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Research paper

Wafer scale fabrication of graphene microelectrode arrays for the detection of DNA hybridization

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ABSTRACT

Since the discovery of graphene, and due to its unique properties, we have witnessed a growing interest in the use of graphene-based devices for applications in the most diverse areas, namely in biosensing, particularly in the detection of genetic material. DNA can bind directly to graphene without the need of a linker and that makes this platform highly interesting for biosensor development.
Here, electrochemical chips consisting of 6 independent gold microelectrode arrays as working electrode, and platinum reference and counter electrodes were fabricated at the wafer scale and, after graphene transfer and patterning, were used in the detection of DNA hybridization. Combining the sensitivity of electrochemical impedance spectroscopy and the selectivity of DNA beacons, we were able to detect DNA hybridization in a linear range between 5 pM and 5 nM, which is in the relevant clinical range for many diseases, with sensitivity to single nucleotide polymorphism.

1. Introduction

The medical community needs detection systems that are fast, reliable and easy to use. DNA biosensors are a technology that is growing rapidly and that presents itself as an alternative to the classical gene assay [1–4]. Apart from the low-cost, simplicity and miniaturization possibility, the ability to detect single nucleotide polymorphism is one of the main advantages that DNA biosensor technologies present. Single nucleotide polymorphism is a variation in a single nucleotide that occurs at a specific position in the genome, and has been correlated with various disorders/diseases, including cystic fibrosis, Alzheimer's, Parkinson's, diabetes and various cancers [5–8].

2D materials are currently used to help lower the limits of detection and to improve the performance of the biosensors. Their relevance stems from their specific electronic, optical and mechanical properties [9,10]. Among 2D materials, graphene is the one to which most of the attention has been dedicated so far. The sheet of sp2-bounded carbon atoms arranged in a honeycomb lattice proves to be in several respects an ideal platform for biosensing, since it provides a chemically and mechanically stable surface for interfacing with molecules, with an exposed 2D electron gas with high carrier mobility. Moreover carrier concentration can be tuned in graphene by simple application of a gate voltage [11,12].

The adsorption of DNA on graphene, or its derivatives such as graphene oxide (GO) or reduced graphene oxide (rGO), has been used for biosensing applications since 2009, when Chen and co-workers [13] used a fluorescent labeled DNA strand to detect target DNA and proteins. They showed that when the dye-labeled single strand DNA (ssDNA) is bound to the GO surface the fluorescence is quenched and that after interaction of the ssDNA with target DNA or with proteins the ssDNA DNA is released from the GO surface and the fluorescence is restored. Bonanni and Pumera [14] also explored the interaction between DNA and graphene to develop an impedimetric genosensor that could detect the single nucleotide polymorphism (SNP) associated with Alzheimer's disease. When the probe DNA is deposited on the graphene surface its bases bind to graphene *via* π-π interactions, and then when target DNA (cDNA) is added there are three possible mechanisms for the formation of the duplex: i) the Langmuir-Hinshelwood model: the cDNA adsorbs on the graphene surface, diffuses and forms duplex, which then leaves the surface; ii) the Eley-Rideal model: the duplex is formed directly on the surface without cDNA adsorption, and iii) the displacement model: the probe DNA is displaced from the surface by cDNA into solution and then reacts with another cDNA in solution [12].

Microelectrode arrays besides possessing all the advantages of the microelectrodes – higher current density, faster mass transport and lower detection limits – allow signal amplification due to geometry [15,16]. Because of these advantages microelectrode arrays create the opportunity for lab-on-a-chip devices and *in-vitro* and *in-vivo* measurements. Microelectrode arrays can be fabricated using i) assembly

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techniques, ii) screen-printing techniques, iii) electrodeposition (also known as electroplating) and, iv) photolithography. Microelectrodes fabricated using assembly techniques include those fabricated using assembly of materials or molecules. The assembly of materials is a physical technique which can be performed by an unskilled laboratory worker, uses materials that are readily available (can be metals: such as gold, platinum, silver and others, or carbon in the form of fiber, paste, graphite or glassy carbon) and does not require special laboratory equipment [15,16]. Molecular assembly includes those methods in which a mixed self-assembled monolayer is deposited and then one of the components is selectively desorbed from the surface, thus creating the microelectrodes [17,18].

The second method for fabrication of microelectrodes is the screen printing technique. It allows the mass production of identical electrodes, by using simple machinery and operation in order to screen-print both the conductors and the insulators, with the desired pattern. The inks used for the printing are based on a suspension of conducive materials. Like in the case of the assembly technique the most common compounds used for the conductors are either metal particles or carbon based materials [16,19].

Electrodeposition is a process that uses electrical current to reduce cations of a desired material from a solution and coat that material as a thin film onto a conductive substrate surface. Electrodeposition, including patterned electrodeposition (electrodeposition performed on a designed array support) presents itself as a very promising alternative microelectrode fabrication technique, especially for the fabrication of nanostructures, and has received an increasing interest in the recent literature [20,21]. Wang et al. [21] proposed a method for the *co*-deposition of PEDOT (poly(3,4-ethylene-dioxythiophene)) and graphene on microelectrodes which led to a biosensor that presents lower impedance and better local field potentials.

Among the techniques of microelectrode array fabrication, photolithography is the most complex but is also among those yielding smallest feature sizes, while retaining the ability to reliably produce a high number of identical arrays at the same time [15,16]. However, photolithography requires access to a clean room which limits its use to cases where the benefits brought by the feature size compensate the higher cost barrier [15,16].

In order to fabricate high quality microelectrode arrays by photolithography there are three steps that are crucial, i) photo mask design, ii) photoresist spinning and exposure and, iii) photoresist development. The photo mask is a nearly optical flat glass or a quartz plate covered with chromium and a photoresist. The photomask design is related to the writing of a specific pattern on a mask into a layer of photoresist using a direct write laser. The pattern is transferred from the photoresist into the chromium by chemical etching. The photomask can be placed in direct contact with the production wafer coated with photoresist while UV light is shined trough the mask. A reduced image of the photomask can also be projected into the photoresist of the target wafer. For applications that do not require a high throughput, or during the optimization of the design and of the fabrication process, the fabrication of the photomask can be replaced by a direct write on the target wafer using a laser. Adequate choice of photoresist and optimization of the process conditions allow the creation of sharp vertical photoresist walls, which in turn leads to the accurate transfer of the photoresist pattern into the materials deposited on the wafer, one of its main advantages [15].

Here we fabricated arrays of 40 μm diameter microelectrodes disks, by employing direct-write laser photolithography to define the pattern in a gold thin film followed by the definition of additional electrodes and passivation layers. The fabricated arrays were covered with a graphene monolayer, which was in turn functionalized with DNA. The resulting sensor was able to detect concentrations of fully complementary DNA in the range between 5 pM to 5 nM, while keeping the sensitivity to single nucleotide polymorphism.

2. Experimental

2.1. Materials

All components of buffer solutions (Na_2HPO_4, NaH_2PO_4, NaCl and $MgCl_2$) and all the solvents were from Sigma–Aldrich. Ultrapure water (18 MΩ cm, Millipore, Bedford, MA, USA) was used throughout the experiments. The probe DNA, (5′-AGC TTC ATA ACC GGC GAA AGG CTG AAG CT-3′) had a C7-amino modification on the 3′ end and a Atto MB2 modification on the 5′, the complementary DNA (5′-AGC TTC AGC CTT TCG CCG GTT ATG A-3′) and the SNP containing target (5′-AGC TTC AGC CTT ACG CCG GTT ATG A-3′) were synthesized by Metabion International AG, Martinsried, Germany. The melting temperature, T_m, of the DNA was estimated using DINAMelt web server [22], and, in the hybridization buffer (10 mM PB/150 mM NaCl/50 mM $MgCl_2$) was 63.9 °C for the probe DNA (beacon), 82.7 °C for the fully complementary duplex, and 80.8 °C for the single nucleotide polymorphism containing duplex, while in the working buffer (10 mM PB) it was, respectively, 42.9 °C,0.58.9 °C and 55.1 °C.

The high purity (>99.99%) 25 μm copper foils used for the graphene growth were purchased from Alfa-Aesar and Goodfellow.

2.2. Design of the microelectrode arrays

Each array is composed of 60 disks of 40 μm diameter arranged in 11 rows of 5 or 6, forming a triangular lattice with a spacing of 400 μm (Fig. 1B) in order to avoid overlapping of the diffusion layers [23], and connected by lines of 12 μm of width, forming a planar truss. Two larger additional rectangular contacts are present (Fig. 1A), with dimensions 11.4 mm × 3.7 mm, used as counter and reference electrodes. The electrical contacts are brought to the edge of the chip, to electricals pads

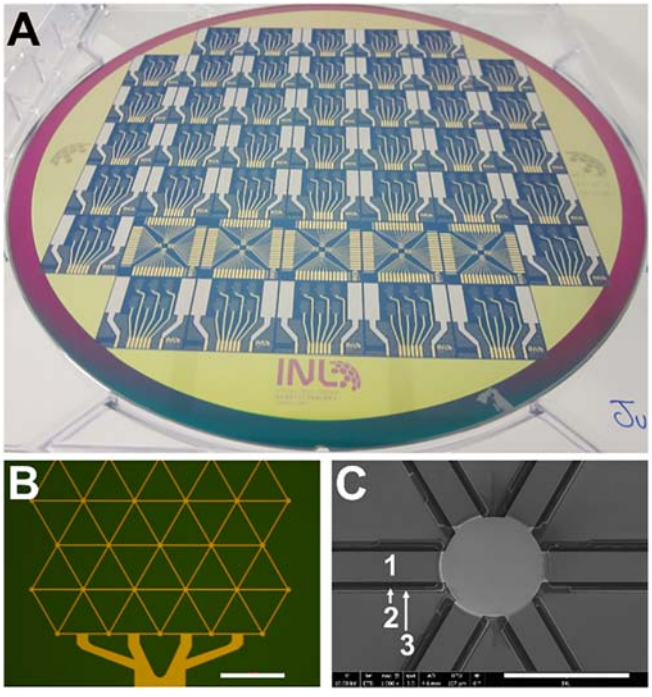

Fig. 1. A) Optical image of the 200 mm full wafer with the electrochemical chips. In each chip two large contacts to be used as reference and counter-electrodes, respectively, are visible. B) Detail of one of the arrays, showing the arrangement of the micro-electrodes in an array and the connection lines, after ion milling of the goldworking electrode. C) SEM image of a single microelectrode. Six gold lines connect the electrode to its neighbors. The gold lines are covered with a stack of thin silicon nitride and silicon oxide films, marked1. The passivation stack overtops the gold layer, over an area marked 2. The edge of the passivation stack is suspended due to the removal of the stopping layer underneath, marked 3). Fig. S1 in ESI is a full size image where 1, 2 and 3 are easily seen. The scale bar in B is 500 μm and in C is 50 μm.