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Design and Fabrication of Ultra-Large Surface Area Self-Driven Microfluidic Chips: Postprint

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Abstract

Objective: To construct a self-driven microfluidic chip for on-site detection featuring simple operation, rapid detection, and high sensitivity by utilizing novel nano-forest materials.

Methods: Quartz nano-forest structured microchannels with excellent optical properties and large surface area were fabricated using MEMS fabrication technology. The height, width/lateral dimension, density, surface area, optical properties, capillary driving effect, and fluorescence enhancement effect of the nano-forest structure were characterized. Ricin toxin detection was performed using a double-antibody sandwich method.

Results: The nanofiber cone base diameter was approximately 200-300 nm, with a height of approximately 1.0 m. The nano-forest density was approximately 10 per m^2, and the estimated surface area to base area ratio exceeded 5:1. The light transmittance at a wavelength of 680 nm reached 89.5%, and the driving flow velocity was approximately 5 mm/s. Compared with planar structures, the saturated fluorescence intensity increased several-fold. The detection limit for ricin toxin was lower than 10 pg/ml, with a good linear relationship within the range of 10-6250 pg/ml.

Conclusion: Based on the nano-forest structure, a capillary self-driven microfluidic chip with ultra-large surface area and high sensitivity was successfully constructed.

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Full Text

Design and Fabrication of a Self-Driven Microfluidic Chip with Ultra-Large Surface Area

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Abstract

Objective: To develop a simple, rapid, and highly sensitive self-driven microfluidic chip for point-of-care testing using a novel nano-forest material. **Methods:** Microfluidic channels with excellent optical properties and large surface area were fabricated on quartz substrates using MEMS processing technology. The height, width/lateral dimensions, density, surface area, optical properties, capillary driving effect, and fluorescence enhancement of the nano-forest structures were evaluated. Ricin toxin detection was performed using a double-antibody sandwich method. **Results:** The nanofiber cones exhibited base diameters of approximately 200–300 nm, heights of about 1.0 μm, and a nano-forest density of approximately 10 per μm^2, yielding an estimated surface-to-base area ratio exceeding 5:1. The transmittance at 680 nm wavelength reached 89.5%, with a driving flow velocity of approximately 5 mm/s. Compared with planar structures, the saturated fluorescence intensity increased several-fold. The limit of detection for ricin toxin was below 10 pg/mL, with good linearity in the range of 10–6250 pg/mL. **Conclusion:** A capillary self-driven microfluidic chip with ultra-large surface area and high sensitivity was successfully constructed based on nano-forest structures.

Keywords: Nano-forest; Microfluidic chip; Point-of-care test

Introduction

Immunochromatographic test strips, developed in the 1980s, represent a simple and convenient rapid detection technology. This method stores detection probes —such as colloidal gold, colloidal selenium, or colored latex microspheres labeled with antibodies or antigens—within glass fiber membranes. When liquid samples migrate via capillary action through the sample pad, probe-containing glass fiber membrane, nitrocellulose membrane with immobilized ligands for target molecules, and absorbent pad, corresponding target molecules are captured and visualized on the nitrocellulose membrane. After decades of development, this technology has gained widespread application in point-of-care testing due to its simple preparation, extremely low cost, and ease of operation [1]. However,

because immunochromatographic test strips rely on nitrocellulose membrane-based detection, color development only occurs on the opaque membrane surface, while captured probes within the membrane interior remain invisible. This results in significant loss of detection signals and consequently low sensitivity, precluding precise quantitative analysis of target molecules.

To address these limitations, microfluidic chip technology has attracted increasing attention for point-of-care testing applications. Microfluidic chips consist of microchannel networks etched using Micro-Electro-Mechanical Systems (MEMS) processing technology, with controlled fluid flow throughout the system to realize various functions of conventional chemical or biological laboratories, thereby achieving miniaturization, portability, automation, integration, low cost, high throughput, and simple, rapid, efficient analysis [2, 3]. Compared with traditional detection methods, microfluidic chips enable precise control of sample and reagent volumes and flow rates, yielding high-precision and high-sensitivity analyte separation and detection [4]. Additionally, microfluidic chips can shorten reaction times, improve analytical efficiency, conserve reagents and samples, and facilitate integration, portability, simplified operation, and automation [5]. Although conventional microfluidic detection systems feature compact chips, they often require complex auxiliary equipment, resulting in cumbersome procedures and high costs that severely limit their application in point-of-care testing [6].

To overcome these challenges, this study utilized mature MEMS processing technology to design and fabricate a capillary self-driven microfluidic chip with ultra-large surface area and high sensitivity, using quartz substrate nano-forest structures as microfluidic channels combined with optical detection methods.

Materials and Methods

Materials and Instruments

Quartz glass (Shanghai Gaishi Optoelectronics), polyimide resin (Beijing Bomei Technology), N-methylpyrrolidone (Jiangyin Jianghua Microelectronics Materials), JAX3038 positive photoresist developer (Jiangyin Chemical Reagent Factory), hot plate (PHP-8, Suzhou Meitu Semiconductor Technology), spin coater (RC-150, Suzhou Meitu Semiconductor Technology), March photoresist stripper (A Nordson Company), reactive ion etcher (Tegal Plasma 903e), six-inch double-sided alignment stepper (SUSS MA6/BA6), Quanta400FEG thermal field emission environmental scanning electron microscope, UV-visible spectrophotometer (Hitachi U-4100), Odyssey infrared fluorescence imaging system (LI-COR, USA), Biodot AD1510 microarray spotting system, 3-aminopropyltrimethoxysilane (APTES) (Sigma, USA), 50% glutaraldehyde (Sinopharm), ricin toxin (self-prepared), ricin polyclonal antibody (self-prepared), goat anti-rabbit IgG (Beijing Dingguo Changsheng Biotechnology), and LinKine AbFluor 680 Labeling Kit (Abbkine, USA).

Microfluidic Chip Design

The microfluidic chip primarily consists of a sample pad, conjugate pad, nano-forest substrate, absorbent material, and PMMA base plate, as illustrated in [Figure 1: see original paper]. Both the sample pad and conjugate pad are glass fiber membranes. The nano-forest substrate is a microfluidic channel with internal nano-forest structures, with both detection and control zones located within the microchannel.

Nano-Forest Substrate Fabrication

The fabrication process for nano-forest structures on quartz substrates is shown in [Figure 2: see original paper]. The procedure includes: cleaning and drying of the quartz substrate; dehydration baking and hexamethyldisilazane (HMDS) vapor priming; spin-coating a 5 μm-thick polyimide (PI) layer; spin-coating a 6 μm-thick phenolic resin photoresist on the PI layer; photolithographic patterning to expose the channel region; bombardment of the patterned PI layer with oxygen and argon plasma to form nanofiber forest structures (oxygen plasma: 200 sccm, 20 min; argon plasma: 150 sccm, 40 min; constant chamber pressure and power at 80 mTorr and 400 W throughout); anisotropic quartz etching via reactive ion etching (RIE) using the nanofiber forest as a mask (SF /CHF /He: 5.5/32/150 sccm, 200 W, 1850 mTorr, 15 min) to form nanofiber-quartz nano-forests; removal of the upper nanofiber forest structure and photoresist coating to obtain microchannels with internal nano-forest structures [7-9].

Nano-Forest Structure Characterization

The nano-forest structures were observed using a Quanta400FEG thermal field emission environmental scanning electron microscope to measure height, width, density, and specific surface area. Optical properties were tested using a Hitachi U-4100 UV-visible spectrophotometer. The driving force was characterized by measuring PBS solution flow velocity in a 1 mm-wide, 40 mm-long channel inclined at 45°.

Surface Modification and Fluorescence Testing

Quartz nano-forest substrates were cleaned in a 2:1 H SO :H O solution for 1 hour, followed by ultrasonic cleaning in deionized water for 5 minutes. Cleaned chips were immersed in 3-aminopropyltrimethoxysilane (APTES) solution (2% v/v in acetone) for 20 minutes, then rinsed with acetone, ethanol, and deionized water, and dried with nitrogen. The aminated chips were subsequently immersed in glutaraldehyde solution (5% v/v in PBS, pH 7.4) for 2 hours, rinsed with PBS and deionized water, and nitrogen-dried. Rabbit anti-ricin antibody (0.5 μL, 1 mg/mL) was spotted onto both nano-forest and planar quartz structures and incubated overnight at 4°C in a humid chamber. After thorough washing with 1% Tween-20 in PBS and nitrogen drying, chips were blocked with 3% bovine

serum albumin in PBS at 37°C for 1 hour. AbFluor 680-labeled goat anti-rabbit antibody (0.5 mL, 1 mg/mL, 500-fold dilution) was added and incubated at room temperature for 15 minutes in the dark. Following washing with 1% Tween-20 in PBS and nitrogen drying, fluorescence was read using the Odyssey infrared imaging system.

Ricin Toxin Detection

Substrate preparation followed the method described in Section 1.2.4. Ricin toxin antibodies were spotted onto the detection line and goat anti-rabbit IgG onto the control line using a Biodot AD1510 microarray spotting system. Secondary antibodies were labeled according to the LinKine AbFluor 680 kit protocol and immobilized on glass fiber membranes. After assembling the microfluidic chip, 100 µL of various ricin toxin concentrations were added and reacted at room temperature for 15 minutes before fluorescence imaging with the Odyssey system.

Results

Microfluidic Chip Assembly

The sample pad and glass fiber membrane with immobilized fluorescently labeled antibodies were cut into the shape shown in [Figure 3: see original paper] and adhered to the sample loading area. Corresponding antibodies were immobilized in the detection and control zones, nitrocellulose membrane was placed at the distal end of the nano-forest substrate, and absorbent material was attached or clamped at the terminal end to complete the microfluidic detection chip.

Characterization Results

Scanning Electron Microscopy SEM results of the nano-forest structures are presented in [Figure 4: see original paper]. After oxygen and argon plasma bombardment, nanofiber forest structures formed on the quartz substrate, with individual nanofibers standing upright, exhibiting diameters of approximately 50–100 nm, heights of about 1.8 µm, and densities of approximately 20 per μm^2 [FIGURE:4(a)]. Using the nanofiber forest as a mask, RIE etching produced dumbbell-shaped nanofiber-quartz nano-forest structures; the etching endpoint was reached when the upper nanofibers began to collapse [FIGURE:4(b)]. After resist removal, uniformly distributed quartz nano-forest structures were obtained with cone base diameters of approximately 200–300 nm, heights of about 1.0 µm, densities of approximately 10 per μm^2, and an estimated surface-to-base area ratio exceeding 5:1 [FIGURE:4(c).

Dry stripping using a March photoresist stripper for 1.5 hours (O flow: 200 sccm, power: 400 W, pressure: 80 mTorr) left substantial nanofiber residue. In wet etching methods, 49% KOH at room temperature for 10 minutes completely removed nanofibers without residue. H SO and H O solution (2:1 ratio) at

room temperature for 10 minutes left partial nanofiber residue visible by SEM. BOE (7:1) solution rinse for 10 seconds removed all nanofibers but corroded the quartz nano-forest structures, reducing pattern fidelity [Figure 5: see original paper].

By adjusting PI coating thickness, oxygen/argon plasma bombardment parameters, and RIE etching time, various nano-forest structures were obtained. Comparing different PI thicknesses and plasma bombardment durations revealed that the structures shown in [FIGURE:6(c) and (h) exhibited optimal density, uniformity, and surface-to-base area ratios exceeding 5:1.

Driving Force Characterization Flow velocity tests using PBS in channels with different nano-forest structures revealed two outcomes: flow or no flow, with flowing structures demonstrating rapid velocities. Considering height, density, and uniformity, the nano-forest structure in [FIGURE:6(h) was selected as the chip channel. As shown in [Figure 7: see original paper], PBS traveled from one channel end to the other within 8 seconds, achieving a flow velocity of approximately 5 mm/s.

Optical Properties The quartz-based nano-forest structures exhibited excellent optical transmittance of 89.5% at 680 nm wavelength. This high transmittance minimizes excitation and emission light loss, facilitating signal capture by the detector and improving detection sensitivity [Figure 8: see original paper].

Fluorescence Enhancement Characteristics Under identical surface modification conditions, planar quartz structures suffered from short-lived surface modification effects and low saturated fluorescence intensity. In contrast, quartz nano-forest structures with ultra-large specific surface area demonstrated significant sensitization effects in saturated fluorescence tests, indicating excellent potential for optical detection applications [Figure 9: see original paper].

Ricin Toxin Detection Results The ultra-large surface area of nano-forests provides abundant binding sites for capture antibodies, enabling increased antigen binding and enhanced sensitivity. Ricin toxin concentrations of 10, 50, 250, 1250, and 6250 pg/mL were tested, with 3% BSA as control. As shown in [Figure 10: see original paper], fluorescence intensity increased with ricin concentration, exhibiting good linearity from 10–6250 pg/mL and a limit of detection below 10 pg/mL.

Discussion

The excellent optical properties of nano-forest structures reduce instrument design requirements and expand fluorescent dye options. The three-dimensional architecture provides ultra-large surface area, dramatically increasing antibody binding capacity compared with planar structures and thereby substantially improving detection sensitivity [10]. Unlike immunochromatographic test strips

that suffer significant signal loss, this microfluidic chip achieves high sensitivity and enables quantitative analyte analysis within a specific range [11]. Furthermore, most microfluidic chips require complex external driving equipment, increasing cost and size. In contrast, the capillary-driven nano-forest chip eliminates bulky and expensive pumping systems, simplifying and accelerating detection. Combined with miniaturized detection terminals, this platform enables miniaturization, portability, and automation for simple, rapid, and efficient analysis, making it ideal for field applications [12].

Quartz material offers advantages including hydrophilicity, facile surface modification, and robust nanofibers. In nano-forest fabrication, nanofiber forest morphology and RIE etching parameters critically influence the final quartz nano-forest structures. Better vertical alignment and stronger substrate adhesion of individual nanofibers enhance masking effectiveness during RIE, improving pattern fidelity. However, nanofiber collapse or clustering during etching can cause quartz fiber agglomeration and suboptimal separation. RIE process effects primarily manifest in etch factor and selectivity—higher values in both parameters yield greater quartz fiber fidelity [13].

Both nanofibers and photoresist cannot withstand subsequent surface treatment processes and must be removed. Among four stripping methods evaluated, dry stripping proved ineffective, time-consuming, and costly; BOE solution etching corroded quartz structures and reduced fidelity; H SO /H O solution required long processing times and risked carbonization of organics; while 49% KOH immersion at room temperature for 10 minutes provided optimal resist removal with high pattern fidelity, simplicity, and low cost.

The nano-forest channels exhibited rapid, difficult-to-control flow velocities with complex structure-dependent effects. Therefore, during chip assembly, nitrocellulose membrane was placed at the channel terminus as a flow-limiting barrier to prolong liquid residence time in the nano-forest channel and ensure adequate antigen-antibody reaction.

The nano-forest-based microfluidic chip demonstrated high sensitivity for ricin detection with a limit of detection below 10 pg/mL, substantially surpassing conventional colloidal gold immunochromatographic test strips [14, 15]. These results indicate promising applications in biomedical diagnostics.

This study fabricated nano-forest channels using standard MEMS processing technology and designed a microfluidic chip combining optical detection. The simple architecture, operational convenience, and high sensitivity overcome the limitations of immunochromatographic strips—such as poor signal uniformity and quantitative difficulty—as well as the complexity and high cost of other microfluidic chips, establishing a solid foundation for future research and product development.

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