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Superwettatable Electrochemical Biosensor toward Detection of Cancer Biomarkers

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Supporting Information Placeholder

ABSTRACT: Bioinspired superwettatable micropatterns that combine two extreme states of superhydrophobicity and superhydrophilicity with the ability of enrichment and absorbing microdroplet are suitable for versatile and robust sensing applications. Here we introduce a superwettatable microchip that integrates superhydrophobic-superhydrophilic micropatterns and nanodendritic electrochemical biosensor toward detection of prostate cancer biomarkers. On the superwettatable microchip, the superhydrophobic area could confine the microdroplets in superhydrophilic microchannels, such behavior is extremely helpful for reducing the amount of analytical solution. In contrast, superhydrophilic microwells exhibit a high adhesive force toward microdroplets, and the nanodendritic structures can improve probe-binding capacity and the response signals, thus greatly enhancing the sensitivity. Sensitive and selective detection of prostate cancer biomarkers including miRNA-375, miRNA-141 and prostate-specific antigen on single microchip is also achieved. Such superwettatable microchip with high sensitivity, low sample volume and upside-down detection capability in a single microdroplet, shows great potential to fabricate portable devices toward complex biosensing applications.

Inspired by extreme states of solid surface wettability in nature, considerable efforts have been devoted to the development of materials with superwettatable properties for diverse applications.1-7 Recently, superwettatable materials have been proved to have outstanding capacity in controlling liquid droplets, such as their directional motion on anisotropic wettable surface and droplets transferring between surfaces.8-10 Importantly, superwettatable micropatterns that combine two extreme states of superhydrophobicity and superhydrophilicity in precisely two-dimensional micropatterns,11-14 exhibit excellent ability of patterning microdroplets toward new possibilities and functionalities in a wide variety of biomedical applications including high-throughput cell patterns.15-18 Drug/cell screening,19-21 and open-channel biochips for separation and diagnostic devices.22-25 Taking advantage of enrichment and anchoring microdroplet ability of such superwettatable microchips, recent efforts have shifted to modify the superhydrophilic microwells with biological entities, toward more versatile and robust sensing applications. As a result, several types of superwettatable microchips have been explored depending on different detection signals such as surface-enhanced Raman scattering (SERS),26-28 colorimetric,29-33 and fluorescence enhancement effect,34-36 to perform demanding sensing tasks.37-39

Electrochemical biosensors, as one of the most common methods of analytical research, have been widely used to sensitively detect biomolecules,40-42 such as glucose,44 nitric oxide,45 hydrogen peroxide,46 hydrogen sulfide,47 DNA,48-50 miRNA and proteins.51 However, electrochemical biosensing are usually carried out in a large solution system. Here we demonstrate a superwettatable electrochemical biosensor that integrates superwettatable substrate for managing microdroplets with conductive nanodendritic electrochemical biosensor for sensitive measurement, toward detection of multiple prostate cancer biomarkers including miRNA-375, miRNA-141 and prostate-specific antigen. Such superwettatable electrochemical biosensor bridges the gap between sensitive electrochemical biosensing and superwettatable droplet management approach, showing great potential in highly sensitive and low-sample-volume detection within a single microdroplet.

Combining electrochemical deposition and template O2 plasma etching technology, superwettatable electrochemical sensors with patterned superhydrophobic-superhydrophilic microarrays were fabricated as detailed in Fig. 1. Firstly, the nanodendritic gold substrate was fabricated according to previous literatures as following steps (Fig. 1a).52, 53 Indium tin oxide (ITO) substrate was cut into small rectangular pieces (1 cm × 2 cm), and ultrasonically cleaned for 10 min in deionized water, acetone, ammonia aqueous, and deionized water sequentially, to remove the organic matters and then dried with a flow of nitrogen. Before electrochemical deposition, a titanium layer and a gold film were sputtered on the conductive side of the ITO substrate to serve as a working electrode for electrochemical deposition processes. Ag/AgCl wire and Pt wire were employed as reference and counter electrodes, respectively. All electrochemical deposition steps were carried out at room temperature (25 °C). Gold nanodendritic structures were then electrodeposited at −1.8 V for 1800 s in an electrolyte composed of HAuCl4 (1 mg/mL) and sulfuric acid (0.5 M). After electrochemical deposition, the superhydrophobic-superhydrophilic micropatterns were prepared as shown in Fig. 1b. Firstly, the dendritic gold nanostructure
Figure 1: Design of superwettable electrochemical microchip towards biosensing. a) Fabrication of nanodendritic gold substrate including vapor deposition of Ti/Au thin film and electrochemical deposition gold nanodendrites. b) Modification of nanodendritic gold substrate to achieve conductive superhydrophobic-superhydrophilic surface. c) Schematic of electrochemical detection of analyte in droplets on the superwettable microchip was treated with oxygen plasma at 100 W for 180 s for removing the organic matters, and then immersed in a dodecanethiol solution (10 vol. % in ethanol) for 24 h at room temperature. The dodecanethiol-modified substrate was cleaned by rinsing in ethanol and ultrapure water, respectively. Oxygen plasma at 100 W was used to irradiate the dodecanethiol-modified substrate through a photomask for 120 s. The non-irradiated region remained superhydrophobic. In contrast, the oxygen plasma irradiated regions became superhydrophilic. Thus, we prepared the superwettable electrochemical biosensor.

The superwettable microchip integrate conductive gold superwettable substrate with electrochemical biosensors, showing great ability of holding microdroplets toward biosensing as shown in Fig 1c. Compared to flat glass without any modifications, the superhydrophilic microwells with nanodendritic structures exhibit a larger adhesive force to hold water microdroplets due to the capillary force of each individual nanostructure. As a result, microdroplets could be captured in the microwell against gravity.\(^\text{32}\) In contrast, the dodecanethiol-modified superhydrophobic area prevents water adhering on surface and confines the microdroplets in superhydrophilic microwells, greatly reducing the amount of analytical solution. In addition, due to the large surface area, multi-directionality and 3D nanostructure of the nanodendritic electrode surface, the probe-binding capacity and the response signals of the sensor are also greatly improved (SI Fig. 2).

The gold electrode fabricated by electrochemical deposition method was confirmed by SEM images as illustrated in Fig. 2a. The as-prepared electrode exhibited a highly branched structure (about 10-100 nm), the height of these branched structure is up to about 10 μm (SI Fig. 1). Fig. 2b illustrates the contact angle of gold nanodendrites with or without dodecanethiol modification. Without any modification, the contact angle of gold nanodendrites is about 0° (Fig. 2b), while the contact angle of dodecanethiol-modified gold nanodendrites is 151.9 ± 1.6°. Methyl blue labelled microdroplets on the as-prepared substrate present two diametrically opposed appearances as shown in Fig. 2c. On the dodecanethiol-modified surface, microdroplet is almost spherical and indicates its superhydrophobicity. In contrast, microdroplet is flattened in microwells, revealing its superhydrophilicity. In addition, we also evaluated the wettability of bare Au before and after the modification of dodecanethiol (Fig. 2d). For Au substrate with or without dodecanethiol modification, the contact angles are 58.6 ± 2.5° and 98.4 ± 2.0° respectively, which shows a relatively small differences.

Figure 2: Physical characterization of the superwettable electrochemical microchip. a) SEM images of the gold nanodendrites. b) Microscope images of water contact angle of dodecanethiol modified gold nanodendrites (left) and after O\(_2\) plasma etching (right). c) Methyl blue labelled water microdroplets behavior on the thiol-modified superhydrophobic gold nanodendrites and on superhydrophilic microwells. d) Comparison of the contact angles of bare Au and Au nanodendrites before and after the modification of dodecanethiol.

To further evaluate feasibility and versatility of superwettable electrochemical microchip as a platform toward practical applications, three key parameters closely related to the performance of our superwettable electrochemical microchips including size of droplet, rotation angle of microchip and size of microwell, were chosen to illustrate the ability of performing biodetection.
Figure 3: Effect of the microdroplets size, tilting angle and microwell size on electrochemical redox reactions. a) Cyclic voltammetry curves (Scan rate: 100 mV/s) of superwettable electrode microwells (diameter: 2 mm) with microdroplets volumes of 1, 4, 8, and 16 µl, respectively. b) Rotate the superwettable electrochemical microchip with an angle of 0°, 45°, 90°, 180° to confirm their stability during the detection processes. c) Effect of microwell size of 0.5, 1.0, 1.5, and 2.0 mm on electrochemical detection. All the measurements were processed in 0.01 M phosphate buffered solution (pH 7.4) containing 0.1 M KCl and 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆].

Fig. 3a illustrates the capability of our superwettable electrode microwells toward electrochemical measurement with different microdroplet volumes. Briefly, microdroplets volumes of 1, 4, 8, and 16 µl were then carefully added in the microwells (Diameter: 2 mm). Due to the superhydrophobic propriety of surrounding area, microdroplets were confined in superhydrophilic microwells and present almost spherical when increasing droplet volume as shown in the optical images. The electrochemical measurement was carried out in 0.01 M phosphate buffered saline (pH = 7.4) containing 0.1 M KCl and 5 mM potassium ferricyanide/ferricyanide. Cyclic voltammetry results (Scan rate: 100 mV/s) of microdroplets with of 1, 4, 8, and 16 µl are just subtly different, revealing the stability of such superwettable microchip toward biodetection regardless of the droplet size. Fig. 3b demonstrates the adhesive property to microdroplet of such superwettable microchip to confirm its capability toward upside-down measurement during the detection process. In brief, the superwettable electrochemical microchip with a microwell of 2 mm was firstly horizontally placed, 5 µl microdroplet which contained 0.01 M phosphate buffered saline and 0.1 M potassium ferricyanide/ferricyanide were then carefully added in the microwells (0°). When the superwettable electrochemical microchip are rotated with angles of 45°, 90°, and 180°, the water droplets can be anchored at the microwells without dropping down due to the large capillary force of such superhydrophilic microwell as demonstrated in optical images. Cyclic voltammetry curves are almost the same with different tilting angles, revealing the stability of such superwettable microchip. Such non-angle dependent electrochemical device shows great potential in fabricating portable device. In Fig. 3c, the influence of the microwell size on the electrochemical response of superwettable microchip was demonstrated. The electrochemical measurement was carried out in the reversed microchip, which is much more conducive to observe. 5 µl microdroplet (0.01 M phosphate buffered saline and 0.1 M potassium ferricyanide/ferricyanide) were carefully added in the reversed microwells. With the microwell sizes decreasing from 2.0 mm to 0.5 mm, the microwells keeps anchoring microdroplets as shown in optic images. However, the contact area between microdroplet and the microwell has a significant influence on probe-binding capacity and the response signals, in which the signal decrease as the contact area decreases.

For electrochemical detection of miRNA and protein, a two-electrode system where Ag/AgCl acts as both reference and counter electrode is chosen to simplify design and suit the small size of microdroplets, which is a common strategy for low-current and low-volume electrochemical sensing. Two kinds of typical prostate cancer biomarkers including miRNAs (miRNA-141 and miRNA-375, 55, 56) and prostate-specific antigen (PSA), 57 were chosen to illustrate the ability of performing biodetection to further evaluate its feasibility and versatility as a platform toward sensitive biosensing as demonstrated in Fig. 4a. Such multivariate analysis of the PSA and miRNA levels are able to enhance the performance in detecting prostate cancer.
The mechanism of superwettable microchip for electrochemical miRNA sensing is illustrated in Fig. 4b. The miRNA sensors are comprised of an electrode-bound, redox-reporter-modified DNA probe (purple-blue-purple ribbon) that undergoes a conformational change on binding to the target miRNA (bluish green ribbon). This conformational change alters the positioning of the reporter (Fc) relative to the electrode surface, thereby producing a target-dependent change in current when the sensor is interrogated by differential pulse voltammetry (DPV). The detailed detection processes were carried out at constant temperature and saturated humidity as follows: a 5 µL water droplet with 10.0 µM DNA probe was dropped in a superhydrophilic microwell (Diameter: 2 mm), and incubated at room temperature for probe DNA self-assembly and automatically ringing on nanodendritic gold surface. Prior to measurement, the microchip was immersed in ultrapure water to remove unfixed DNA. Then, 5 µL water microdroplet (pH 7.0) contains 140 mM NaCl, 100 mM NaClO₄, and target miRNA concentrations of 0 M, 10 nM, 100 nM, 1 µM and 10 µM were dropped in microwells for 30 min to ensure the sufficient hybridization of DNA probe and target miRNA. The detection of miRNA-141 and miRNA-375 was performed by DPV (voltage varies from -0.0 V to +0.14 V) as demonstrated in Fig. 4d and 4e, respectively. The response signals decrease as the concentration of target miRNA increase. A linear plot of the signal peak point (0.08 V) versus concentration of target protein is ranging from 0.014 V to concentration of target protein is ranging from 0.014 V to 10 pm to 10 nM with a detection limit of 1.0 pM.

To evaluate the practical application and the selectivity of the biosensor, the goat serum (containing various proteins, 1:10 diluted with 0.1 M PBS) was used as electrolyte to detect the PSA and miRNA (Detailed in supporting information). Such results revealed the potential of this superwettable biosensor toward practical application.

In conclusion, the superwettable microchip sensor described in this study represents a hybrid system that integrates superwettable substrate for microdroplets management with nanodendritic gold electrochemical biosensor for sensitive measurement into single microdroplet detection platform, which overcome limitations of conventional electrochemical sensors. Such microchip is realized via electrochemical deposition for nanodendritic gold structure, dodecanethiol modification for superhydrophobic surface, and template O₂ plasma etching for superhydrophilic microwells. Microliter of analytical solution can be utilized during detection processes due to the superhydrophobicity of the surface. The conductive nanodendritic structures in superhydrophilic microwells can effectively enhance the sensitivity and exhibit a high adhesive property. Such capability allows the superwettable microchip as a biosensing platform in sensitive detection of routine prostate cancer biomarkers including miRNA-375, miRNA-141 and prostate-specific antigen (PSA) (Fig. 4f).
specific antigen. Such superwettability microchip represents a great opportunity to manipulate microdroplets toward precise disease detection based on multivariate analysis on a single microchip.

ASSOCIATED CONTENT

Supporting Information

Experimental details, oligonucleotides sequences, electrochemical performance comparison of nanodendritic Au and bare Au electrode, additional figures. This material is available free of charge on the ACS Publications website via the Internet at http://pubs.acs.org.

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