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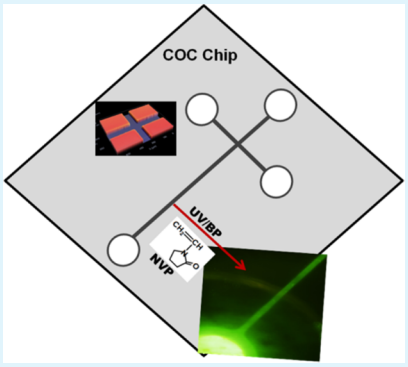
High Performance of Cyclic Olefin Copolymer-Based Capillary Electrophoretic Chips

Sunanda Roy,[†] Tanya Das,[‡] and C. Y. Yue*[,‡]

[†]School of Materials Science and Engineering, Nanyang Technological University, Singapore 639798

[‡]Singapore-MIT Alliance, Manufacturing Systems and Technology Programme, School of Mechanical and Aerospace Engineering, Nanyang Technological University, Singapore 639798.

ABSTRACT: This paper demonstrates a simple, one step, and low cost surface modification technique for producing cyclic olefin copolymer (COC) polymer-based microcapillary electrophoresis chips consisting highly hemocompatible microchannels by UV-photografting with *N*-vinylpyrrolidone (NVP) monomer. An optimal condition has been identified to achieve the best surface grafting process. It has been found that this surface treatment enables extremely high surface wettability, hemocompatibility, and bond strength to the microchannels. The surface grafting was confirmed by attenuated total reflection Fourier transform-infrared spectroscopic (ATR-FTIR) study. In vitro protein adsorption using fluorescent labeled bovine serum albumin (FITC-BSA) into the COC microchannel results indicates that the modified chips have excellent protein resistance ability because of the increase of surface hydrophilicity. Hence, the modified chips showed fast, reproducible and high efficient separations of proteins (up to 51 000 theoretical plates per meter). Moreover, this surface modification process show no loss in the optical transparency to the modified microchannel surfaces: an important requirement for real capillary electrophoresis since the fluorescent intensity is directly related to the amount of adsorbed protein on the surface. Therefore, we believe that this simple and promising route of surface modification could be very useful for developing high performance COC microfluidic devices for the separation of proteins, amino acids, and other biomolecules.



KEYWORDS: *capillary electrophoresis, hydrophilic, hemocompatibility, grafting degree, COC*

INTRODUCTION

Polymer-based microfluidic devices are becoming more important for a huge range of applications including immunoassay, drug discovery, DNA detection, cell sorting, pharmaceuticals, protein separations,[1−5] and many more[6−8] because of their low cost, flexibility, disposability, excellent optical properties, easy fabrication, and capacity for mass production.[9] Recently, COCs have become the most popular material in the microfluidic community because of their high transparency,[10] excellent mold transferability, low water absorption rate, low dielectric loss, and high glass transition temperature. Also, COCs are soluble in toluene and hexane which are very rarely utilized solvents in the applications of microfluidic devices. Furthermore, COCs have excellent resistance to acid, alkali and hydrolysis.[11] Despite of many merits of COCs, a microfluidic device made by COCs may not be useful in many bioanalytical applications because of its intrinsic hydrophobic surface, which makes it susceptible to cell adhesion and proteins adsorption when come in contact with any biological molecules. This could be harmful in the performance of the devices as because uncontrolled cell adhesion and protein adsorption may cause blockage to the microchannel and eventually disrupt the flow of the fluids. Moreover, the hydrophobic surface hinders introducing fluids into microchannels and inconsistencies in flow dynamics. Thus, it requires reproducible and efficient surface modification before it can be adapted for widespread commercial applications.

To minimize the above problems, several methodologies, including plasma treatment,[12−14] UV-photografting of hydrophilic monomers,[15−18] dynamic coating with hydrophilic polymers,[19] and UV/ozone oxidation,[20,21] have been introduced in COC microchannels for suitable surface modification. Among the strategies above, UV-photografted surface modification is of great importance because it is facile, cost-effective, industrially favorable, and renders hydrophilic surface with high stability at a faster reaction rate. Apart from that it also modifies the surface properties of the solid materials without significantly affecting the bulk characteristics. In addition to that, UV-grafting technique provides stable and durable surfaces as compared to the most extensively used plasma treatments or physical coatings that can be delaminated. Gaudioso et al.[22] demonstrated the surface modification of COC by oxygen plasma treatment to increase the surface hydrophilicity. However, they noticed that the modified COC surfaces were not stable and showed faster hydrophilic loss, that is, aging only after six days time. Hence, this modification might not be appropriate for durable applications. Li et al.[19] used

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polyacrylamide coating on COC microchannels by UV-grafting method to create the protein separation COC chips. Stachowiak et al.[20] have modified the surfaces of COC microchannel using poly-(ethylene glycol) methacrylate (PEGMA) monomer by two-step UV-grafting process. But the water contact angle results showed less improvement in hydrophilicity after grafting (from 88° for the native COC to 45° for modified surface). Nevertheless, a significant reduction of protein adsorption (~78%) was reported for modified surfaces as compared to the unmodified COC surface. Zhang et al.[17] have demonstrated surface modification of COC microchannel by dynamic coating with hydroxyethyl cellulose (HEC). The modified microchips showed good protein resistance, low electroosmotic flow (EOF), and efficient protein separation. Nevertheless, there could be a chance of delamination of the coating layer with aging.

More recently, Ma et al.[23] have demonstrated the preparation of SiO_2−COC hybrid material suitable for Bio-MEMS applications. The process was although new and interesting, but their main attention was only on the material preparation and characterization rather than to investigate the real microfluidic device. Jena et al.[21] have described UV-grating of hydrophilic, biocompatible 2-methacryloyloxyethyl phosphorylcholine (MPC) monomer on the COC surface for the preparation of COC biomicrofluidic devices. But here also, most of the attention was given to analyze the result on flat substrate rather than on real microchannels. From the above, it is clear that despite of many attempts looking into surface modification of COC microchannels, many important factors, such as burst pressure, biocompatibility, protein separation, surface transparency, etc., that are directly related to the shelf life and device performance; have not been studied yet.

In this work, we describe a novel surface modification technique for production of smart, robust and high performance LOCs for the Bio-MEMS applications. NVP was opted as grafting monomer due to its outstanding property of water solubility, high hydrophilicity, and great extent of biocompatibility. Moreover, NVP grafted surfaces retain the optical transparency similar to the pure COC.

EXPERIMENTAL METHODS

Materials and Reagents. COC, a trade name of Topas 5013 with T_g of 130 °C, purchased from Ticona (U.S.A.) was used for the device fabrication. NVP (99+% purity), benzophenone (BP, 98% purity), FITC-insulin, and FITC-BSA were procured from Sigma Aldrich. All other analytical grades of chemicals, such as methanol, ethanol, and acetone were purchased from Merck.

Chip Fabrication. Both injection molding and hot embossing processes were used to fabricate microchannels on the COC substrates. Microfluidic chips that were used for burst pressure test were prepared by injection molding process, whereas the microcapillary electrophoresis chips (μCE) for protein separation were fabricated by hot embossing on the injection molding polymer sheets (1 mm thick) using a silicon die. Finally, to form the closed microchannels, the patterned COC substrates were thermal bonded with another flat piece of Topas 5013 substrate. The fabrication and hot embossing process was same as described previously.[24]

UV-Grafting Process. UV grafting was carried out under a metal halide lamp equipped with strong UV light source (UV flood curing system, TechnoDigm, Singapore) at a wavelength of λ = 365 nm. The intensity of the UV light was adjusted by varying the lamp and the COC substrate positions. The UV-grafting was performed by the same process as reported in previous publications.[25,26] Various formulations were prepared with different percentages of monomer solutions in water (2.5−22.5 wt %) and at a fixed BP concentration in acetone (10 wt %), to optimize the best grafting results. After irradiation, the grafted polymer films were rinsed in a series of solvents such as acetone, mixture of methanol and hot water, and aqueous sodium hypochlorite (NaOCl) solution to remove the excess BP, homopolymer and unreacted monomers prior to drying for 8hrs at normal room temperature. The grafting degree (G_d) was calculated by the following equation:[27]

$$G_d = (W_1 - W_0)/W_0 \times 100\%$$

where W_1 is the weight of the film after grafting and W_0 is the initial weight of polymer film.

Characterization. Attenuated total reflection Fourier transform infrared spectroscope (ATR-FTIR, Perkin-Elmer GX spectrometer) spectra were used to identify the chemical changes between the unmodified and modified surfaces. During scanning, the resolution and total accumulation scans were set at 4 cm^{-1} and 32, respectively, for each spectrum.

The surface wettability of the pure and unmodified films was evaluated by static water contact angle measurement with the help of a FTA 200 video Series instrument. The experimented values were an average of six different measurements for single a sample over which the deionized water droplets (~1−2 μL) were placed on each single COC substrate.

Atomic Force Microscopy measurement (AFM, Nanoscope III Scanning Probe Microscope, California, U.S.A.), operated in the tapping mode was used to determine the surface roughness (R_a) of the pure and grafted COC surfaces. Scanning electron microscope (SEM, JSM-5600) was used to measure the geometrical features of the microchannels. The UV−vis absorbance spectra of the pure and modified COC films were measured with a UV-2501PC Spectrophotometer (Shimadzu, Japan) in the range from 200 to 800 nm.

Burst Pressure Test. The burst pressure test was conducted to evaluate the integrity and the performance of the thermally bonded microdevices. The detail of the procedure was described previously.[24] Briefly, the burst pressure was performed by connecting the thermally bonded COC chip containing of a straight microchannel to a micro pump connected to a pressure transducer. To promote the test and to generate the internal pressure into the microchannel, either one of the two ports were sealed by plug fitting using a PEEK nanoport assembly glued by epoxy. The burst pressure is the maximum pressure up to which the chip can withstand before failure. The chips used are 50 μm deep, 200 μm wide, and 6 mm in length.

Adsorbed Protein Measurement. In vitro protein adsorption tests into the unmodified and modified microchannels were performed carefully in phosphate buffered saline (PBS) solution. Prior to the test both were hydrated with 1% PBS buffer solution for 30 min. To acquire maximum adhesion of BSA molecules to the walls of the microchannels, the FITC-BSA solution was incubated at 37 °C for 2 h, followed by a thorough rinse with PBS buffer solution to get rid of unwanted BSA molecules that are not adhered to the surface. However, more details of the process was given previously.[26]

CE Separation of Proteins. The electrophoretic separation of proteins was performed on a simple cross type (T-shaped) microchannel. The design of the T-shaped chip is presented in Figure 1. There are total four reservoirs in the chip, represented by A, B, C,

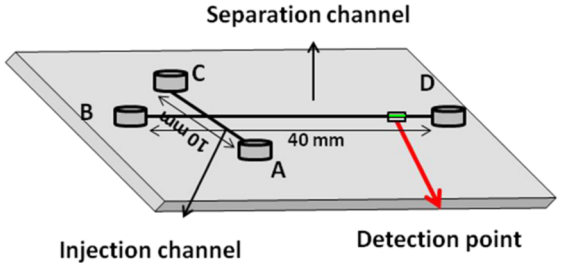

Figure 1. Schematic representation of the microchips used for capillary electrophoresis. Reservoirs are defined by A, B, C, and D.

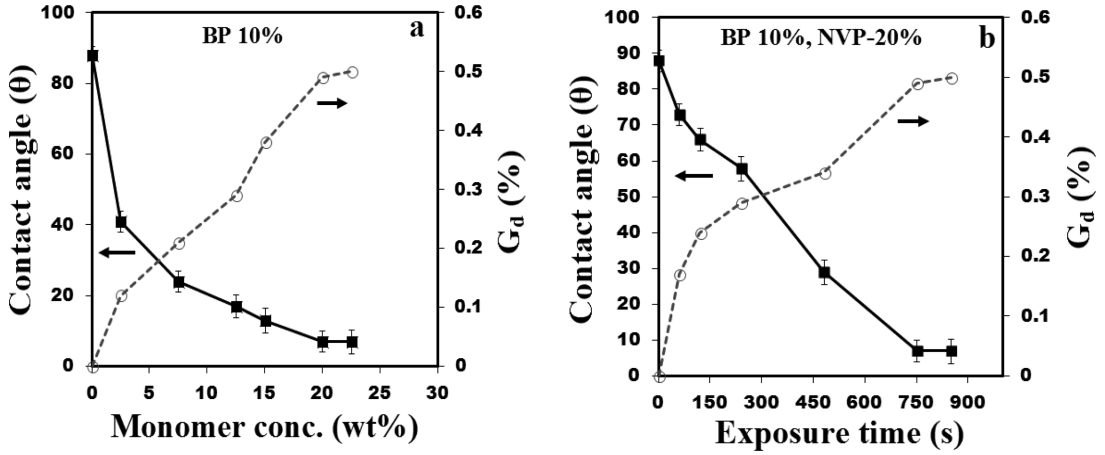

Figure 2. Effects of (a) monomer concentration and (b) UV exposure time on average surface contact angle and grafting degree on the on the NVP-photografted COC surfaces.

and D. The injection and separation channels were 40 and 75 μm wide, respectively, and 50 μm deep. The holes are punched to create access to the reservoirs for loading analytes and applying the voltages. The lengths of injection and separation channels are 10 and 40 mm, respectively. The effective separation distance from the intersection is 28 mm. The protein separation analysis were performed based on the same approach described by Liu et al.,[28] where 60 μL protein was introduced into the reservoir A and then electrophoresed toward the opposite reservoir (reservoir C) of the injection channel by applying 600 V voltage across the full injection channel. Platinum electrodes were inserted to provide electrical contact. Pinch injection was utilized to load the experimented sample in the injection channel. During sample injection, reservoirs A, B, and D were grounded, and reservoir C was adjusted at +600 V. While during separation process, reservoirs A and C were set at +600 V, reservoir B was grounded, and reservoir D was fixed at +2.0 kV. The laser-induced fluorescence (LIF) detection was used in this system. The Tris-HCl buffer with pH 8.50 was used as the testing buffer solution.

RESULTS AND DISCUSSION

To know the influence of monomer concentrations and exposure time on improving the surface hydrophilicity, the water contact angles of modified surfaces were determined, and the values were represented in Figure 2a and b respectively. It is observed from Figure 2a that the contact angle sharply decreases with increasing the monomer concentration and reaches the lowest values of 7−8° from 88° (for unmodified COC) at 20 wt % NVP. This decrease of water contact angle with an increase of monomer concentration can be explained due to grafting of more number of hydrophilic polar NVP chains as a result of increasing grafting degree (G_d) values. The result of irradiation time on the contact angle and G_d for 20 wt % NVP grafted COC is observed in Figure 3b. It is being noticed that with the increasing exposure time, the contact angle diminishes and the G_d value increases, reaching an ultimate value of 0.49% and 8°, respectively, at 750 s and stabilizes thereafter. This clearly indicates that the wettability/hydrophilicity of the COC microchannel can be significantly increased by UV-photografting of NVP monomer and the lowest contact angle can be achieved with 20 wt % NVP at 750 s treatment.

ATR-FTIR spectra of the untreated and poly-NVP (PNVP)-grafted COC films with an increase of grafting degree due to different monomer concentrations are presented in Figure 3. The peaks appearing at 1291 and 1664 cm^{-1} corresponds to the C−N bond and C═O group in the pyrrolidone ring of PNVP,

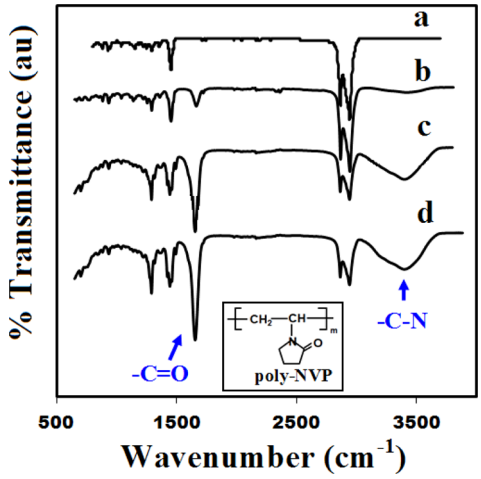

Figure 3. Typical FTIR-ATR spectra of (a) untreated COC, (b) 7.5%, (c) 12.5%, and (d) 20% of NVP grafted COC surfaces.

respectively. The symmetric and asymmetric stretching of methylene ($-CH_2$) can be seen at 2866 and 2935 cm^{-1} respectively. The broad absorption peak in the regions of 3200−3600 cm^{-1} can be ascribed to the C−N group. It is apparent that the intensity for the characteristic peak of the carbonyl group and C−N stretching increases with increasing the grafting degree (Figure 3b−d). The presence of such new peaks in the modified samples validates the effective grafting of the PNVP chains on the surface of COC.

The surface roughness of the grafted samples is considered subsequently as it gives an impact to the wettability and fluid flow. The variations in surface roughness with exposure time are shown in Figure 4. We observe that the unmodified COC have very smooth surface with surface roughness of 14 nm (±5). It can be seen from Figure 4 that the surface roughness increases while increasing the irradiation time. But the surface roughness did not show much change up to 480s but rises sharply thereafter. The values for 750s and 850s were found to be 168 and 188 nm respectively. Nevertheless, these values are much smaller than the values reported earlier.[29] The increase of surface roughness with exposure time can be explained by the increase of grafting degree.

Since thermal bonding of polymer microfluidic channel without deformation is considered to be a big challenging issue

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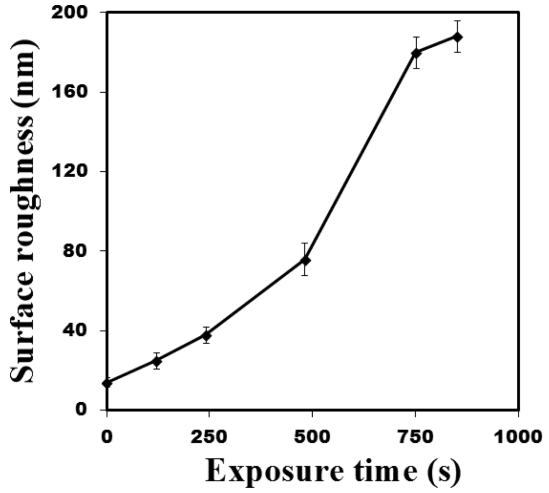

Figure 4. Variation of surface roughness with UV-exposure time. Each point represents an average value of the four different measurements.

in the microfluidic chip fabrication process, thus studied next. Thermal bonding is basically carried out near the polymer glass transition temperature (slightly higher than T_g) under a certain load. However, a small variation in temperature or pressure can lead to collapse the whole microchannel network. Table 1 show

Table 1. Effect of Monomer Concentration on the Burst Pressure Strength for the Various Monomer Grafted COC Chips

sample	irradiation time (s)	bonding temperature (°C)	burst pressure [bonding pressure = 2.2 MPa, t = 6 min]
untreated COC		120	1.68 ± 0.25
NVP-7.5%	750	100	2.59 ± 0.32
		110	2.97 ± 0.30
		120	3.33 ± 0.30
NVP-12.5%	750	100	2.86 ± 0.31
		110	3.26 ± 0.32
		120	3.80 ± 0.28
NVP-15%	750	100	3.03 ± 0.33
		110	3.77 ± 0.30
		120	
NVP-20%	750	100	3.81 ± 0.35
		110	4.02 ± 0.31
		120	

the burst pressure test results for NVP grafted COC chips that had been bonded at typically low temperature, that is, T_g = 30−10 °C. It was observed that the unmodified chips can only be bonded at 120 °C or higher, whereas the lowest possible bonding temperature for all grafted samples was found to be 100 °C that is 30 °C below the T_g of the COC polymer. It is apparent from Table 1 that modified chips withstand very high burst pressure, and the values increases with increasing the monomer concentration and temperature. The COC chip modified with 20 wt % NVP monomer solution and bonded at 110 °C exhibits extremely high burst pressure of 4.04 MPa that is ∼2.4 times higher than the unmodified chip (1.68 MPa) bonded at 120 °C. Moreover, the modified chips that were bonded at 120 °C with 15 and 20% concentrated monomer solutions, fails at the connector joints of the testing equipment because of tremendously strong interface strength, thus unable to collect the test results.

Figure 5 shows the effect UV-exposure time on the of burst pressure values at a constant monomer concentration (20 wt %) and temperature (110 °C). It is apparent that the burst pressure continuously increases with increasing the exposure time. A greater improvement in the burst pressure is observed for 720 s, probably because of the increased grafting degree (see Figure 2). With exposure time, the burst pressure increases because of the appearance of greater polymer chain entanglement because of increasing graft concentration between the two thermally bonded grafted plates constituting the chip.

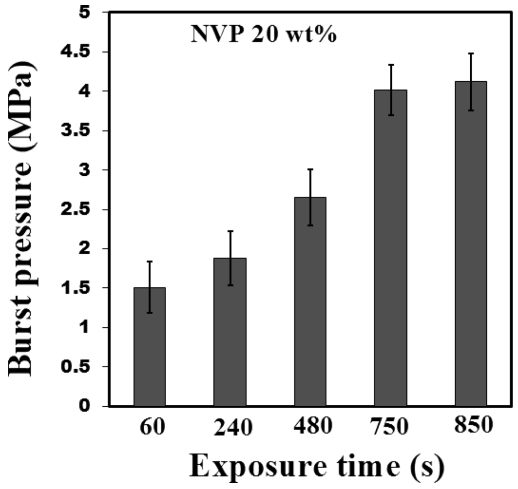

Figure 5. Effects of UV-exposure time on burst pressure strength of thermally sealed COC chips [Bonding temperature 110 °C, pressure 2.2 MPa, and time 6 min].

Figure 6 shows the typical SEM micrographs of different parts of embossed and closed COC microchannel. It is apparent from Figure 6a−c that perfect replication of microstructures can easily be achieved by hot embossing process. Furthermore, the smooth and vertical side walls of the embossed channel confirm the precision of the embossing process.[24] Figure 6d shows the micrograph of a closed microchannel (NVP grafted). It can be clearly seen from Figure 6d that the appearing integrity (dimensional and geometrical) of the microchannel were well maintained even after thermally bonding. The very smooth sidewalls and distinct edges of the bonded microchannel obtained clearly indicate the benefit of low temperature thermal bonding property as a consequence of the novel grafting technique. This image again indicate that high-quality microchip without any shape distortion can be constructed using UV-grafting of NVP.

After thermal bonding, the next important step is to verify the surface biocompatibility/hemocompatibility of the channel walls. Thus, to examine the biocompatibility of the modified COC surfaces, protein adsorption test was performed within the chip. In this study, fluorescently labeled BSA is selected as model protein for the adsorption test. The amount FITC-BSA present on the surface of different NVP-grafted COC microchannels is shown in Figure 7. It is important to note that in case of capillary electrophoresis the microchannel surfaces of the modified chip have to be transparent even after surface modification because the concentration of adsorbed protein on the surface is directly proportional to the fluorescent

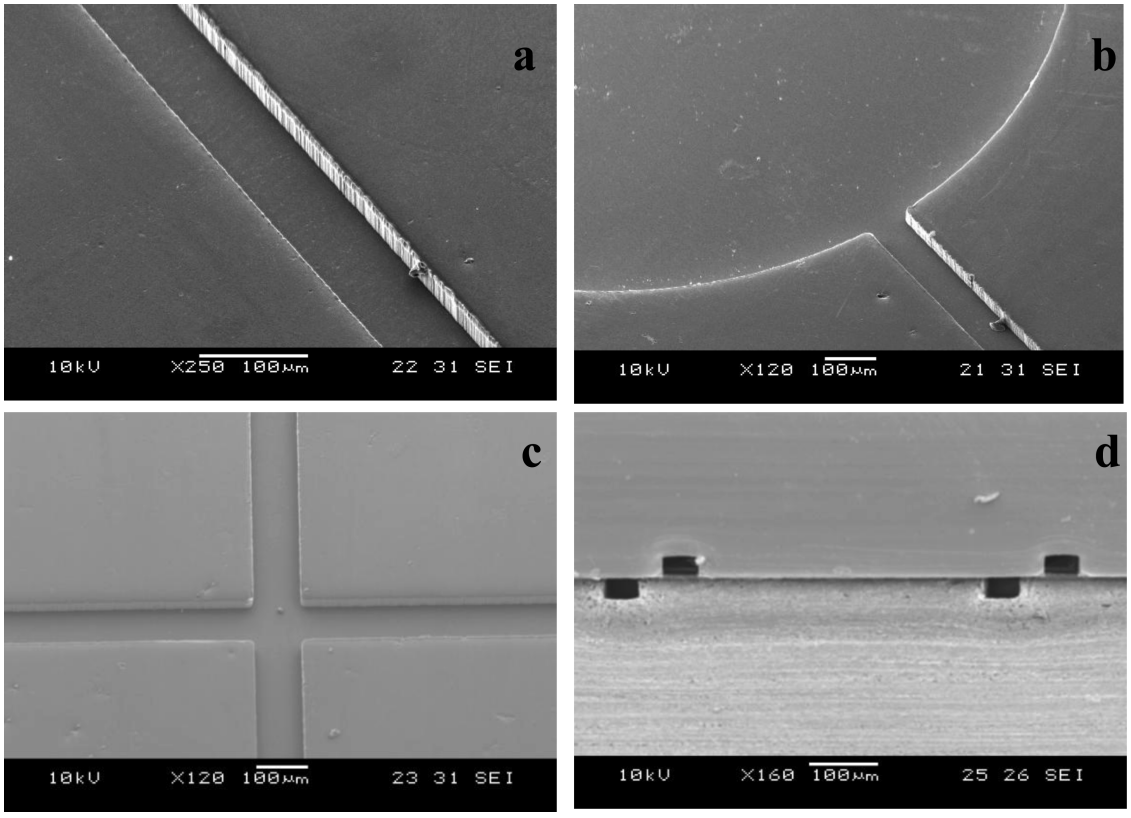

Figure 6. SEM images showing the (a) hot embossed microchannel, (b) reservoir, (c) junction of the capillary chip, and (d) cross section of thermally sealed microchip. The bonding was performed at 110 °C for 6 min under 2.2 MPa bond pressure.

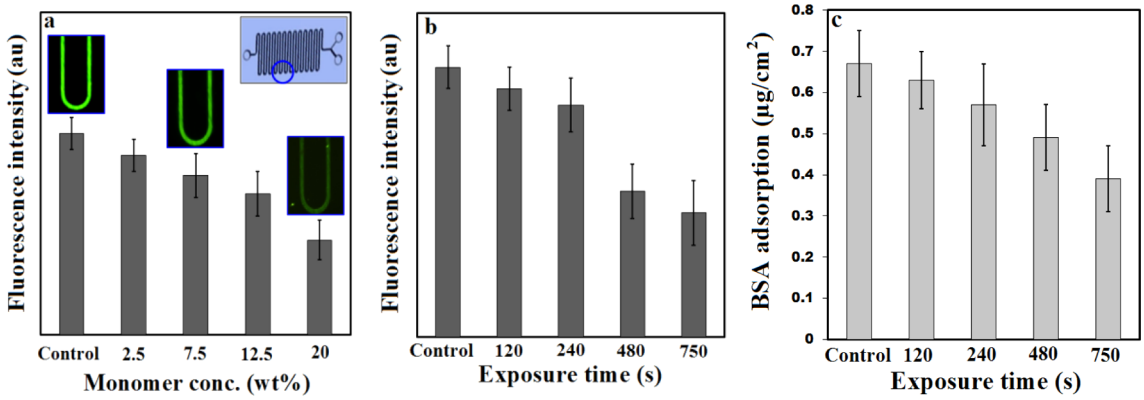

Figure 7. Measure of relative fluorescent intensity of FITC-BSA adsorbed on various NVP grafted COC surfaces (20% NVP).

intensity. Otherwise, the results may not demonstrate the actual surface character. It can be seen from both Figure 7a and b that the intensity of fluorescent light decreases with an increase in the monomer percentage and UV-exposure time, thus indicating that the modified surfaces became more resistance or less sensitive to protein adsorption. To support more of the above facts, fluorescence microscopy images of the unmodified and modified microchannel, taken after incubation with FITC-BSA solution were also presented (see the inset). It is apparent that (inset images) the untreated COC microchannel exhibits strong fluorescence light due to substantial adsorption of FITC-BSA protein on its surfaces. In contrast, the modified chips show extremely low intensity fluorescence light, indicating less protein adsorption on theirs surfaces. We are also able to quantify the adsorbed BSA by desorption of the proteins from the polymer surface using SDS solutions, followed by micro BCA protein assay kit for measurement (Figure 7c) as described previously.[25] It is clear from Figure 7c the adsorbed BSA amount decreases markedly with an increase in the UV-exposure time. The amount of adsorbed BSA decreases from 0.67 (±0.10) $\mu g/cm^2$ for the unmodified to 0.39 (±0.08) $\mu g/cm^2$ for the 20 wt % NVP grafted sample. The suppression of adsorbed protein with irradiation time can probably be explained due to the improvement of the surface hydrophilicity as a result of increase grafting degree of the PNVP chains onto the COC. Moreover, the NVP ring, which acquired a bulky structure, can obstruct the protein adsorption onto the modified COC surfaces, predominantly at higher degree of grafting.

To manifest the performance and efficiency of our best COC chips in bioanalytical applications, capillary electrophoresis separation was performed. Figure 8 represents the electropherograms of FITC-insulin conducted in the 20 wt % NVP-grafted COC chip. More than 40 runs were conducted in the same chip over few days time to ensure that the modified channels are stable, hydrophilic, and did not suffer from any degradation or aging with time. Figure 8 illustrates the results of four electrophoretic separations (run 1, run 2, run 4, and run 20) using the same chip. It can be seen that the four results are absolutely repeatable and have the same migration times. Moreover, the peaks are very sharp and distinguished. Peaks first and third were selected to test the performance and stability of the NVP modified chip. Table 2 represents the RSD of migration times and separation efficiencies. On the basis of the above findings it is very clear that UV-grafting using NVP on the COC microchannel surface is a very effective and robust method for fabricating high performance COC microfluidic devices.

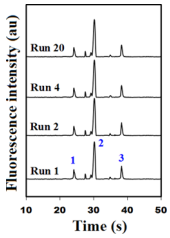

Figure 8. μ-CE separations of FITC-insulin. Electropherograms shows repeatability of the separation results. Run 1 and 2 were conducted on the first day. Run 4 and Run 20 were conducted on the 2nd day and 5th day of the experiment, respectively.

Table 2. Column Efficiencies, Migration Times, and Theoretical Plate Measurements of FITC-Insulin Conducted in NVP-Grafted Capillary Electrophoresis COC Chips

peak	migration time (s)	total plates	relative standard deviation (RSD) (%)
1	24.08	5.1×10^4	0.87
3	38.22	4.8×10^4	1.04

Figure 9 shows the results of UV–vis absorbance spectrum for the unmodified and various NVP-grafted COC samples. It can be seen that transparency of unmodified COC was very high over the wavelength range of 400–800 nm and the light transmittance at 550 nm was measured to be 84%. It is also apparent from the above figure that after surface grafting with NVP, even with the highest concentration of 20 wt % solution, the COC surface remains to be same/equally transparent as the pure COC film. The light transmittance was found to be 82.3% at the same visible region, that is, 550 nm, which indicates less than 2% loss in surface transparency than the pure COC film.

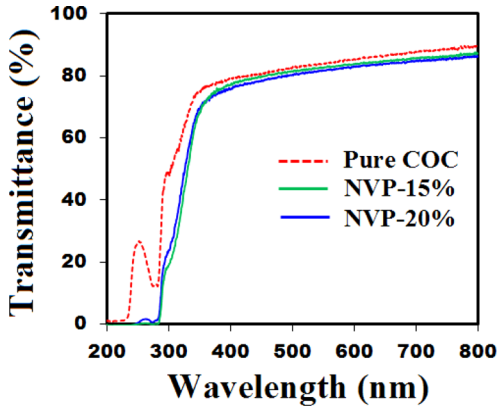

Figure 9. UV–vis transmittance spectra of pure COC and NVP grafted COC samples.

CONCLUSIONS

This study described the feasibility of the UV-grafting of hydrophilic NVP monomer for the fabrication of COC microfluidic devices for capillary electrophoresis separations. The grafting degree was controlled by the monomer concentrations and UV-irradiation time. A remarkably high surface hydrophilicity was achieved using this process. Moreover, this surface treatment can facilitate to achieve exceptionally high thermal bond strength (burst pressure) even at temperature 30 °C lower than the T_g of the pure COC polymer. This in turn result the modified chips to be perfectly shaped, smart, and robust. In vitro protein adsorption results revealed that COC channels grafted with NVP have excellent biocompatibility as observed by the low fluorescent light intensity and amount of protein adsorption. As a result, the modified chips showed fast, reproducible, and high efficiency electrophoretic separations of proteins. Furthermore, the light transmittance measurement results showed no loss in optical transparency after surface grafting with PNVP even with 20 wt % solution for 750 s. On the basis of the above findings, we believe that surface modification with PNVP by UV-photografting is an extremely efficient method capable of producing a high performance COC-μCE chips.

AUTHOR INFORMATION

Corresponding Author

*E-mail: mcyyue@ntu.edu.sg. Tel: +65-6790-6490. Fax: +65-6792-4062.

Notes

The authors declare no competing financial interest.

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