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News & Views

All-printed nanophotonic biochip for point-of-care testing of biomarkers

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Under the global pandemic of COVID-19, point-of-care testing (POCT) strategy as a fast, simple, and low-cost biological detection method has drawn much attention for achieving accurate on-site testing [1]. The China Association of Medical Equipment defines it as a detection method that is carried out at the sampling site and uses portable analytical instruments and biochips to quickly obtain test results [2]. In terms of space, POCT at the patient's side does not require a professional laboratory and the doctors. In terms of time, the diagnostic accuracy and test efficiency are high. At present, the market size of domestic POCT accounts for more than 10% of the overall *in vitro* diagnosis market, with a growth rate of more than 25%. However, the current POCT mainly focuses on the rapid detection of biomarkers such as blood glucose, pregnancy and ovulation, blood and urine biochemistry, drugs and alcohol, coagulation and thrombolysis [3]. There has been a tremendous demand for marker proteins via POCT strategy, instead of complex enzyme linked immunosorbent assay (ELISA) processes [4].

With the rapid development of nanophotonics and the continuous improvement of micro and nano-fabrication technology, biological detection methods based on the interaction between micro/nanostructures and photons have been rapidly developed [5]. Among them, photonic crystal (PC) is composed of periodic nanostructures that have alternatively low and high refractive index to affect the propagation of electromagnetic waves inside the structure [6]. PC is providing an accurate sensing platform because of the strong confinement of light inside the device, which is adopted for POCT within DNA, glucose and so on [1]. At present, the current fabrication of nanostructured PC mainly relies on photolithography-based technology, which is a complex and time-consuming process. Thus, the development of low-cost, large-scale, green manufacturing technology is essential for the practical application of nanophotonic-based ultra-sensitive biological sensing and detection devices [7]. As an additive manufacturing technology, printing is expected to have a great impact on preparing nanostructured devices, benefiting from its advantages of the low processing temperature, low energy consumption, and high material universality. Previous studies have reported that printed PC-based biochip can be used as the ordered and structured substrate to absorb the target sample for biological testing [8]. The fluorescence from the labels can be enhanced on the PC to improve the detection sensitivity and selectivity. However, the sample preparation, biological label injection and signal detection are still complex, which is hard to achieve the practical application for POCT [9].

To address these challenges, we developed an all-printed nanophotonic biochip via the fully integrated process, termed as the photonic crystal-fluorescence immunosorbent assay (PC-FLISA) chip for point-of-care testing of biomarkers. As the demonstration, the N-protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is chosen as the target of quantitative detection, which is an abundant and highly immunogenic protein among four major structural proteins of the coronavirus. Previous studies demonstrate that detecting N-protein in serum can be used as an early diagnostic marker for SARS-CoV-2 [10]. The 10-min quantitative detection of N-protein of SARS-CoV-2 is achieved on the printed nanophotonic biochip for the early detection. Printed nanophotonic arrays (3 mm × 3 mm) on a hydrophobic plastic substrate are self-assembled from nanospheres. The surface is full of carboxy groups, which is easy to be modified with anti-SARS-CoV-2 N-protein for specific recognition. PCs with ordered three-dimensional microporous structures also act as the hydrophilic sites for providing the capillary attraction to the trace target sample. The fluorescent probes are consecutively printed around the PC arrays, whose fluorescence wavelength locates at the PC stopbands for augmenting fluorescence intensity [11]. One drop (20 μL) of liquid sample, covering the PC array and probes, is sufficient to achieve the antigenic specification analysis. The value of N-protein can be directly calculated from the fluorescence intensities after establishing the standard curve of fluorescence intensity on PC-FLISA chip via N-protein concentration, which are of great significance for the diagnosis and investigation of COVID-19 [12].

We have designed a one-step PC-FLISA chip to simplify the detection process, which is convenient for users. The ink is prepared before the preparation of the PC-FLISA chip. Different biological materials are printed step by step by changing different needles (Fig. 1a). Firstly, the hydrophilic poly(styrene-methyl methacrylate-acrylic acid) spheres are printed on the hydrophobic polyethylene terephthalate (PET) substrate. Photography of PC arrays and the corresponding scanning electron microscope

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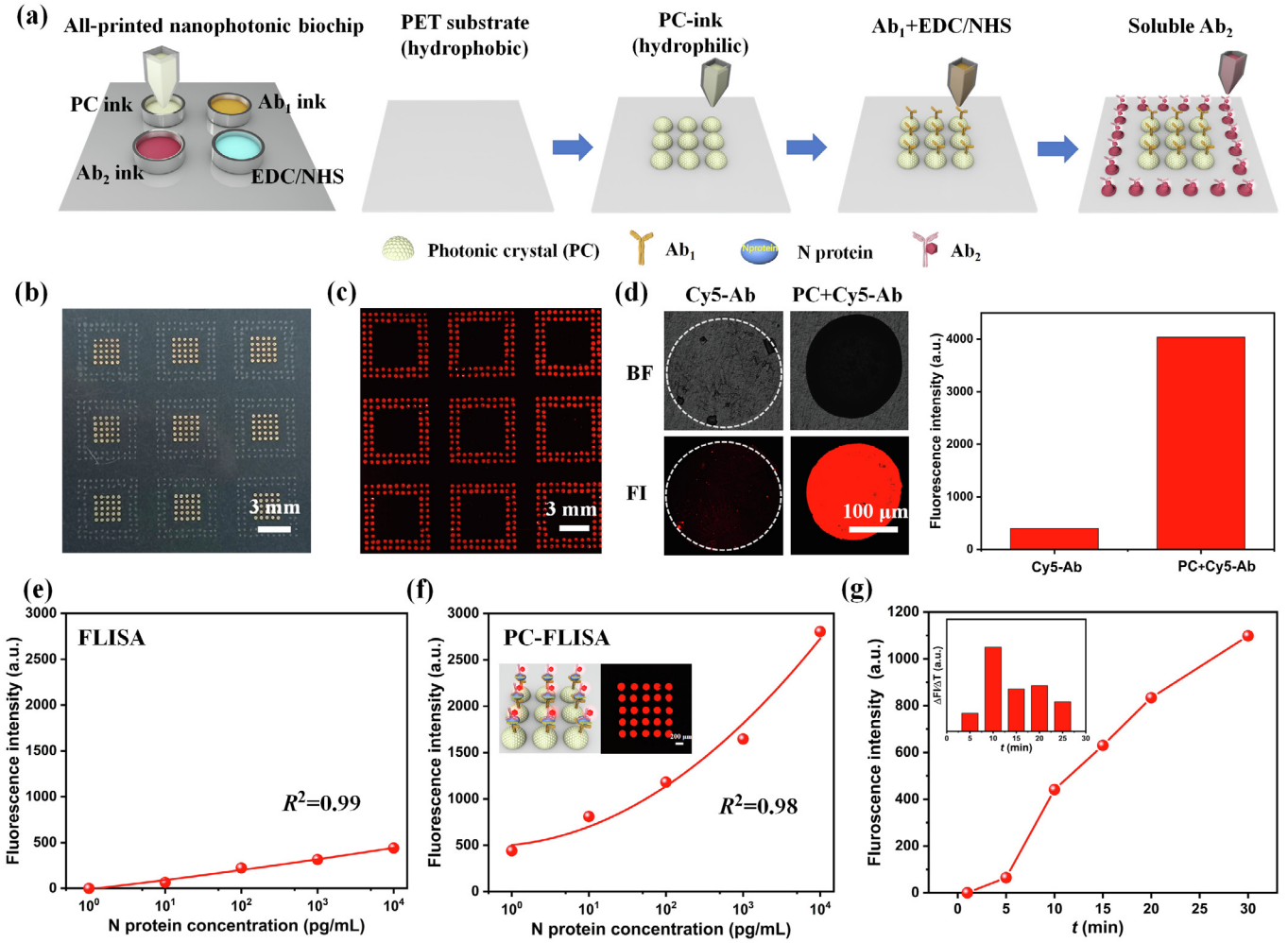

Fig. 1. Fabrication of the nanophotonic biochip for the diagnosis of biomarkers. (a) Schematic process of all-printed nanophotonic biochip. (b) Photography of the nanophotonic biochip with PC dots coupling capture antibody and soluble detection antibody dots. (c) Fluorescence imaging of nanophotonic biochip before the detection. (d) Fluorescence images and corresponding intensity analysis with Cy5-anti-SARS-CoV-2 (0.5 μL, 10 μg/mL) on PC dots and the blank PET substrate. (e) The standard curve of fluorescence intensity on FLISA chip vs. N-protein concenration. (f) The standard curve of fluorescence intensity on nanophotonic biochip vs. N-protein concenration. The inset shows the imaging of N-protein detected by one nanophotonic biochip unit. (g) The change of fluorescence intensity with the detection time for N-protein at 1 pg/mL. The inset shows the ratio of fluorescence change to time for N-protein (1 pg/mL) tested by the PC-FLISA chip.

(SEM) image of the PC periodic structure are shown in Fig. S1a and b (online). Secondly, the SARS-CoV-2 capture antibody named as Ab_1 is immobilized on the PC surface by the 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) coupling reaction. Thirdly, the SARS-CoV-2 detection reagent named as Ab_2 is inkjet-printed around the PC array. The detection reagent includes SARS-CoV-2 detection antibody with Cy5 fluorescent tags and trehalose. The presence of trehalose can make the detection antibody with Cy5 fluorescent tags arrays rapidly dissolved during the detection. Finally, a PC-FLISA chip can be prepared via the all-printing process. One drop (20 μL) of blood sample is sufficient to achieve antigenic specification analysis and POCT detection, which benefits from the hydrodynamic behavior of droplet on the hydrophilic PC array and the relative hydrophobic substrate. The contact angle (CA) of the PCs dot is 20° ± 4°, whereas the CA of PET substrate is 80° ± 3° (Fig. S1c online). When the blood sample dropped onto the PC-FLISA chip, it can quickly dissolve the Ab_2. The SARS-CoV-2 Ab_1 recognizes and captures N-protein by the antibody-antigen specific binding function. Next, the SARS-CoV-2 Ab_1 immobilized on the PC array captures the SARS-CoV-2 Ab_2 bound to N-protein to form the double-antibody sandwich immunoassay detection. The PCs amplify the fluorescence signal, which improve the detection sensitivity and achieve POCT detection. Fluorescence images are displayed before and after PC-FLISA chip detection (Fig. 1b and c). According to the emission wavelength range of Cy5-anti-SARS-CoV-2, PCs with the particle size of 300 nm are selected to match the photonic band gap position. Cy5-anti-SARS-CoV-2 is added to the PC_{300} dots and blank PET substrate, respectively. After fully dried, the fluorescence imaging and fluorescence intensity are measured via the confocal microscope (Fig. 1d). Conventional fluorescence immunosorbent assay (FLISA) involves a standard sandwich immunoassay, whose capture antibody is immobilized on the substrate after the surface-functionalization. The recognition and capture of N-protein followed by the binding of detection antibody will report the fluorescence. To ensure the sensitivity and limit-of-detection (LOD) of PC-FLISA, serial dilutions of N-protein (1 pg/mL to 10 ng/mL) are used as the standards to be tested. After reacting, the PC-FLISA chip is washed away by the deionized water to avoid the nonspecific adsorption. The fluorescence imaging on the photonic crystal and the fluorescence intensity values are shown in Fig. 1f. Signals from the PC-FLISA chip reveal nearly 10-fold fluorescence enhancement compared with conventional FLISA (Fig. 1e). The LOD of the N-protein with the PC-FLISA chip is found

to be around 1 pg/mL, while the FLISA reports extremely weak fluorescent signal (Table S1 online). The one-to-one relationship of the PC-FLISA chip between fluorescence intensity and concentration was established, which can achieve the N-protein detection. Further, we optimize the detection time for the LOD of 1 pg/mL N-protein (Table S2 online). When the detection time is 5 min, the fluorescence signal of N-protein is collected (Fig. 1g). Further, we have calculated the ratio of fluorescence change to time. It is shown that the fluorescence change is the maximum after 10 min, which indicates that the N-protein detection performance in 10 min is sufficient.

The all-printed nanophotonic biochip provides an effective technical platform by combining the manufacturing capacity of large-scale printing and the simplicity of photonic crystal-based fluorescence enhancement [13–15]. Specific identification and quantitate detection of biomarkers are achieved. The present study still has some limitations: (1) the biometric recognition relying on the double antibody methods which need the fluorescence labeled antibody and recognizing antibody immobilized on the nanophotonic structure; (2) a novel operation protocol for one droplet of sample detection insteads of the immunochromatographic assay; (3) the portable test system is needed including the mini device and processing software compatible with mobile phones; (4) the practicality and efficiency of printed biochips should be verified on the detection of multiple biomarkers for practical applications.

In summary, we have demonstrated an all-printed nanophotonic biochip, combining with the characteristics of integrated chip and photonic crystal nanostructure-enhanced fluorescence, for point-of-care testing of N-protein of SARS-CoV-2 in 10 min. Moreover, this printed biochip can be expanded as a general and easy-to-use approach for fast quantitative detection of various biomarkers. In the future, with the further exploration of nanophotonic structure and theory, and the continuous improvement of printing technology, nanophotonic biochip will be developed into a portable, low-cost, and efficient method for point-of-care testing of biomarkers in the trace sample.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary materials

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