

Patterned Photonic Nitrocellulose Membrane for Bio-detection Based on Coffee-Ring Effect

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Abstract. In this work, we report a method for the fabrication of nitrocellulose (NC) membrane with photonic crystal (PC) pattern for bio-detection. The membrane is prepared by imprinting the corresponding PC pattern into it through thermal Nano-imprint method. Aptamer beacon marked with fluorescein is used for detection of target molecules. Only one droplet (2-3 μ l) containing the aptamer-target complexes is dripped onto the nitrocellulose membrane. After evaporation (15-20min), the droplet is completely dried and the complexes form a coffee ring on the membrane. The ring keeps growing with the increase of complexes concentration. As the aptamer beacon is marked with fluorescein and PC pattern has a fluorescence enhancement effect, the fluorescent signal on the coffee ring for detection is considerably improved. Fluorescent detection of adenosine triphosphate (ATP) is used for testing the application of this method. Owing to fluorescence enhancement effect of PC, the fluorescent signal for detection is improved and the limit of detection is 80.2 μ M for ATP. Therefore, we believe this method is promising for the fabrication of patterned photonic nitrocellulose membrane for bio-detection and point-of-care testing.

1. Introduction

Recently, photonic crystals (PCs) are attracting increasing interest in a wide range of research fields for their unique optical structures and properties.[1] Especially, PCs with controlled micro/nanostructures have emerged as promising materials for lots of bioanalytical applications.[2, 3]

On one side, photons with wavelengths near the stop band of PCs will propagate at lower speed owing to resonant Bragg scattering, which enhances optical gain thus amplifying the excitation of incident light. [4] It is because of the fluorescence enhancement effect, PCs have been used for improving the sensitivity of various kinds of fluorescent bio-analysis.[5, 6]

Here, we demonstrate a method for the preparation of a nitrocellulose (NC) membrane with photonic crystal (PC) pattern. As the pseudo paper substrate, NC has the characteristics of simple, easy-prepared and inexpensive.[7] Moreover, we can get NC membrane with PC structure by imprinting specific pattern into membrane through thermal Nano-imprint method, which will enhance the detection sensitivity of fluorescence based biological detection.[8-10]

The process of detection is closely related to a natural phenomenon-coffee ring effect. A coffee ring is a pattern left by drying a drop of coffee on a substrate. Because of the different evaporation rate between liquid near the edge and center in a droplet, a capillary force arises which brings solutes from the center of the droplet to the edge. After droplet is completely dried, it forms ring-shaped stain on the substrate.[11, 12]

First, the PC patterned NC membrane is soaked in poly dopamine solution for surface modification to improve the formation of coffee ring.[13, 14] For detection of the target molecule (Figure 1), we design an aptamer beacon consisting a fluorophore-tagged anti-target aptamer and a secondary oligonucleotide tagged with a quencher. Initially, the aptamer and oligonucleotide are

hybridized and the fluorophore and quencher are adjacent to each other, inhibiting the beacon from fluorescing. When target molecule is present, a shift in binding equilibrium causes the target to bind to the aptamer thus dissociating the oligonucleotide and form the complexes. Eventually, the fluorophore is no longer quenched and release fluorescence. The complexes diffuses in the droplet and finally forms a coffee ring after the droplet completely dried. [15]The concentration of target is determined quantitatively by detecting fluorescence signal. For testing the application of this detection method, adenosine triphosphate (ATP) is used as experiment for quantitative assay.

The photonic NC membrane is important for the following reasons. First, the process of the fabrication is simple and low cost. The PC pattern is obtained by simple Nano-imprint instead of traditionally using SiO₂ nanoparticles or polystyrene microspheres for assembly. [9, 16]Second, the NC membrane with PC structure can effectively enhance the detection sensitivity.[17] By adjusting the initial mode, corresponding PC pattern with different shape and photonic band gap can be get which will enhance the fluorescent intensity of fluorescein with different excitation wavelengths. Finally, the process of detection is rapid, the volume of the liquid needed for detection is small. Only one droplet (1-2 L) is needed and it will be dried completely in 15-20 minutes. Therefore, we believe the method for fabrication of photonic NC membrane has a significant advantage, which will be useful for point of care testing.

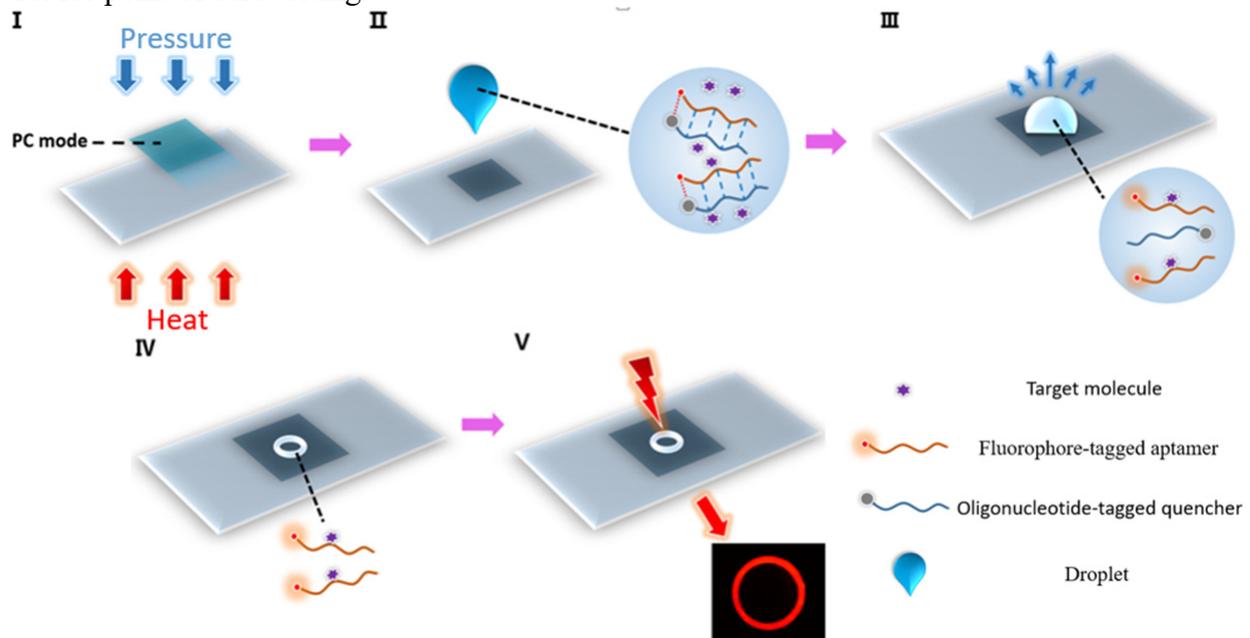


Figure 1. Schematic illustration showing the procedure to fabricate the NC membrane with PC pattern for fluorescent aptamer-based assays. (I) NC membrane with specific PC pattern is fabricated by thermal imprint (II) Droplet containing target molecule and aptamer beacon is dropped onto the NC membrane, the aptamer beacon is consisted of a fluorophore-tagged anti-target aptamer and a secondary oligonucleotide tagged with a quencher. (III) Target molecule will bind to aptamer thus dissociating the oligonucleotide and finally forms the complexes. Droplet will be completely dried in a short period of time. (IV) The complexes diffuses in the droplet and finally forms a coffee ring after the droplet is completely dried. (V) Quantitative detection of target by detecting fluorescence signal.

2. Experiment section

To obtain the photonic NC membrane, an acetone and DMF (1:1 v/v) solution containing 7.5% (w/v) NC was prepared. After stirring the solution at room temperature (25 °C) for 12h, the solution was then drop cast onto the glass slowly until the whole glass is filled. After baking the glass at 60 °C for 2h, the cellulose solution is dried and turned into flexible NC membrane. Tear the NC membrane slowly from the glass and wash it with deionized water. Place the NC membrane, PC mode, silicon wafer and coating film sequentially on thermal Nano-imprint device. Imprint the PC pattern of the mode into the NC membrane. The NC membrane with PC pattern is obtained after rinsed.

NC membrane were dipped into a 20 mL aliquot of 10mM Tris-HCl solution (PH 8.5) containing 0.50 mg/mL dopamine for surface modification and allowed to react for 12 h. [13]Then the membrane were rinsed and dried.

The aptamer beacon is obtained by hybridizing the sequences (Cy3-5'-CACTGTACCTGGGGGAGTAT-3')and (5'-AGGTACAGTG-BHQ-2-3') 2 μ M respectively .Then ATP solution with different concentration is mixed with aptamer beacon (10:1 v/v) and heated in water-bath 37 $^{\circ}$ C for 30min.[15] One droplet (2-3 μ L) of mixture solution is dripped onto NC membrane and completely dried after 15-20min. Finally fluorescence detection is carried out.

3. Result and discussion

The NC membrane reported here has PC pattern due to thermal Nano-imprint. Therefore, it can be used for quantitative fluorescent detection based on coffee ring effect. As shown in in Figure 2a, The NC membrane shows different colors because light with corresponding wavelength was reflected by the periodic photonic structure. [8]By changing PC mode before Nano-imprint, NC membrane with different shapes of PC pattern can be obtained (Figure 2b). As shown in Figure 2c, NC membrane with hexagon hole has peak reflection wavelength at 500nm.The fluorophore we use to detect ATP is Cy3 whose excitation wavelength is 514nm. The fluorescence signal is enhanced because the stop band of the NC membrane (500nm) overlap with excitation wavelength of Cy3 (514nm) which result in resonant enhancement.[4, 16]

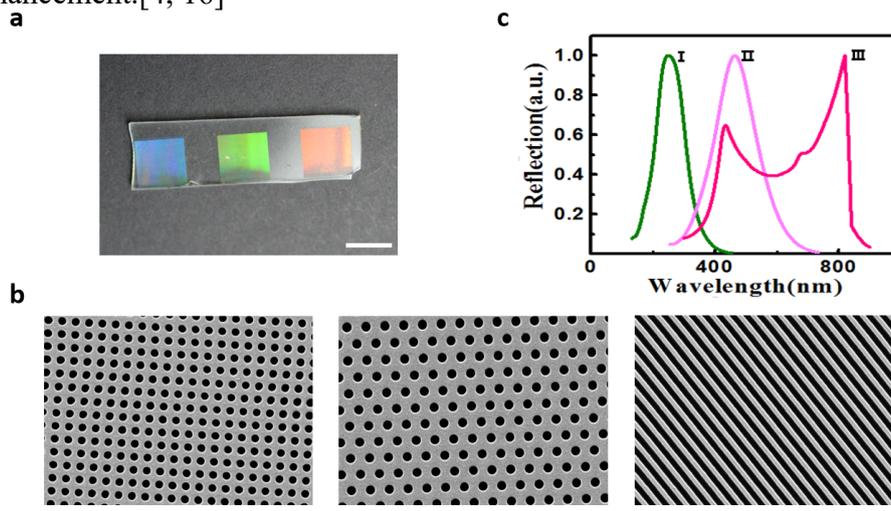


Figure 2. (a) Photograph of prepared NC membrane with different PC pattern. The NC membrane exhibited different colors due to reflection of the various PCs (Scale bar: 1 cm). (b) Scanning electron micrographs of NC membrane with different PCs (scale bar: 2 μ m). Square hole, hexagon hole and grating respectively from left one. (c) Reflectance spectra of PCs from NC with corresponding square hole, hexagon hole and grating.

The result of fluorescent detection is shown in Figure 3. The NC membrane with PC structure which has 500nm peak reflection wavelength is used for detection. Fluorophore used here is Cy3 (excitation wavelength is 514nm). NC membrane with no PC pattern is used as control .By matching the excitation wavelength of the stop band of the photonic structure, the fluorescence of dye is significantly enhanced. [16]After introduction of droplet containing analyte and aptamer beacon, the assay is completed until the droplet is dried.[15]Fluorescence from both NC membrane were measured and correlated to concentration of ATP. As shown in Figure 3b (I), a linear relationship between fluorescent intensity and ATP concentration was observed from 200 μ M to 5mM. What's more, Coffee ring with concentration of 100 μ M ATP can be observed in the fluorescence image. The limit of detection (LOD), which was calculated as 3 times the standard deviation of the testing results of the blank divided by the slope of the calibration curve, was 80.2 μ M for ATP. The LOD is lower than those of many complex bio-detection sensor with tedious operation which demonstrates that this NC membrane with PC structure can enhance the fluorescence signal and lower the detection limit.

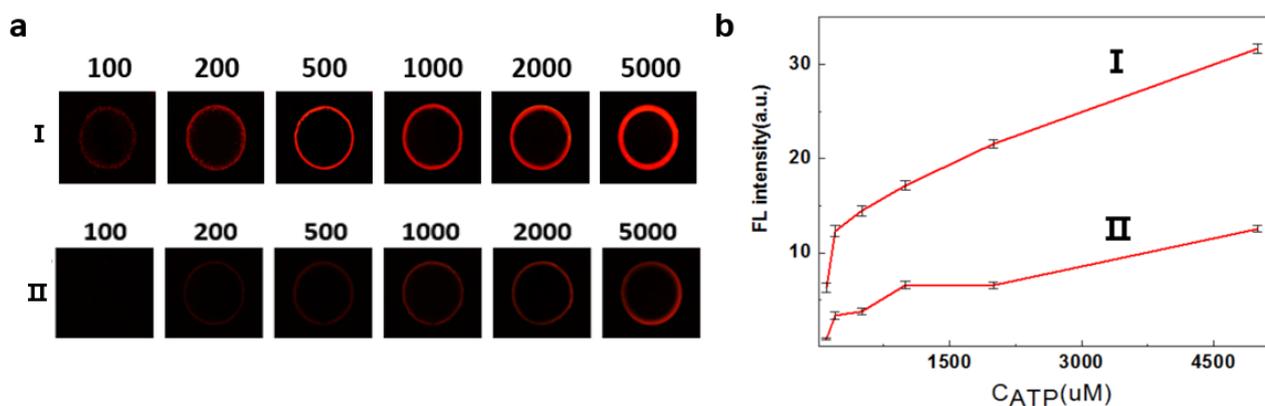


Figure 3. (a) NC membrane for fluorescent detection of ATP based on coffee ring effect. I: Fluorescent signal as a function of ATP concentration of NC membrane with hexagon hole PC pattern mentioned above. II: Fluorescent signal as a function of ATP concentration of single NC membrane with no PC pattern.

(b) Relationship curve of ATP Concentration and fluorescence intensity. Error bars represent the standard deviation for six replicated tests.

4. Summary

To summarize, we have reported the method for fabrication of nitrocellulose membrane with photonic crystal structure through thermal Nano-imprint. The NC membrane is used for bio detection based on coffee ring effect. The NC membrane with PC pattern were utilized for enhancing the fluorescence signal. Upon matching of the excitation wavelength of fluorescent dye with the stop band wavelength of photonic structure, fluorescence was amplified and detection sensitivity was improved. The NC membrane were further modified by aptamer beacon for bioanalysis. ATP was used as experimental target molecule which were quantitatively detected with LOD 80.2 μ M. Therefore, we believed this method is promising for fabrication of bioanalytical materials for POCT.

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