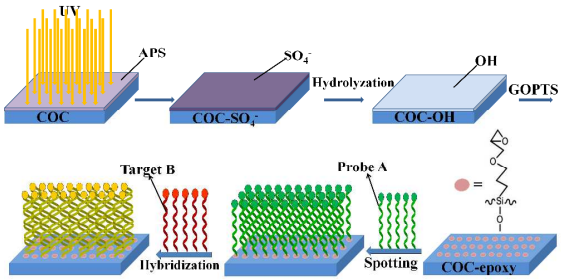
RSC Advances

Fabrication of DNA Microarrays on Cyclic Olefin Copolymers Surfaces Based on Confined Photo-Catalytic Oxidation

Journal:	*RSC Advances*
Manuscript ID:	RA-ART-07-2014-007442.R1
Article Type:	Paper
Date Submitted by the Author:	31-Aug-2014
Complete List of Authors:	Chen, Ruichao; State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology Ma, Yuhong; Beijing University of Chemical Technology, Zhao, Changwen; Beijing University of Chemical Technology, College of Materials Sciecne and Engineering Lin, Zhifeng; State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology Zhu, Xing; State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology Zhang, Lihua; Beijing University of Chemical Technology, State Key Laboratory of Chemical Resource Engineering Yang, Wantai; Beijing University of Chemical Technology,

A table of contents entry.

* Colour graphic: maximum size 8 cm x 4 cm



* Text: A novel strategy for DNA immobilization on cyclic olefin copolymers surface

Fabrication of DNA Microarrays on Cyclic Olefin Copolymers Surfaces Based on Confined Photo-Catalytic Oxidation

Ruichao Chen[a,b], Yuhong Ma[c], Changwen Zhao*[a,b], Zhifeng Lin[a,b], Xing Zhu[a,b], Lihua Zhang[a,b], Wantai Yang*[a,b]

[a]State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing, 100029, China

[b]Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, Beijing, 100029, China

[c]Key Laboratory of Carbon Fiber and Functional Polymers, Ministry of Education, College of Materials Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, China

* E-mail: yangwt@mail.buct.edu.cn.

E-mail: zhaocw@mail.buct.edu.cn.

Abstract:

In recent research on fabrication of DNA microarray, polymers have been intensively investigated as substrate to immobilize oligonucleotide due to their low cost, disposability and excellent process flexibility. Among them, cyclic olefin copolymer (COC) was of special interest because of its many favorable properties, such as high glass transition temperature, low auto-fluorescence, optical clarity and resistance to organic solvents etc. In this work, a novel strategy was developed to introduce epoxy groups on COC surface based on confined photocatalytic oxidation (CPO) method. Firstly, sulfate anion ($-SO_4^-$) was introduced onto COC film in a short time (120 s) irradiation of UV light through CPO. These sulfate anion groups subsequently were hydrolyzed to hydroxyl groups (-OH), and thus forming glass-like surface (containing surface OH groups), which could easily react with silane coupling agent. Here, (3-glycidoxypropyl) trimethoxy silane was used as model to introduce epoxy groups onto COC film. The successful introduction of epoxy groups on COC film was characterized by X-ray photoelectron spectroscopy (XPS), water contact angle measurement and atomic force microscopy (AFM). DNA probes were subsequently spotted and immobilized on COC surface via the reaction between epoxy groups and amino groups attached on DNA strands. The immobilization efficiency on COC surface for different concentration of probe DNA was range from 45% to 65%, which is comparable to the traditional epoxy-functionalized glass slide. The hybridization with complementary strands of this microarray was successfully achieved and the

fluorescence intensity after hybridization could be facile tuned by adjusting the probe immobilization density or target DNA sequence concentration in hybridization solution. This simple approach has great potential application in fabrication of low cost polymeric biochips.

Key words:

Confined Photocatalytic Oxidation (CPO), DNA Microarray, Cyclic Olefin Copolymer (COC), DNA Immobilization and Hybridization

Introduction

Recently, the use of plastic materials as substrate of miniaturized biochips has attracted increasing interest due to their low cost, availability of various materials with different physical properties, easily integration with microfluidics and amenability to high volume manufacturing processes [1-11]. Comparatively, polymeric materials can provide unique surface properties such as surface and chemical heterogeneity that fairly different from traditional extremely flat inorganic substrates, which may be favorable for biomolecules immobilization [12-16]. Recently, several polymers that have been tested as alternative supports including cyclic olefin copolymer (COC), poly (dimethylsiloxane) (PDMS), poly (methyl methacrylate) (PMMA) and polycarbonate (PC) [17] have been reported. COC is a relatively new polymer material and is highly suitable for the biochip application because of their many favorable properties, such as high glass transition temperature, low auto-fluorescence, optical clarity, better chemical resistance than that of any other thermoplastic polymer, optical transparency

close to that of glass (for wavelengths over 300 nm), low water uptake and mold ability [18, 19]. Moreover, COC are very well suited for both rapid prototyping and low cost mass production [11]. However, due to the low surface energy and chemical inert of COC surface, it is hard to directly immobilize DNA on it, which requires the development of a new modification method for its surface functionalization.

The strategies for the immobilization of biomolecules can be divided into three categories: covalent, physical, or affinity based binding interactions [20]. Covalent immobilization is the most commonly used methods due to its stable binding with biomolecules. Generally, the inert surface of COC can be altered in several ways to render them reactive for covalently binding biomolecules: e.g. by photografting, ozone oxidation or plasma treatment [21]. For example, Pu and coworkers reported a photografting method to regionally introduce functional polymers containing reactive carboxylic groups onto COC microfluidic chips using photomasks. Based on this modification, micropatterns of proteins, DNA, and biotinlated conjugates were readily obtained by surface chemical reactions in one or two subsequent steps [22]. Diaz-Quijada *et al.* realized the surface oxidation of COC and PMMA slides by treating with O_3 and demonstrated the feasibility to covalently immobilize DNA microarrays on the modified plastics. They integrated the plastic-based DNA microarrays with microfluidics and presented a proof-of-concept microfluidic device for rapid hybridization of DNA arrays. COC surface can also be pretreated by oxygen plasma to produce oxidized surface (covered by ether and carboxyl groups)

subsequently functionalized with aminopropyl triethoxysilane (APTES), then labeled antibody or solid-phase PCR primers could be introduced on amine-covered surface by use of a cross-linking agent, such as 1,4-phenylene diisothiocyanate (PDITC) [21, 23]. However, there are also some limitations for the application of these methods. The main disadvantage of photografting was that low grafting density will result in a nonuniform modification causing inhomogeneous immobilization of biomoleccules, while high grafting density can obtain a uniformly modified surface but will greatly change the surface morphology of substrate. Moreover, the coverage of photografting is not suitable for all polymer substrate because the activity of the substrate surface was greatly influenced by a combination of several factors such as the reactivity of hydrogens as well as the degree of crystallinity and regularity [24]. For ozone oxidation and oxygen plasma treatment, although they are applicable for surface modification of most of polymer substrate, they also resulted in the introduction of multiple functional groups, etching, and morphological alterations. For example, plasma treatments involve complex sources containing a variety of energetic components such as charged or neutral particles (electrons, ions, excited molecules, radicals, metastable species) and photons (infrared to soft Xrays), which easily give rise to multiple reaction pathways and products on soft matter surfaces.

The immobilization of biomolecules on inorganic substrates such as glass and silica has been well established. For inorganic substrates, there have been a series of chemistries by grafting of silanes to introduce various functional groups such as

amine, epoxy, thiol, etc [25, 26]. Therefore, a robust and industrially scalable avenue to the attachment of biomolecules to COC surfaces is the adaption of the established chemistry mentioned above to its surfaces. The main barrier to this approach is the creation of surface containing -OH groups which is capable of reacting with silane coupling agent like on glass surface. In this work, different from the strategies mentioned above, a facile, effective and chemoseletive modification protocol is demonstrated to immobilize DNA probes on COC surface. Firstly, the C-H bonds on COC surface were converted to pure sulfate anion ($-SO_4^-$) groups by confined photocatalytic oxidation (CPO) method [27]. After subsequent hydrolysis, sulfate anions were further transformed into hydroxyl groups. Thus a hydroxylated COC surface which could perform silane coupling reaction was obtained easily. The increases in surface hydrophilicity can produce more functional groups-epoxy groups, then more DNA can be introduced on COC surface. Then (3-glycidoxypropyl) trimethoxy silane (GOPTS), a widely used reagent that could covalently attach oligonucleotide probes on surface of glass in the field of DNA microarrays, was employed to functionalize the modified COC slide with reactive epoxy groups. Finally, 5′-NH_2 modified oligonucleotide probe was covalently attached on this epoxy-modified COC surface through nucleophilic opening of epoxide rings by amines. The probe DNA was designed from a segment of IGFBP2 gene (overexpressed in grade IV astrocytoma [28, 29], which is the most common type of brain tumor). The traditional way for pathological classification of gliomas need long time and is not entirely accurate due

to extensive heterogeneity of astrocytic tumors, while the DNA microarray analysis could make the diagnosis more accurate and effective. The successful hybridization of the prepared DNA microarray with complementary strand demonstrated that this method has great potential in production of low cost and disposable biochips with process similar to traditional glass-based microarrays.

Experimental

Materials

Topas Cyclic-olefin Copolymer (COC) was purchased from Ticona (NJ, USA) and was injection molded to 0.5mm thickness slide. Ammomium persulfate (APS) and sodium dodecylsulfate (SDS) were purchased from Xilong Chemical Co., Ltd. (Beijing, China). (3-Glycidoxypropyl) trimethoxy silane (GOPTS) was from Alfa Aesar (Tianjin, China). The ultrapure water was obtained from the Millipore Direct-Q5 equipment. Glass slides attached with epoxy groups were purchased from GencBio Biological Technology Co., Ltd. (Shanghai, China). Saline sodium citrate (SSC) was from Beijing Chemical Works (Beijing, China). Albumin from bovine serum (BSA) was from Sigma-Aldrich. The probe A (5′-$(T)_{15}$ CAT GTT CAT GGT GCT GTC CAC G-3′) was Cy3-labeled at the 3′ end for visualization and modified at the 5′ end with amino groups to facilitate the attachment to COC surface. DNA B (5′-CGT GGA CAG CAC CAT GAA CAT G-3′) complementary to probe A was used as the target DNA and Cy5-labled at the 5′ end. Probe A and target B were synthesized by Invitrogen.

Instruments

Contact angle system OCA20 equipped with SCA20 software was from Dataphysics Instruments GmbH. The measurements were done in quintuplicate at room temperature with a volume drop of 2 μL. Microarray printing was carried out by Personal Arrayer in contact dispensing mode from CapitalBio Corporation (Beijing, China). The fluorescence images and signals of the spots were registered with LuxScan-10K/A from CapitalBio Corporation (Beijing, China). X-ray photoelectron spectra (XPS) were obtained by using ESCA Lab 220i-XL (VG scientific) instrument and Al K-alpha excitation at one 15° angle. Atomic force microscopy (AFM) was carried out with Nano Scope Ⅲa (DI, USA) microscope.

Epoxidation of COC films

COC film were cut into pieces of 7.5 × 2.5 cm^2 with thickness of 0.5mm and cleaned with acetone and air-dried. Briefly, a predetermined amount of APS aqueous solution (the concentration of APS was 30 wt%) [27] was sandwiched between biaxially oriented polypropylene (BOPP) film and COC film and irradiated by a high-pressure UV lamp (UV intensity at λ =254 nm is 9000 μW/cm^2) at room temperature for 2 min. After the irradiation, the modified COC film (denoted as COC-SO_4^-) were rinsed by deionized water and soaked into ultrapure water at 50 ℃ for above 16 h, and then rinsed by ultrapure water and dried in air at room temperature. The final substrate is named a hydroxlated surface (COC-OH). The hydroxylated COC films were subsequently silanized in ethanol containing 2% (3-glycidoxypropyl) trimethoxy silane (GOPTS)

for 4 h at room temperature. Finally, the films were baked in drying oven at 50 ℃ and stored under inert atmosphere at room temperature. The final substrate is named an epoxydized surface (COC-epoxy).

DNA Immobilization and Quantification

The Cy3-labeled Probe A with amino groups (-NH_2) was diluted in ultrapure water to a final concentration from 0.1 to 10 μM. A certain concentration of probe A solution was spotted on COC-epoxy slides through a contact spotting pin. Ambient humidity within the printing chamber was ~45%, each dilution was spotted in 3 × 3 arrays, and every array contains 10 × 10 spots of oligonucleotide probes. Oligonucleotide probes were spotted on each slide as spots of about 80 μm diameter with spot center-center distance of 400 μm.

After spotting, the slides were immediately scanned by microarray chip scanner to obtain the fluorescence intensity before incubation. Then, the slides were incubated 12 h at room temperature in a dark place. After incubation, slides were thoroughly rinsed with ultrapure water for 10 min to remove the unbounded DNA strands and air-dried. The microarrays on COC slides were immediately scanned by scanner after incubation and quantified by calculating the average pixel intensity of each spot. A standard curve for probe A immobilization was prepared by diluting fluorescent Cy3-labeled probe A in ultrapure water to a final concentration ranging from 0.05 μ M to 10 μM. Each dilution was spotted in 60 replicates with volume of 0.45 μL/spot and the fluorescence intensities were recorded before washing away unbound probes

[30].

The immobilization efficiency was calculated from the ratio of surface intensities of immobilized DNA and initially spotted DNA, and the immobilization density was calculated from the calibration curve. The above operations were conducted in a dark environment to prevent photo bleaching of the dyes.

DNA hybridization

Cy5-labeled complementary strand was used as target B and was diluted to a final concentration of 5 μM and 10 μM in ultrapure water. Mix 99 μL of 4 × SSC for each 1 μL of 5 μM and 10 μM labeled nucleic acid respectively (hybirdization solution), then add 1 μL 10% SDS per 100 μL of hybridization solution. Before hybridization, the COC slides attached with probe A were treated with a blocking buffer (10 g/L BSA, 1 L blocking buffer containing 0.25 L 20 × SSC and 0.01 L 10% SDS) at least 4 h to block the remaining active sites. After that, the slides were immersed in ultrapure water and dip 15-20 times, then rinsed in 4 additional change of ultrapure water and air-dried for further hybridization.

About 40 μL hybridization buffer containing target B was loaded directly on microarray slides and the cover slips were mounted to seal the reaction droplets. The slides were placed in a gene microarray chip hybridization box (CapitalBio) where the hybridization was performed overnight at 45 ℃. After hybridization, the slides were washed with 1 × SSC and 0.1% SDS 5 times, followed by a short rinse in ultrapure water. After rinsing and drying, the fluorescence images and intensity of the spots was

obtained by the scanner.

Results and discussion

Surface Characterization

The chemical routes to introduce epoxy groups on COC were summarized in Scheme 1. The key step was the transformation of inert C-H bond of COC surface into functional groups by CPO treatment. Compared to plasma and UV/O_3 treatment, CPO approach exhibited excellent advantages of fast, mild to environment and substrate (needs no complex facility related to high vacuum or high voltage or produces strong oxidizing gas such as O_3), facile, low cost and no discernible morphology changes which is more suitable for large-scale production. And we have proved that CPO can be used for most of commercial polymeric materials but not limited to COC. Moreover, the introduction of hydroxyl groups on polymer surface offers a great opportunity to directly tailor polymer surface property through engineered surface chemistry. The significance of this method is that it can directly benefit from the well-developed silane chemistry, thereby rendering grafting any functionalities on inert alkyl surfaces by changing the terminal groups in silanes possible, which should instantly stimulate the development of many domains such as microarrays, immunoassays, biosensors, filtrations and microseparation as well as fast-developing organic electronics [31].

The modification process was firstly monitored by the water contact angle measurement. Figure 1 shows the variation in surface static water contact angle (CA)

with irradiation time. The curve indicated that in very beginning, CA of the samples decreased significantly to 30-40° in a few seconds. Then the CA decreased gradually and leveled off to minimum values about 34.8° after about 120 s [27]. All the results verified the $-SO_4^-$ was successfully introduced by the confined photocatalytic oxidation treatment. And the successful hydrophilic modification in a few seconds also demonstrated the rapidity of CPO method.

The further modifications of COC surface were also characterized by the static water contact angle test. Since the contact angle is determined by the outermost atoms, its value could be considered as a sensitive indication of the surface chemical nature. Figure 2 shows the typical images of water contact angles of various surfaces during the modification process. Significant change of the CA was observed after each modification step. As we can see, the surface of COC blank sample before treatment showed a CA of about 99.1°. After CPO treatment, the CA decreased to 32.6° showing obvious hydrophilicity. The hydrolysis of $-SO_4^-$ to -OH resulted in CA increasing to 62.4°. After reacted with GOPTS, epoxy-activated COC slides showed a CA of 53.9°.

The chemical composition change of the COC surfaces after functionalization was also investigated by XPS and shown in Figure 3. The related results of elements composition are presented in Table 1. For CPO modified COC, the peaks at 168.6 eV (S 2p) (Figure 3 (b)) and 399.8eV (N 1s) (Figure 3 (d)) were attributed to S (SO_4^-) and N (NH_4^+) elements respectively, and the corresponding atom percentage (At%) were

5.93 (S 2p) and 8.45 (N 1s), which is much higher than that of original COC. The results obtained by XPS indicated that a thin single molecular sulfate salt group layer was introduced covalently onto COC surface. For DNA immobilized COC, from Figure 3(e) we can see a strong peak appeared in the N 1s region (399.8 eV) and the At% of N element was 10.12 on DNA attached surface, which is much higher than that on original COC surface (Figure 3 (c)), proving the presence of DNA on COC surface.

For functionalized surfaces, a superposition resulting from the different chemical environments of the corresponding C atom was observed in the C1s peak spectra (Figure 3). The C1s signal for the epoxy-functionalized surface can be divided into three components at 284.6, 286.2 and 288.2 eV (Figure 3 (f)), assigned to C-C,C-O and O-C-O carbon atoms, respectively [32]. As a sensitive surface analytical tool, XPS has been used for detailed studies of DNA interfacial chemistry on surfaces. For the DNA-functionalized surface, the C1s spectrum was decomposed into four different carbon positions with varying intensities, showing further increased intensity at 284.6, 286.2, 287.5 and 288.5 eV (Figure 3 (h)). Especially, the peaks at 287.5 eV and 288.5 eV represent carbon species to the DNA bases [33] once again indicating that the successful binding of oligonucleotides on COC surface.

Table1. XPS elemental surface composition (At%) of COC surface before and after sequential modification steps from survey scans.

Sample	C	O	N	S	Si
COC	89.91	5.95	3.91	-	2.89
COC- SO_4^-	58.75	14.74	8.45	5.93	7.78
COC-OH	73.52	14.64	1.06	1.78	9.01
COC-Epoxy	71.51	12.85	1.94	1.52	11.18
COC-DNA	73.03	10.71	10.12	2.73	3.41

For the accurate quantification and measurements repeatability of the immobilized molecules on polymer surface, great change for surface topography is unfavorable. However, physical strategies commonly used for polymer surface modification, such as plasma and UV/O_3 treatment, often create undesirable physical and chemical changes on the exposed soft surface, e.g., cracks, pitches and overoxidized products. In our work, CPO produces a way to form high quality smooth modified COC surface. In more detail, the surface morphology change of COC after CPO modification was investigated by AFM. As shown in Figure 4, original COC slides were flat and smooth, and only a few scratches and particles can be observed which is resulted from the process of machining. After the GOPTS treatment, no observable changes on surface topography were found. In order to compare the surface roughness of original and modified surface quantitatively, the RMS values of the surface were calculated. The roughness of the original COC surface was evident from its RMS value of 4.48 nm, while the average RMS value after GOPTS treatment was 6.69 nm. This minor

difference of the RMS roughness indicated that there was little surface etching effect after the introduction of epoxy groups on COC surface.

DNA Immobilization

Probe A with amino groups (-NH_2) was immobilized on COC-epoxy slides by the covalent bond formed via reaction of -NH_2 and epoxy groups. To evaluate the immobilization efficiency, Cy3-labeled Probe A with concentration from 0.1 to 10 μM was spotted on COC-epoxy surface. The DNA immobilization efficiency was investigated by comparing the surface signal intensities of immobilized DNA (after incubation overnight and washing away unbound probes) and initially spotted DNA (before incubation), which represented the ratio of the amount of attached probes to the actual spotted probes. As we can see from Figure 5, the immobilization efficiency on COC-epoxy surface was between 45% and 65%. For comparison, the DNA immobilization efficiency on commercially available epoxy-activated glass slide was also investigated on which probe A was spotted in the same way as on COC surface. From Figure 5 we can see the immobilization efficiency on epoxy-activated glass surface was also between 45% and 65%, indicating that the DNA immobilization method used in this work is feasible and can obtain higher immobilization efficiency than the immobilization efficiency (between 30% and 50%) of the method previously reported by Sun [30, 34]. This should be attributed to the fact that CPO could introduce higher density of hydroxyl groups on COC surface, which is favorable to obtain a higher density of epoxy groups to react with the -NH_2 on DNA probes.

In order to visually verify the immobilization of DNA on the COC-epoxy, the fluorescence images were recorded and the immobilization efficiency of three COC films (COC-epoxy, original COC and original COC immersed in GOPTS) was measured, which are shown in Figure 6 and Table 2. The 5 μM probe A was spotted on unmodified COC surface and incubated overnight in the same way for comparison. From Figure 6, we can see the fluorescence intensity of COC-epoxy surface after incubation (Figure 6 (a′)) was the maximum among the three COC films. The original COC surface (Figure 6 (b′)) exhibited nearly no fluorescence of captured probe and virtually no background fluorescence and the final immobilization efficiency was 1.0% (Table 2), suggesting little probe A immobilized on unmodified COC surface. Figure 6 (c′) showed slight fluorescence signal of DNA probes after incubation which was due to the physical adsorption of epoxy groups on COC surface and the immobilization efficiency was 2.9%, further illustrating the immobilization method used in this work is feasible and effective.

Table 2. The fluorescence intensity of probe A immobilization on different slides and the resulting immobilization efficiency of each slides.

	COC-epoxy	Original COC	Original COC in GOPTS
Before incubation	43491.7	42956.9	43829.8
After incubation	19385.4	429.5	1271.8
Immobilization efficiency (%)	44.6	1.0	2.9

To look for a better modification condition for DNA immobilization, the relationships between UV irradiation time and relevant immobilization efficiency were investigated. As seen from Figure 7, the immobilization efficiency increased significantly with the increase of irradiation time before 120 s, then increased gradually and leveled off to maximum immobilization efficiency about 45%. This tendency is easy understandable by the fact where the number of surface hydroxyl groups of COC film would increase with UV irradiation time.

In order to further evaluate the immobilization performance of our COC-epoxy substrate, we measured the immobilization density of the COC-epoxy and epoxy-activated glass by calibration curve, and the results are shown in Figure 8. The immobilized density increased with the concentration of the spotting solution, the difference of immobilization density on epoxy-activated glass surface and COC-epoxy surface was small when the concentration was below 2.5 μM. However, the difference of immobilization density on epoxy-activated glass surface and COC-epoxy surface became larger when the probe concentration was above 2.5 μM. When the concentration reached 10 μM, the immobilization density on glass surface and COC surface was around 78 pmol/cm^2 and 57 pmol/cm^2, respectively. The higher immobilization density of glass should be attributed to its higher epoxy groups density, which was derived from its higher hydroxyl groups density nature than modified COC surface. Although the immobilization density on COC surface was lower than that on glass surface, the immobilization density on COC surface was relatively higher than

that previously reported by Sun [30, 34] when the concentrations are the same.

DNA Hybridization Assay

In order to test whether the hybridization can be achieved on the modified surfaces, COC-epoxy DNA microarray slides with different probe spotting concentrations (1 to 20 μM) was prepared. Then, the slides were blocked with blocking buffer to deactivate the remained active sites. In the process of hybridization, two concentrations of target DNA (5 μM and 10 μM) were used. After hybridization with target B overnight at 45 ℃ and wash, the Cy5 channel was firstly used to verify whether the Cy5-labeled target B was introduced on COC surface (Figure 9 (a)). Then, the Cy3 channel was scanned and the green fluorescence (Figure 9 (b)) is ascribed to the Cy3-labled probe A attached on COC surface. The merged fluorescence images show the yellow light (Figure 9 (c)) due to the precisely superimposition of red light and green light, indicating the successful hybridization between probe A and target B introduced on COC slides.

For two different concentrations of target B, the hybridization intensity increased with the concentration of the spotted probe solution until 15 μM (Figure 9 (d)). The binding capacity of the COC surfaces was nearly saturated at the spotting solution concentration of 20 μM, showing that high surface coverage of DNA probes did not consequently lead to the formation of more hybrids. This might due to the repulsive electrostatic and steric interactions which were increasing with probe intensity [34]. In addition, hybridized target density increased with target concentration, the

hybridization of 10 μM concentration of target B is higher than that of 5 μM.

Conclusions

In this paper, a practical method that introducing epoxy groups on COC surface based on confined photocatalytic oxidation was reported. A series of characterization including water contact angle measurement, XPS and AFM verified the successful introduction of epoxy groups on COC surface without altering its surface morphology. The DNA probes were immobilized on COC via the reaction between the epoxy groups and the amine groups on DNA 5′ end. The DNA immobilization efficiency for different concentration of probe A was between 45% and 65%, which are comparable to commercial available epoxy-activated glass slide and relatively higher than that on original polymer surface previously reported. Successfully hybridization was also conducted on COC surface modified with DNA probe microarrays, indicating our protocol has great potential application in large scale biochip manufacturing, especially for the diagnose of glioma tumor lesion grades.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Grant No.51033001, 51221002, 51103009) and the Fundamental Research Funds for the Central Universities (ZY1311).

References:

1. G. A. Diaz-Quijada, R. Peytavi, A. Nantel, E. Roy, M. G. Bergeron, M. M. Dumoulin and T. Veres, *Lab on a Chip*, 2007, **7**, 856-862.
2. D. M. Dankbar and G. Gauglitz, *Analytical and Bioanalytical Chemistry*, 2006, **386**, 1967-1974.
3. Z. Zhang, P. Liang, X. Zheng, D. Peng, F. Yan, R. Zhao and C.-L. Feng, *Biomacromolecules*, 2008, **9**, 1613-1617.
4. A. V. Vasiliskov, E. N. Timofeev, S. A. Surzhikov, A. L. Drobyshev, V. V. Shick and A. D. Mirzabekov, *BioTechniques*, 1999, **27**, 592-600.
5. K. Kato and Y. Ikada, *Biotechnology and bioengineering*, 1996, **51**, 581-590.
6. A. Macanovic, C. Marquette, C. Polychronakos and M. F. Lawrence, *Nucleic Acids Research*, 2004, **32**.
7. H. Peng, C. Soeller, N. A. Vigar, V. Caprio and J. Travas-Sejdic, *Biosensors & Bioelectronics*, 2007, **22**, 1868-1873.
8. N. Kimura, *Biochemical and Biophysical Research Communications*, 2006, **347**, 477-484.
9. S. Taira and K. Yokoyama, *Biotechnology and Bioengineering*, 2004, **88**, 35-41.
10. S. Sabella, G. Vecchio, R. Cingolani, R. Rinaldi and P. P. Pompa, *Langmuir*, 2008, **24**, 13266-13269.
11. Y. Ladner, F. d'Orlye, C. Perreard, B. Da Silva, C. Guyon, M. Tatoulian, S. Griveau, F. Bedioui and A. Varenne, *Plasma Processes and Polymers*, 2013, **10**,

959-969.

12. P. D. Sawant and D. V. Nicolau, *Materials Science and Engineering B-Solid State Materials for Advanced Technology*, 2006, **132**, 147-150.
13. T. Brandstetter, S. Boehmer, O. Prucker, E. Bisse, A. zur Hausen, J. Alt-Moerbe and J. Ruehe, *Journal of Virological Methods*, 2010, **163**, 40-48.
14. S. Laib and B. D. MacCraith, *Analytical Chemistry*, 2007, **79**, 6264-6270.
15. S. H. Lee, S.-W. Kim, J. Y. Kang and C. H. Ahn, *Lab on a Chip*, 2008, **8**, 2121-2127.
16. Z. Zhao, R. Peytavi, G. A. Diaz-Quijada, F. J. Picard, A. Huletsky, E. Leblanc, J. Frenette, G. Boivin, T. Veres, M. M. Dumoulin and M. G. Bergeron, *Journal of Clinical Microbiology*, 2008, **46**, 3752-3758.
17. K. Kuroda, T. Ishida and M. Haruta, *Journal of Molecular Catalysis a-Chemical*, 2009, **298**, 7-11.
18. S.-J. Hwang, M.-C. Tseng, J.-R. Shu and H. H. Yu, *Surface & Coatings Technology*, 2008, **202**, 3669-3674.
19. D. Sung, D. H. Shin and S. Jon, *Biosensors & Bioelectronics*, 2011, **26**, 3967-3972.
20. M. Rendl, A. Boenisch, A. Mader, K. Schuh, O. Prucker, T. Brandstetter and J. Ruehe, *Langmuir*, 2011, **27**, 6116-6123.
21. J. Raj, G. Herzog, M. Manning, C. Volcke, B. D. MacCraith, S. Ballantyne, M. Thompson and D. W. M. Arrigan, *Biosensors & Bioelectronics*, 2009, **24**,

2654-2658.

22. Q. Pu, O. Oyesanya, B. Thompson, S. Liu and J. C. Alvarez, *Langmuir*, 2007, **23**, 1577-1583.
23. J. Hoffmann, S. Hin, F. Stetten, R. Zengerle, G. Roth, 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences, Seattle, 2011.
24. J. P. Deng, L. F. Wang, L. Y. Liu, W. T. Yang, *Progress in polymer science*, 2009, **34**, 156-193.
25. P. Wu, P. Hogrebe and D. W. Grainger, *Biosensors & Bioelectronics,* 2006, **21**, 1252-1263.
26. J. Escorihuela, M. Jose Banuls, R. Puchades and A. Maquieira, *Chemical Communications*, 2012, **48**, 2116-2118.
27. P. Yang, J. Y. Deng and W. T. Yang, *Polymer*, 2003, **44**, 7157-7164.
28. G. N. Fuller, C. H. Rhee, K. R. Hess, L. S. Caskey, R. Wang, J. M. Bruner, W. K. Yung, W Zhang. *Cancer Res*, 1999, **59**, 4228-4232.
29. S. L. Sallinen, P. K. Sallinen, H. K. Haapasalo, H. J. Helin, P. T. Helen, P. Schraml, O. P. Kallioniemi, J Kononen. *Cancer Res*, 2000, **60**, 6617-6622.
30. Y. Sun, I. Perch-Nielsen, M. Dufva, D. Sabourin, D. D. Bang, J. Høgberg, A. Wolff, Transducers'11, Beijing, 2011.
31. P. Yang, W. T. Yang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 3759-3770.
32. J. Escorihuela, M.-J. Banuls, R. Puchades and A. Maquieira, *Bioconjugate*

Chemistry, 2012, **23**, 2121-2128.

33. H. O. Ham, Z. Liu, K. H. A. Lau, H. Lee and P. B. Messersmith, *Angewandte Chemie-International Edition*, 2011, **50**, 732-736.

34. Y. Sun, I. Perch-Nielsen, M. Dufva, D. Sabourin, D. D. Bang, J. Hogberg and A. Wolff, *Analytical and Bioanalytical Chemistry*, 2012, **402**, 741-748.

Figure Legends:

Scheme 1. Schematic illustration of the processes of DNA immobilization on COC surface and subsequent hybridization reaction.

Figure 1. Changes in water contact angle of COC surface with irradiation time. (UV intensity: 9000 μW/cm^2; $(NH_4)_2S_2O_8$, 30 wt%.)

Figure 2. Images of water contact angle on COC surface before and after modification. (a) COC; (b) COC-SO_4^-; (c) COC-OH; (d) COC-epoxy.

Figure 3. XPS spectra of untreated COC surfaces: (a) S 2p peak, (c) N 1s peak and treated COC surfaces: (b) S 2p peak, ((d), (e)) N 1s peak; XPS high-resolution spectra of carbon C 1s for (f) original COC surface, (g) COC-epoxy and (h) COC immobilized with DNA oligonucleotides.

Figure 4. AFM topographic images (5 × 5 μm) of (a) the original COC surface and (b) the epoxy-functionalized COC surface.

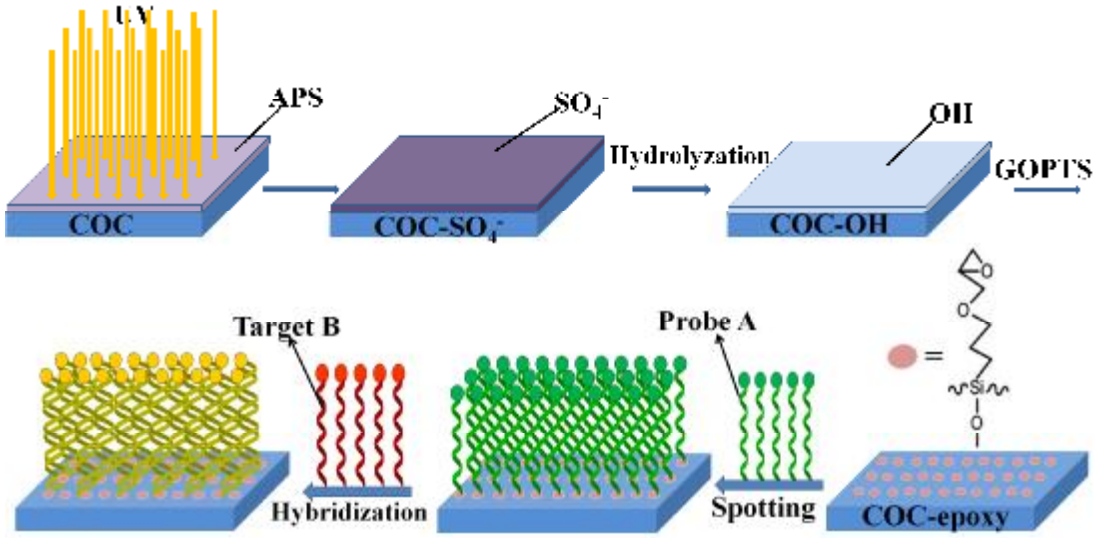
Figure 5. Immobilization efficiency on COC films and epoxy-activated glass surfaces as functions of spotted probe A concentrations. The immobilization efficiency was calculated from the ratio of surface intensities of immobilized DNA and initially spotted DNA. Each experiment was repeated three times and error bars represented standard deviations.

Figure 6. Fluorescence images of DNA immobilization on (a, a′) epoxy functionalized COC surface before and after incubation; (b, b′) original COC surface and (c, c′) original COC surface after immersing in GOPTS ethanol solution.

Figure 7. Evaluation of DNA probe immobilization efficiency with UV irradiation time of CPO process on COC surface. (UV intensity: 9000 μW/cm^2; $(NH_4)_2S_2O_8$, 30 wt%; DNA probe, 5 μM)

Figure 8. Immobilized density of probe A on COC-epoxy and epoxy-activated glass surface with probe A spotting concentrations ranging from 0.1μM to 10μM.

Figure 9. Representative hybridization results of DNA hybridization on epoxy functionalized COC slides spotted with probe A (5 μM) in a 5×5 microarray pattern. The concentration of target DNA and Probe DNA was 5 μM. (a) the scanning image of Cy5 channel, (b) the scanning image of Cy3 channel, (c) merged fluorescence images of (a) and (b), (d) the hybridization intensity on epoxy functionalized COC slides. Amine-modified Cy3-labeled probe A was spotted at different concentrations (1-20 μM) and hybridized with Cy5-labeled target B (5 μM and 10 μM).


UV
APS
SO_4^-
Hydrolyzation
OH
GOPTS
COC
COC-SO_4^-
COC-OH
Target B
Probe A
Hybridization
Spotting
COC-epoxy

Scheme 1.

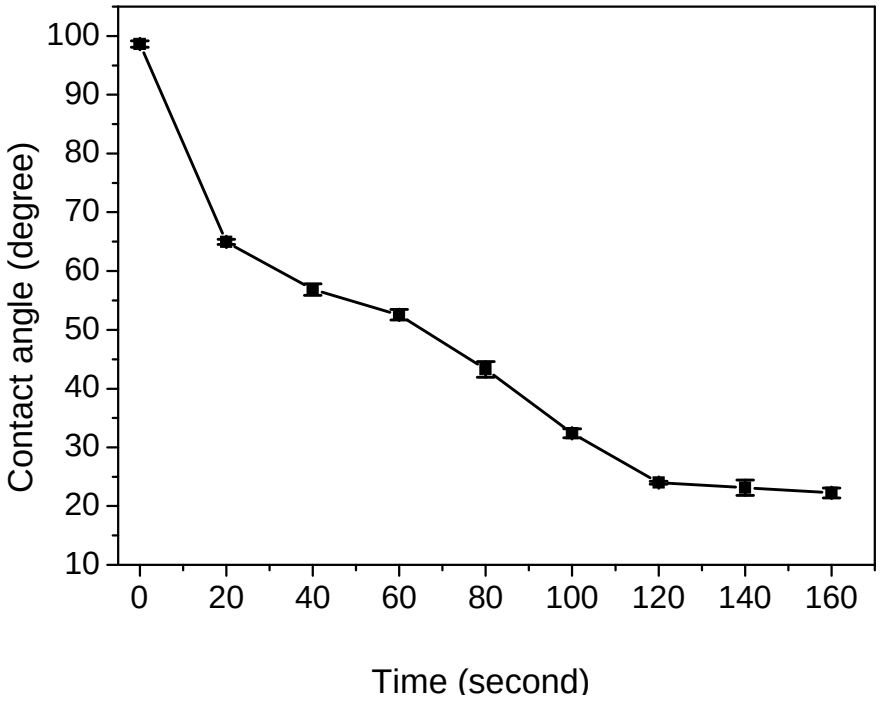

Contact angle (degree)
100
90
80
70
60
50
40
30
20
10
0
20
40
60
80
100
120
140
160
Time (second)

Figure 1.

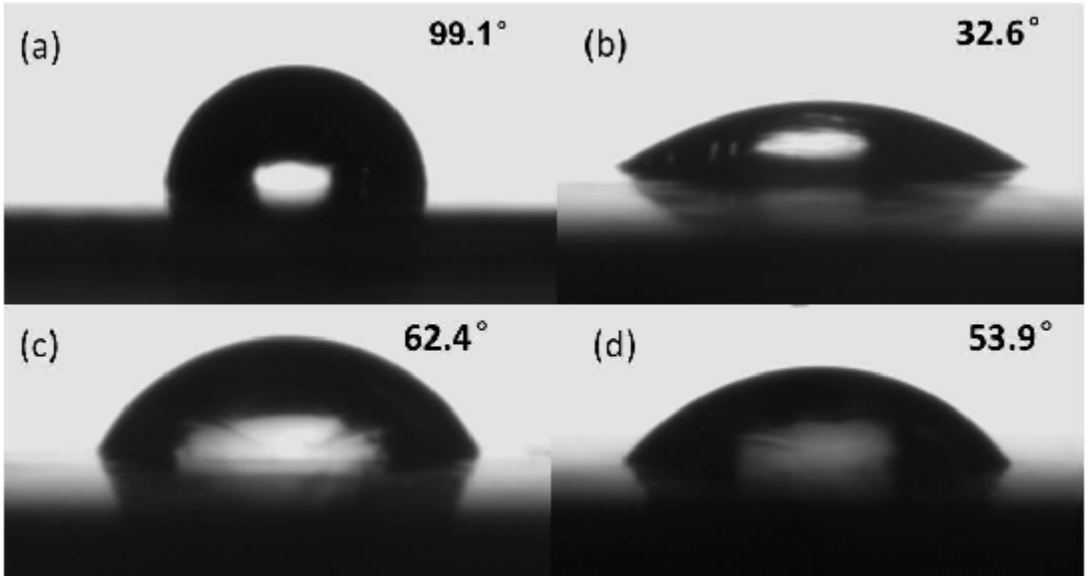


Figure 2.

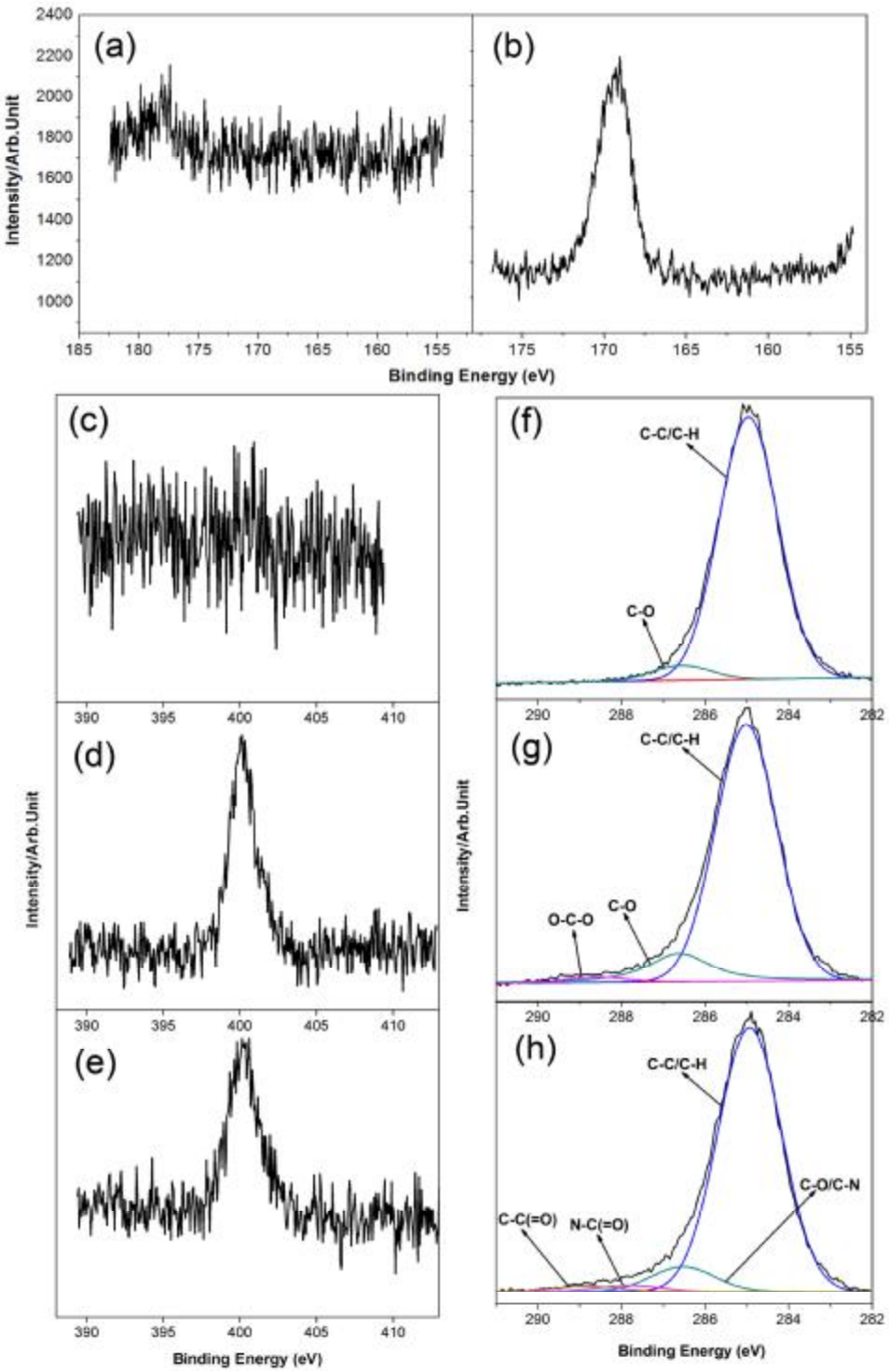

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
Intensity/Arb.Unit
Binding Energy (eV)
C-C/C-H
C-O
O-C-O
C-O/C-N
C-C(=O)
N-C(=O)

Figure 3.

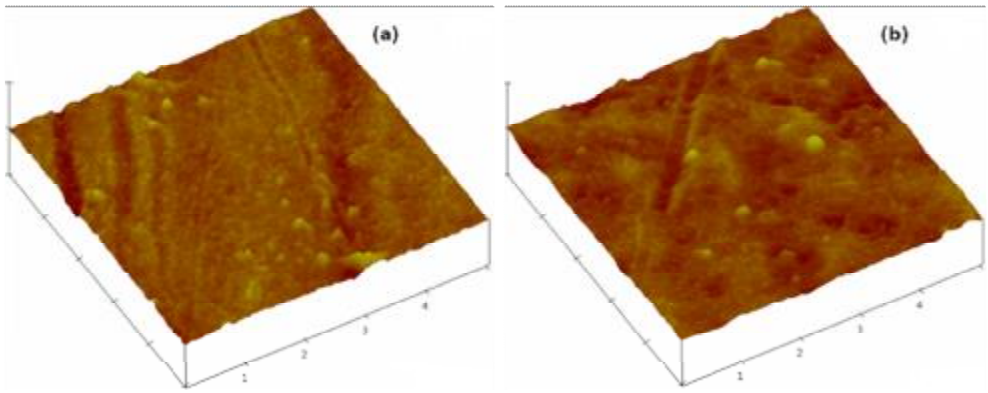


Figure 4.

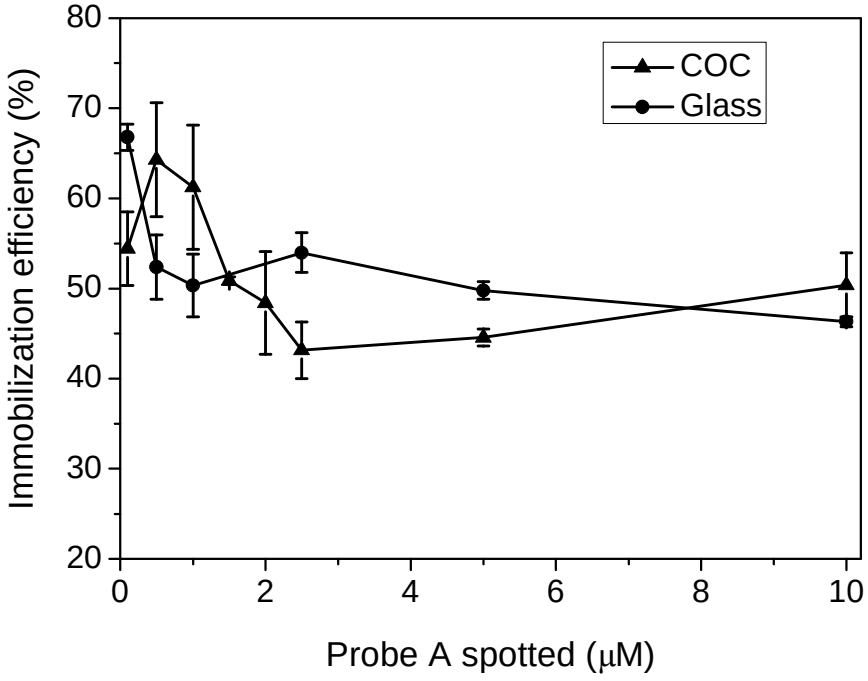

Immobilization efficiency (%)
80
70
60
50
40
30
20
0
2
4
6
8
10
Probe A spotted (μM)
COC
Glass

Figure 5.

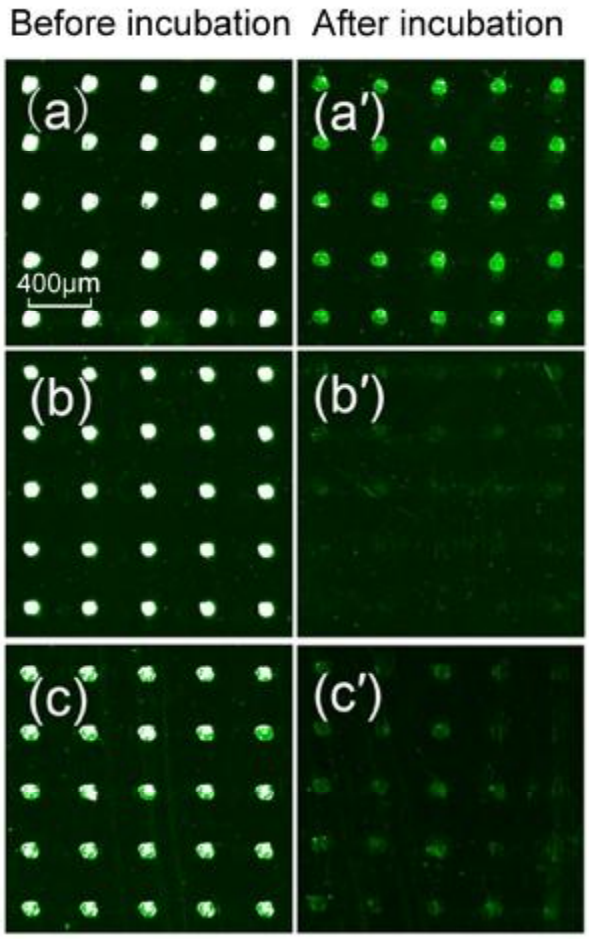


Figure 6.

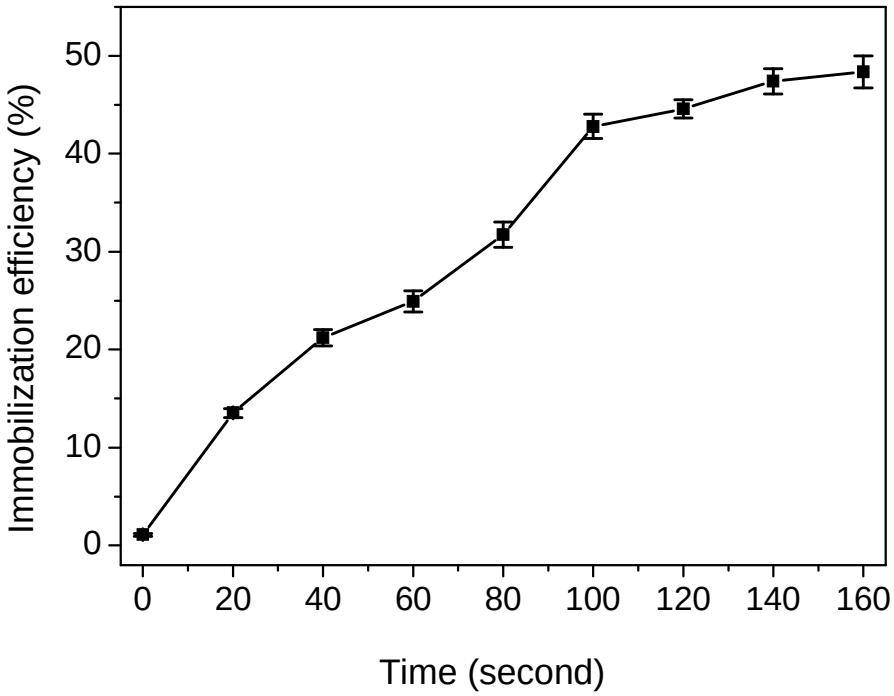

Immobilization efficiency (%)
0
10
20
30
40
50
0
20
40
60
80
100
120
140
160
Time (second)

Figure 7.

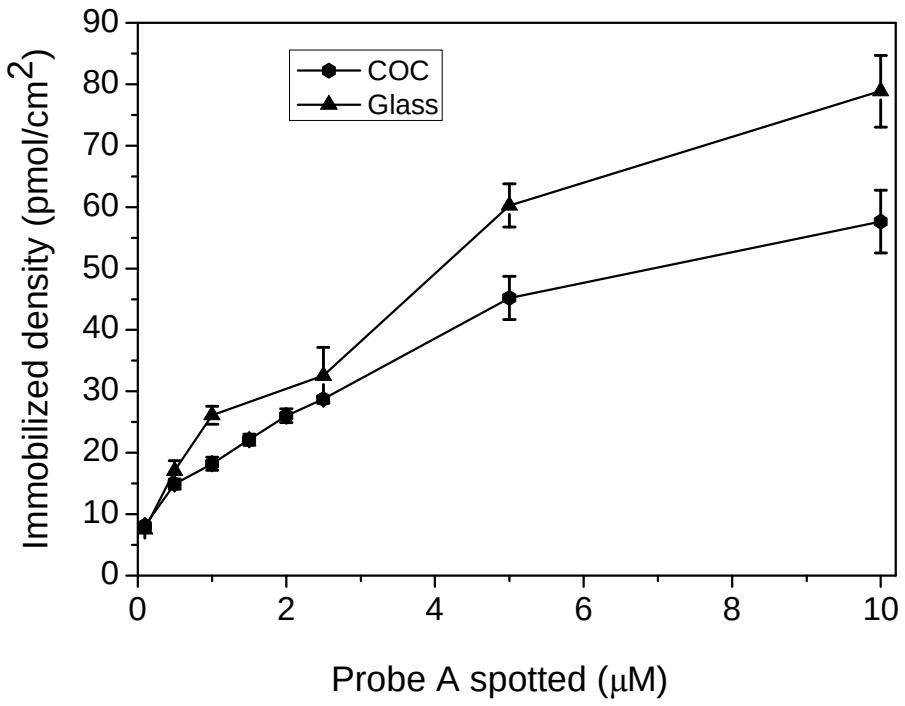

COC
Glass
Immobilized density (pmol/cm2)
Probe A spotted (μM)
0
10
20
30
40
50
60
70
80
90
0
2
4
6
8
10

Figure 8.

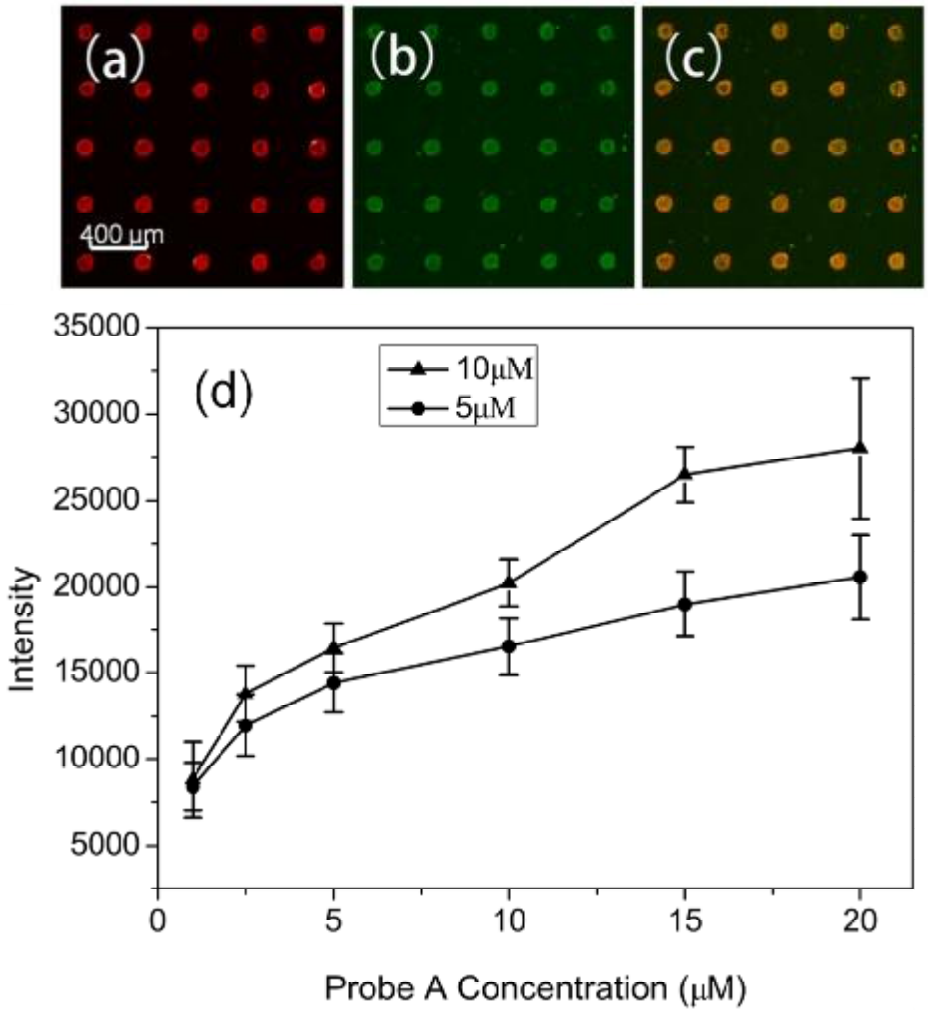

(a)
(b)
(c)
400 μm
(d)
10μM
5μM
Intensity
35000
30000
25000
20000
15000
10000
5000
0
5
10
15
20
Probe A Concentration (μM)

Figure 9.