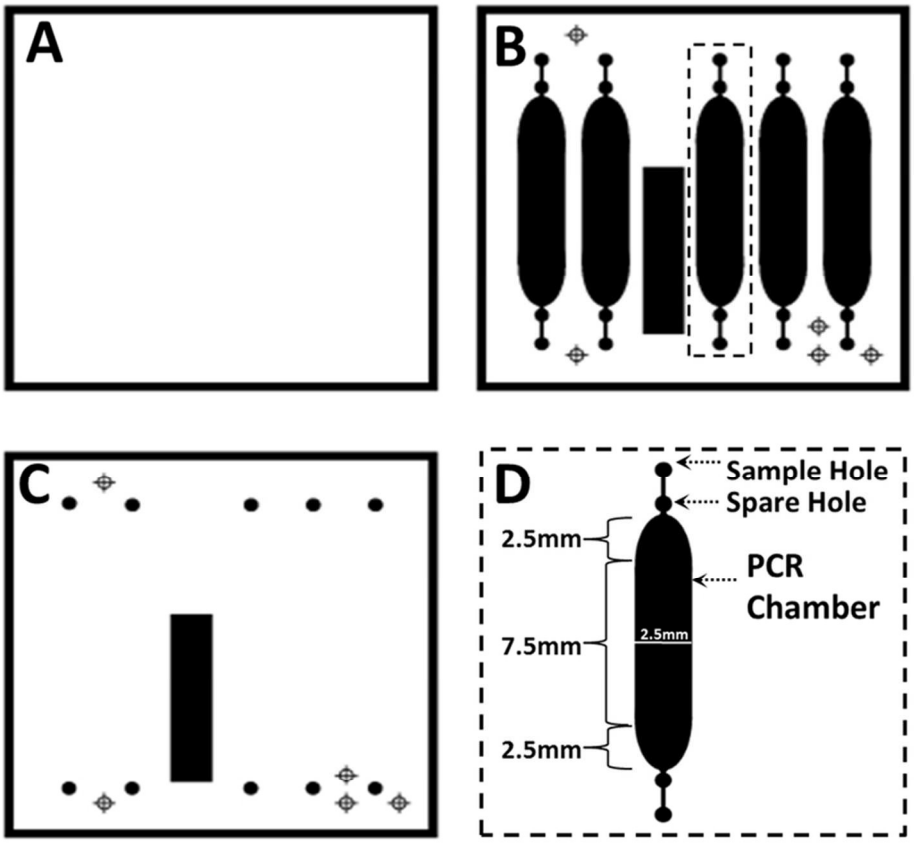

Fig.1.The design schematic of NP-chip
75x67mm (300 x 300 DPI)

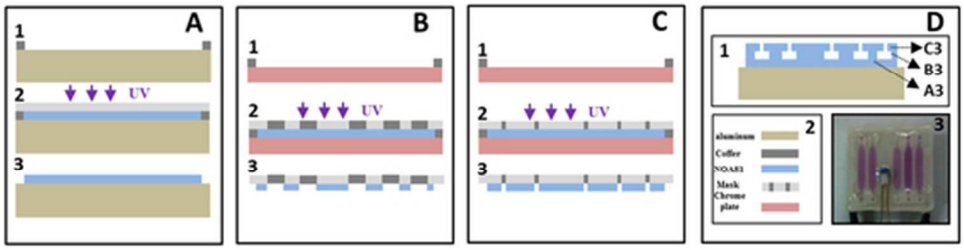

Fig.2.The fabricating process of the NP-chip
49x14mm (300 x 300 DPI)

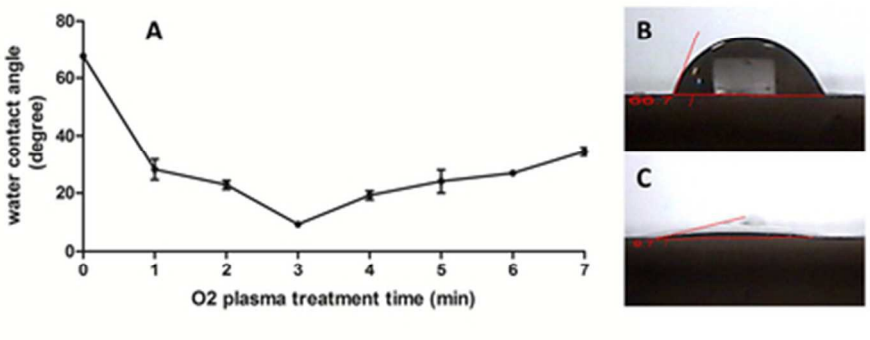

Fig.3.The relationship between water contact angle and O2 plasma treatment time on the surface of NP-chip
39x19mm (300 x 300 DPI)

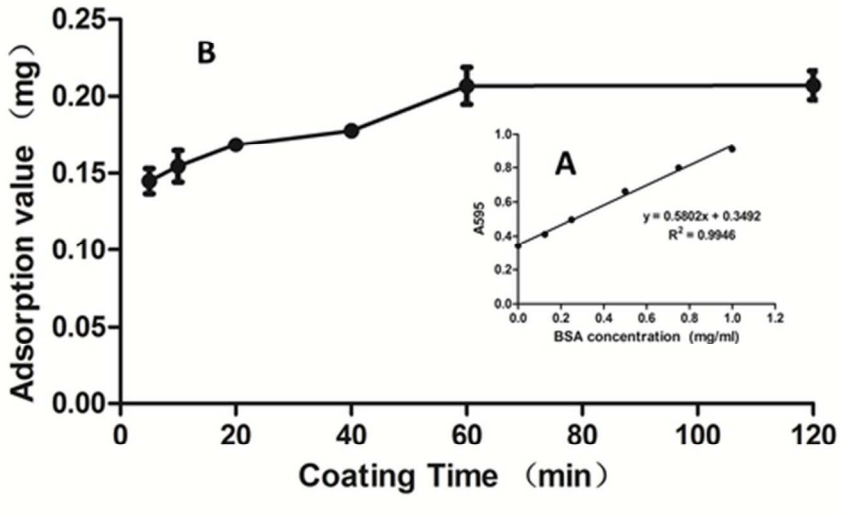

Fig.4.The relationship between BSA adsorption value and BSA treatment time on the NP-chip
49x30mm (300 x 300 DPI)

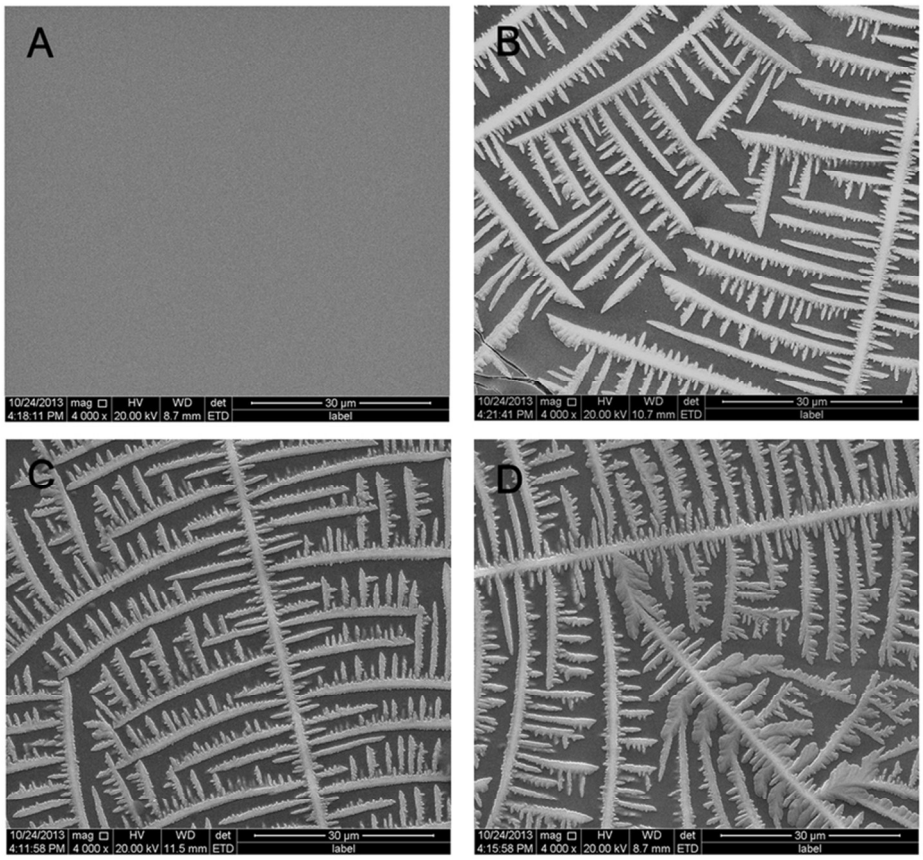

Fig.5. SEM pictures of the NP-chip with different BSA coating time.
77x73mm (300 x 300 DPI)

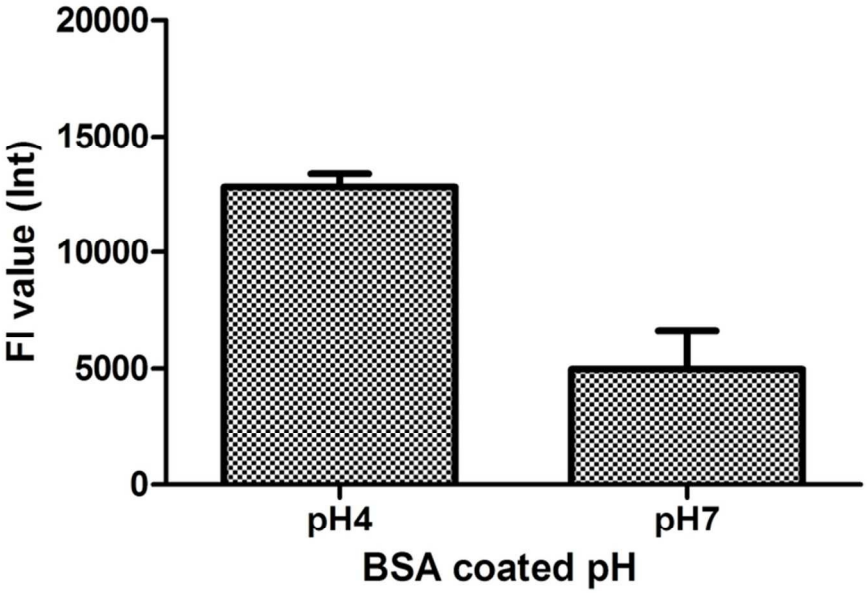

Fig.6.The FI value of HLA-DRB1 PCR products with different BSA coated pH.
72x48mm (300 x 300 DPI)

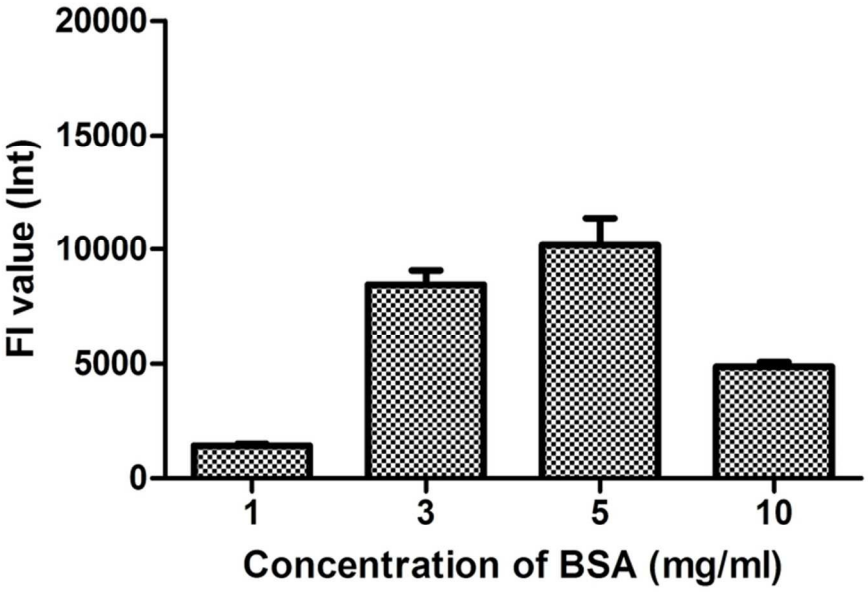

Fig.7.The FI value of HLA-DRB1 PCR products with different BSA coated concentration.
72x48mm (300 x 300 DPI)

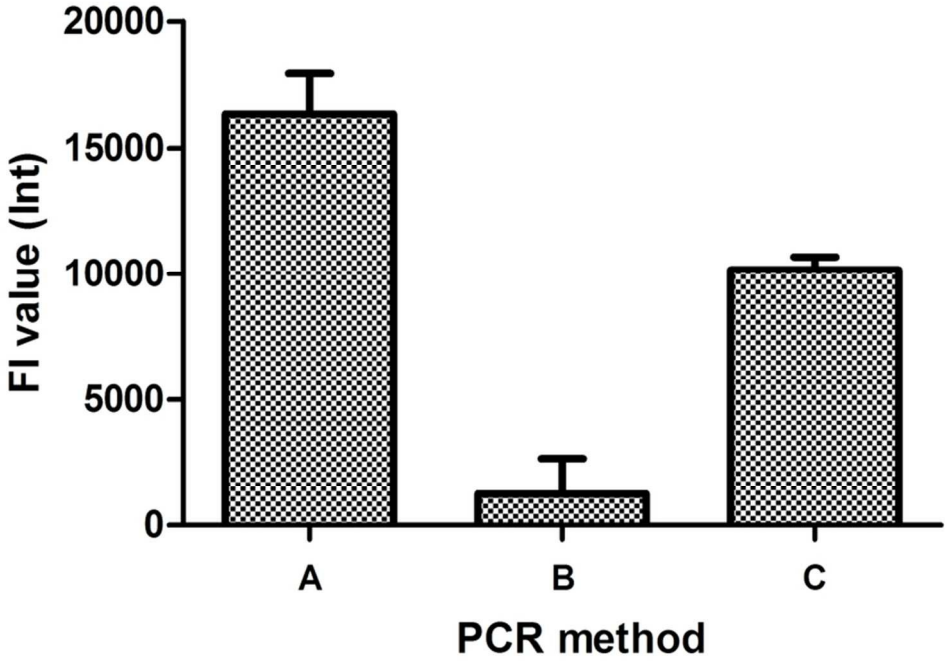

Fig.8.The FI value of HLA-DRB1 PCR products with different BSA coated method
75x56mm (300 x 300 DPI)

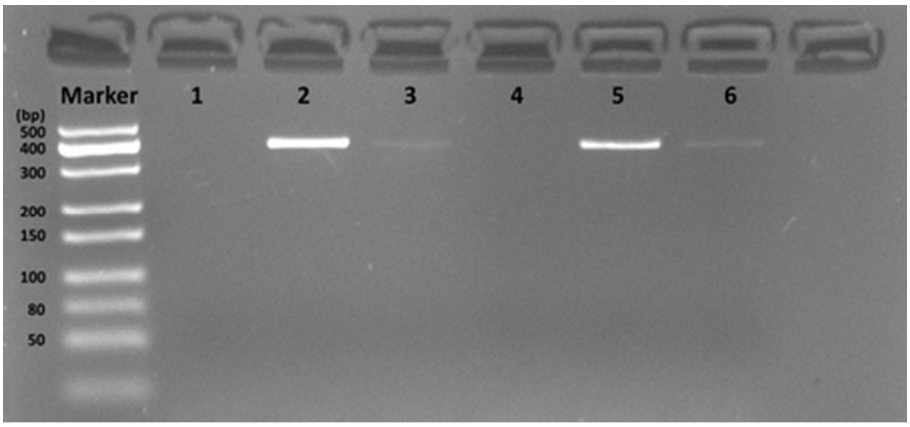

Fig.9.The agarose-gel electrophoresis results of AEB-HC6-6.
38x17mm (300 x 300 DPI)

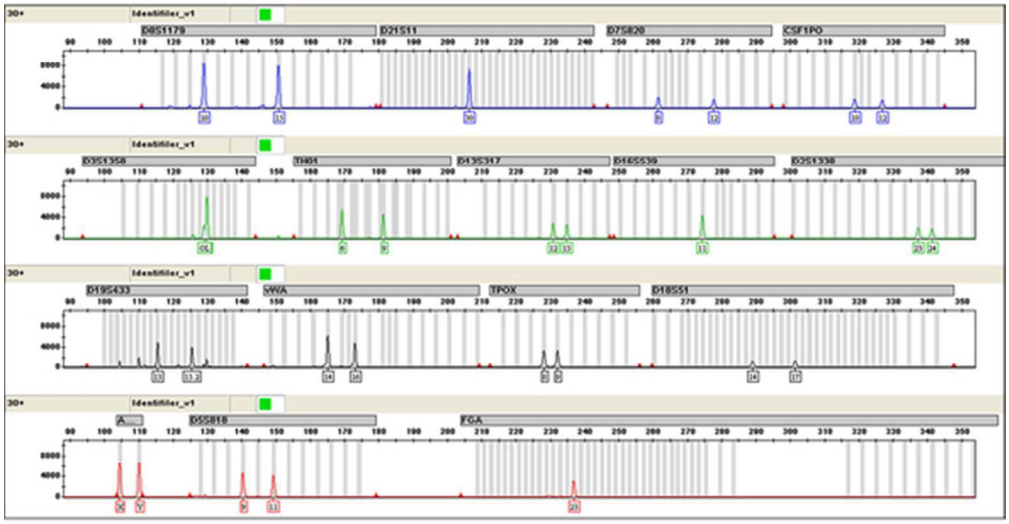

Fig.10. The STR results with the in-tube STRs PCR method.
49x30mm (300 x 300 DPI)

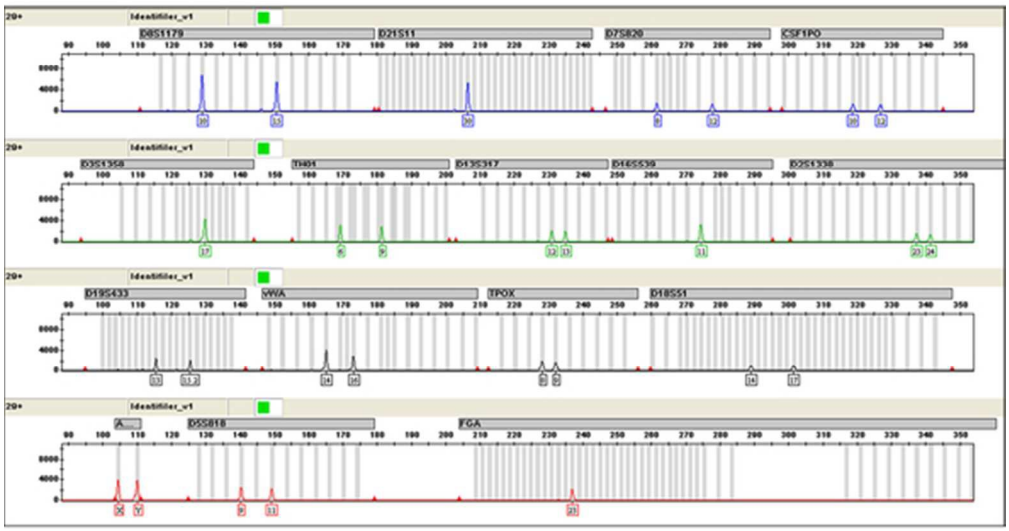

Fig.11. The STR results with the on-chip STRs PCR method.
49x30mm (300 x 300 DPI)

TITLE: BSA Coated NOA81 PCR Chip for Gene Amplification

Analytical Methods: AY-ART-12-2015-003233.R1

Tab.1. The primers of HLA-DRB1 gene and AEB-HC6-6 gene in detail

Template	Primer no.	Sequence(5'-3')	Primer length (bp)	Amplification segment length(bp)
HLA-DRB1	Forward	GTTCGTGTCCCCACAGCACGTTT	23	283
	Reverse	GCCGCTGCACTGTGAAGCTCTC	22	
AEB-HC6-6	Forward	CAGGTCCAGCTGGTGCAATC	20	416
	Reverse	TGAGGAGACGGTGACAGGGTT	21	

Tab.2. The PCR programs of three different PCR in this paper

Programs	HLA-DRB1	AEB-HC6-6	STR
Initialization step	94 °C, 5min	94 °C, 5min	95 °C, 11min
Denaturation step	94 °C, 30s	94 °C, 30s	94 °C, 1min
Annealing step	60 °C, 30s	54 °C, 30s	59 °C, 1min
Extension step	72 °C, 30s	72 °C, 30s	72 °C, 1min
Cycles number	25	20	29
Final elongation step	72 °C, 10min	72 °C, 5min	60 °C, 60min

Graphical Abstract

We fabricated a NOA81 PCR chip (NP-chip) and first proposed a simple BSA coated method to successfully overcome the low PCR efficiency induced by the inhibition of the NP-chip which serious impacted on its application in the area of gene amplification.