Biosensors and Bioelectronics 107 (2018) 145–152

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

All-carbon suspended nanowire sensors as a rapid highly-sensitive label-free chemiresistive biosensing platform

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ARTICLE INFO

Keywords:
Suspended carbon nanowire
Chemiresistive sensor
Label-free biosensing
Foodborne pathogen detection
Salmonella detection

ABSTRACT

Nanowire sensors offer great potential as highly sensitive electrochemical and electronic biosensors because of their small size, high aspect ratios, and electronic properties. Nevertheless, the available methods to fabricate carbon nanowires in a controlled manner remain limited to expensive techniques. This paper presents a simple fabrication technique for sub-100 nm suspended carbon nanowire sensors by integrating electrospinning and photolithography techniques. Carbon Microelectromechanical Systems (C-MEMS) fabrication techniques allow fabrication of high aspect ratio carbon structures by patterning photoresist polymers into desired shapes and subsequent carbonization of resultant structures by pyrolysis. In our sensor platform, suspended nanowires were deposited by electrospinning while photolithography was used to fabricate support structures. We have achieved suspended carbon nanowires with sub-100 nm diameters in this study. The sensor platform was then integrated with a microfluidic chip to form a lab-on-chip device for label-free chemiresistive biosensing. We have investigated this nanoelectronics label-free biosensor's performance towards bacterial sensing by functionalization with *Salmonella*-specific aptamer probes. The device was tested with varying concentrations of *Salmonella* Typhimurium to evaluate sensitivity and various other bacteria to investigate specificity. The results showed that the sensor is highly specific and sensitive in detection of *Salmonella* with a detection limit of 10 CFU mL^{-1}. Moreover, this proposed chemiresistive assay has a reduced turnaround time of 5 min and sample volume requirement of 5 μL which are much less than reported in the literature.

1. Introduction

Carbon, the building block life, is now poised to become the building block of next-generation electronics. Carbon nanomaterials such as graphene and carbon nanotubes (CNT) offer superior electrical, thermal and mechanical properties as well as energy efficiency than current silicon-based electronics (Peng et al., 2014; Shulaker et al., 2013). Furthermore, their fast electron transfer kinetics, excellent conductivity, and ease of biofunctionalization give rise to numerous sensor applications (Barsan et al., 2015; Hernández et al., 2014; Qiu et al., 2015). However, these two allotropes of carbon, graphene and CNTs, are heterogeneous and hard to handle (Yang et al., 2010). Moreover, they are difficult to fabricate into any desired shapes, for instance, a suspended nanowire for electronic sensors. Graphene and CNT based bio transistor sensors have been synthesized by chemical vapor deposition or chemical reduction methods which are still hard to integrate with established large-scale manufacturing techniques for electronics. Currently, nanowires in electronic sensors are mainly fabricated by focused ion beam (Fujii et al., 2017) and electron beam lithography (Cui et al., 2001; Juhasz et al., 2005). While these techniques are capable of high-resolution patterns down to tens of nanometers, they are relatively expensive and slow for mass-manufacturing. In this report, we fabricated highly defined suspended carbon nanowires by simple electrospinning process combined with standard Carbon-

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https://doi.org/10.1016/j.bios.2018.02.024
Received 8 December 2017; Received in revised form 4 February 2018; Accepted 8 February 2018
Available online 09 February 2018
0956-5663/

Microelectromechanical Systems (C-MEMS) techniques for rapid, ultrasensitive, label-free electronic biosensor application.

C-MEMS offers a way to control fabrication of carbon materials in desired shapes from macro to nanoscale dimensions. A typical C-MEMS fabrication involves patterning polymer structures and subsequent carbonization to convert them into carbon structures. Patterning can be performed by standard photolithography techniques on photosensitive polymers with high carbon content. SU-8 photoresist is a typical polymer used in C-MEMS because they are patternable to high aspect ratio and 3-dimensional structures (Martinez-Duarte, 2014). Carbonization is performed by pyrolysis of patterned polymer structures in an inert atmosphere at an elevated temperature ranging from 800° to 1200°C. This process converts polymer patterns to conductive carbon while retaining their shape. Carbon structures obtained from this technique are found to have glassy carbon like properties (Martinez-Duarte, 2014). Glassy carbon is a widely used electrode material for electrochemical analysis because of its inert nature, stability and wide electrochemical windows (Bollella et al., 2017). Using C-MEMS fabrication techniques, researchers have fabricated high aspect ratio carbon micro 3D electrodes for various applications. These include next-generation battery electrodes (Long et al., 2004), electrochemical sensors (Kamath and Madou, 2014), gas sensors (Sharma and Madou, 2012) and glucose sensors (Xi et al., 2013).

While photolithography in C-MEMS offers a way to fabricate high aspect ratio microstructures, electrospinning provides a simple and cost-effective way to fabricate nanostructures. In electrospinning, a high voltage (5–30 kV) is applied between syringe tip and collector. The electric field stretches polymer droplet to nanoscale as it ejects from syringe tip towards collector and deposits as a nanofiber mat. Electrospinning has been very widely explored in a variety of applications such as filtrations (Gopal et al., 2006), tissue engineering scaffolds (Li et al., 2002) to polymer field effect transistors (FET) (Babel et al., 2005; Manuelli et al., 2014). However, a major obstacle of integrating electrospinning into electronics and sensor fabrication is that electrospinning inherently is a random process. Thus, improving control of electrospinning process, with the focus on positioning single nanowires and integrating with support structures, is an active research area (Pramanick et al., 2016). Recently, Prakash et al. electrospun multi-walled carbon nanotubes (MWCNT) embedded SU-8 polymer fibers on micro copper poles for biosensing (Prakash et al., 2016). The authors reported controlling electrospinning time for deposition of one single fiber on the support structure. This way of controlling, however, tends to require mostly manual observation of resultant fibers (Sharma et al., 2012). In a more recent study, Paul et al. (2017) have reported deposition of MWCNTs-zinc oxide nanofibers as stretchable biosensors deposited between gold electrodes. Although fibers were fabricated by electrospinning, fiber mats were collected by dissolving in a solvent and subsequent drop-casting of the solution onto support structures. In this report, we describe on-site suspended carbon nanowire fabrication by integration of electrospinning and photolithography. By electrospinning photoresist polymer, the resulting mat can be photopatterned and crosslinked selectively in the desired area. Thus, this can be integrated with standard photolithography methods for suspended nanowire sensor fabrication. By converting the resulting electrospun photoresist nanowires to carbon by the C-MEMS fabrication, we can achieve photo patternable fabrication of carbon nanowires while pushing C-MEMS forward into nanodomain as Carbon Nanoelectromechanical Systems (C-NEMS).

On-chip electrochemical biosensors technologies utilize varieties of analytical methods to probe molecular bindings at the electrode surface to liquid interface. These include cyclic voltammetry (Zhao and Liu, 2016), chronoamperometry (Lin et al., 2017) and electro impedance spectrometry (Jiang et al., 2014). Polymeric and metal nanowire electrochemical biosensors which use above sensing methods can be fabricated by electrochemical deposition of nanowires on a planar substrate (Lin et al., 2012; Spain et al., 2015; Zhuang et al., 2016). These methods employ current sensing in electrodes through a liquid medium and analyze changes in charge transfer characteristics. On the other hand, in chemiresistive biosensors, the conductivity of sensor material changes in response to surface binding. As the dimension of sensor components in chemiresistive sensors are comparable to Debye length, a slight change in surface properties can vary the material's electronic properties significantly (Prakash et al., 2016). Here, sensing took place in the sensor substrate and the current doesn't need to flow through the liquid medium. Hence, unlike other electrochemical biosensing platforms, chemiresistors do not require conductive medium and redox species to perform analysis, resulting in shorter time, simpler assay protocol and reduced reagents. Besides, chemiresistive sensors require simpler instrumentation for analysis as only the conductivity of substrate need to be measured (Janata, 2009) making them more suitable for point-of-care diagnosis. Hence, they have been widely applied in biosensing applications such as DNA sensing, protein sensing and bacteria sensing (Paul et al., 2017; Prakash et al., 2016; Rajesh et al., 2013). Our on-site electrospun deposition enables suspension of nanowire sensors to the height of tens of micrometers, which has additional advantages of having better contact with the analyte as target molecules can bind from all sides and reducing electronic interface from the substrate surface (Pramanick et al., 2016).

Among biorecognition elements for sensors, aptamers have become recent alternatives for the traditional usage of antibodies as biorecognition element. Although antibodies were used as conventional probes in biosensing due to their high affinity and high specificity to a broad range of analytes, some limitations and drawbacks still hinder their development as biosensors (Zhou et al., 2014). For instance, antibodies are susceptible to chemical modification and high temperatures (Han et al., 2010). The production of antibodies is also an expensive and complex process as compared to in-vitro selection and amplification process of the aptamer (Han et al., 2010). Aptamers are single-stranded nucleic acid (DNA or RNA) that interacts and binds the target analyte with high affinity, selectivity, and sensitivity (Hamaguchi et al., 2001). Aptamers can be easily tailored and chemically modified to bind with various target analytes such as antibodies, nucleic acids, cells, and microorganisms with dissociation constants of aptamer-target complexes ranging from picomolar to micromolar (Hamula et al., 2006). Owing to these advantages, extensive research and effort have been channeled to the application of aptasensor for foodborne pathogen detection. Besides offering high-stability to chemical modifications, aptamers are also attractive candidates for novel, label-free and direct detection of whole-cell bacteria using electronic biosensing platform. Aptamers can interact and undergo conformational changes with outer membrane proteins of a bacterial cell to form a unique 3D-structure, and thus enable them to discriminate between protein isoforms of another bacterial cell (Davydova et al., 2016; Joshi et al., 2009; Muniandy et al., 2017). Another major advantage of aptamer to antibodies as biorecognition probes is that aptamers are much smaller than antibodies and hence, aptamer probes reduce distance between the electrode surface and bio-analyte. This results in higher sensitivity in FET sensors and chemiresistive sensors by reducing Debye screening effect (Maehashi et al., 2007; Zhang et al., 2016).

In this work, we report the application of suspended carbon nanowire based aptasensor as an innovative platform for chemiresistive biosensing. The suspended carbon nanowires were fabricated by integrating electrospinning and photolithography with C-MEMS techniques. The performance of the sensor platform was evaluated for the label-free detection of *Salmonella* Typhimurium (*S.* Typhimurium), a common food-borne pathogen, using a specific aptamer probe. Amine-ended aptamers were immobilized on suspended nanowire surface by means of carbodiimide crosslinker chemistry with carboxylic groups on the carbon nanowire. This nanoelectronics biosensor platform enables rapid, real-time, highly-sensitive and specific label-free detection. By functionalization with other suitable aptamer probes, this platform can also be used for label-free detection of various analytes such as DNA,