A low cost and flexible carbon nanotube pH sensor fabricated using aerosol jet technology for live cell applications

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ABSTRACT

Minute pH changes impact all living organisms, and thus facile pH monitoring in environment and on the cellular level is important. A low cost and flexible carbon nanotube based pH sensor is fabricated in this work using Aerosol jet printing technique. The chemiresistive pH sensor is fabricated with a CNT-based miniaturized serpentine sensing element printed on top of the silver electrodes. The trace width of the serpentine sensing element is accurately controlled to about the same size as the gaps between the traces. Aerosol jet parameters are optimized to achieve high resolution printing of 20 μm. The fabricated pH sensor shows good sensitivity (up to 59 kΩ/pH) and repeatability (coefficient of variance <1.15%) with a response time of 20 s the sensor demonstrates excellent biocompatibility required for live cell applications.

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1. Introduction

Detection and control of pH is important for many environmental, biological and chemical processes that impact human lives [1]. Water and food quality is judged by the change in the pH value. The pH level in a chronic wound bed is a key indicative parameter for assessment of the healing progress [2]. Many kinds of pH sensors exist such as potentiometric [3,4], capacitive [5], chemiresistive [6], luminescence [7,8], optical [9], and shape/mass [10,11]. Different pH-sensitive materials such as metal oxides for solid state electrodes [12]; epitaxial graphene for solution-gated electrode [5]; gold-coated polymer for dimension-changing materials [13]; polymers that change their optical properties [9] have been employed and tested. Commonly, pH sensing is done by using glass electrodes [14] and ion-selective field-effect transistor (ISFET) [15]. However, these pH sensing techniques require a reference electrode, which in turn restricts the miniaturization of the device; and also suffer from leakage of electrolyte [14,15]. Chemiresistive pH sensors are the most popular amongst all as they have the simplest design and are thus easy to fabricate. Chemiresistive sensors are two-terminal microsensors, which work without a reference electrode and can detect many analytes like NOx, O2, NH3, SO2, O3, hydrocarbons, or CO2 [16]. For pH sensing, carbon nanotubes (CNTs) are the most suitable material due to their good mechanical reliability, high surface-to-volume ratio, ease of chemical functionalization, and tunable electrical properties [17]. CNT-based pH sensors are explored through unpatterned CNT sheet [18] and by drop casting [19] method. However, unpatterned CNT films increase the overall sensor dimensions and drop casting processes are not reproducible [20], thus making patterning of CNT films an important research area.

Printing techniques have been widely explored for patterning of materials especially CNTs. Conventionally, CNT-based electronics are fabricated using screen printing [21,22], and gravure printing [23,24] through the use of homogeneous paste-like ink. Transfer-printing [25], inkjet printing [26] and photolithography [27] have also been widely researched for printed electronics applications. However, most of these printing techniques are limited to flat surfaces and provide moderate resolution [28]. New age printed electronic applications put additional demands on design architectures and printing parameters, and need to be met through new technique [29]. Aerosol jet printing is an additive manufacturing technique, which has recently gained interest. It’s 5-axis and non-contact capability with substrate-to-nozzle standoff distance between 1 and 5 mm provides freedom to fabricate novel designs and explore complicated designs on arbitrary surfaces [30]. Aerosol jet offers high resolution and conformal printing [31].

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Herein, we report fabrication of flexible pH sensor based on CNT material. Some research has been done to print nanoparticle inks via aerosol jet printing [32–35], but study of CNT printability via the same technique is not well-explored [36,37]. The pH sensor reported here consists of a miniaturized serpentine sensing element with an effective sensing area as small as 500 μm × 100 μm printed on polyimide substrate. The effect of various aerosol jet process parameters is investigated and optimized for the printing of the sensing element. Both qualitative and quantitative analysis have been carried out to investigate the process window for controlled, repeatable and good quality line trace. The sensor is tested in different pH conditions and its response is discussed. Lastly, an in vitro biocompatibility test of the fabricated pH sensor is conducted to check its suitability for live cell applications.

2. Experimental section

The substrate used is Kapton® polyimide film with a thickness of 150 μm. Substrate is cleaned by sonication in ethanol bath for 10 min. For the fabrication of pH sensor, the silver electrode (PRELECT TPS®, Clariant™) is first printed on the polyimide film. The serpentine CNT sensing element is then printed on top of the silver electrode. NINK single-walled CNT aqueous dispersion purchased from Nanolab® is used. NINK has concentration of 2 g/L with reported average CNT length of 1.5 μm. The printed pH sensor is then sintered 200°C for 2 h. Finally, the connecting wires are attached to the pH sensor by using conductive epoxy. In order to investigate the printing parameters, a benchmark coupon with three lines of length of 2 cm is used. To precisely study the effect of each parameter on printing, only one parameter is varied at any given time. For this work, the range of variation for each parameter is AF 20–35 sccm, SF 10–40 sccm, UC 0.4–0.7 A, and PS 4–10 mm/s. Other working parameters are kept constant with nozzle size = 150 μm, substrate temperature = 40°C, print speed = 10 mm/s and ultrasonic current = 0.6 A.

The pH sensor is tested with pH buffer (Fisher Scientific) of pH 4, 7, and 10. The pH and the conductivity of the pH buffers are measured using Fisher Scientific Accumet AR20. The sensor is connected to a Fluke digital multimeter (DMM) via crocodile clips to measure its change in resistance. First, a few drops of pH 10 buffer is first added to CNT sensing element to ensure the sensing element is fully covered with the pH buffer (similar to Fig. S4B). The response of the pH sensor is then recorded by the DMM. Once the sensor readout reaches steady state, the pH buffer is then removed with Kimiwipes®. The sensor is then cleaned with DI water and the DI water is removed before adding new pH buffer for testing. Similar steps are repeated for the subsequent tests for pH 4 and 7. In the experiment, it is found that the pH sensor response is not consistent in the first few cycles (1–3) but gradually become stable after several cycles. Similar observation is also reported by Li et al., which the pH sensor become stable after approximately 10 cycles [38]. This can be explained by the removal of the loosely bonded CNT from the sensing element due to the test cycles [38]. The pH sensor becomes stable when the sensing element is left with strongly bonded CNT. The results presented in the Section 2 excludes the inconsistent result for the first few cycles (1–3).

A resistivity coupon is designed to evaluate the electrical property of the printed CNT film. The resistivity coupon has a center line of length (L) 1 cm and width (W) 1 mm with two contact pads at both ends for the probes. The resistance (R) of the printed CNT film is measured using a digital multimeter. The sheet resistivity, Rs is calculated by R x W/L. The effect of print passes (10, 20 and 30 passes) on the conductivity of the printed CNT film is also investigated. Scanning electron microscopy (Joel JSM-5600LV) is used to evaluate the surface morphology and film thickness. The contact angle measurements (Attention Theta optical tensiometer) are done to determine the wettability of CNT ink on polyimide surface. The surface roughness of substrate and printed films is also measured using Keyence VK-X series 3D laser scanning confocal microscope. Optical images are taken using Olympus CKX41 microscope. All physical measurements are performed at least five times to achieve statistically significant results.

Murine myoblasts C2C12 was cultured and expanded before experiment. The cells were cultured in the cell culture media of high-glucose DMEM supplemented with 10% FBS and 1% antibiotic-antimycotic, incubated under 5% CO2 at 37°C. For the experiment, the C2C12 cells were seeded at a density of 5 × 104 cells per well in a 24-well plate, and cultured for 1 day before gently placing the 1 × 1 cm2 pH sensor (n=3) onto the cells. The polyimide surface faces the cells, mimicking the wearing of the device on skin. The cells were maintained in an incubator and the medium (2 mL in each well) was changed every two days. The cells were observed on day 0, 1, 3 and 7 under an inverted microscope (Zeiss Axio Vert. A1). The viability of the cells was imaged using the live/dead cell viability kit (Molecular Probes) after 7 days of culture. Briefly, the cells together with the sensors were rinsed twice with DPBS and incubated in a DPBS solution containing 5 nM/mL propidium iodide and 2 μM/mL calcein acetoxymethyl ester for 20 min at 25°C before examining via an inverted fluorescent microscope (Zeiss Axio Vert. A1). The percentage of live cells (reported as the cell viability) was computed from the fluorescence readings.

3. Results and discussion

The pH sensor is basically made up of CNT-based sensing element which connects two silver electrodes. The fabrication process of the pH sensor is schematically illustrated in Fig. 1a with detailed descriptions presented in the experimental section. Fig. 1b shows the printing parameters of the aerosol jet printer that will affect the line resolution and overall printability. Fig. 1c shows the design of the pH sensors. The printability of the CNT ink on the polyimide substrate depends on the surface tension of the ink, the surface free energy and the surface roughness of the polyimide substrate. The average surface roughness (Ra) of the polyimide substrate is measured to be 0.60 μm. The average contact angle of the CNT ink on the polyimide substrate’s surface is measured to be 47.6°, which signifies hydrophilic nature of the ink.

Several process parameters are involved in printing ink via aerosol jet process, namely atomizer flow (AF), sheath flow (SF), ultrasonic current (UC) and process speed (PS). All these parameters play a part in influencing the quality and accuracy of the printed features. Fig. 2 demonstrates the variation in the printed line width with changing trends of SF, AF, UC and PS. Fig. 2a shows that the line width decreases with increasing shear flow. The decreasing line width is due to the narrowing of the aerosol jet stream when more shear flow going through the nozzle. Fig. 2b depicts increase in line width with increasing atomizer flow, whereas Fig. 2c shows the line width decreases when the process speed is increased. The change in line width due to the process speed, Vf and atomizer flow can be explained by the continuity Eq. (1) [39].

\[
\rho_e A_e V_e = \rho_a A_a V_a \tag{1}
\]

where \( \rho_e \) and \( \rho_a \) are the densities of the aerosol through the nozzle and the coalesced print line, \( V_e \) and \( V_a \) are the velocities of the aerosol exiting the nozzle and stage velocity, \( A_e \) and \( A_a \) are the cross sectional areas of the aerosol jet stream and printed line.

The printing process can be explained with the help of the continuity equation. The line width, \( wt \) is proportional to the aerosol