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Highly target-specific graphene devices for biomedical applications

The importance of biosensors in biomedical research is increasing fast, as they are routinely used in a wider range of applications, from prognosis and diagnosis to personalized medicine. Graphene low-dimensionality, high carrier mobility and chemical stability, allow to fabricate relatively simple and highly sensitive biosensors with transducing capability, based on different types of devices. Ion-sensitive field-effect transistors (ISFETs), where the graphene channel is directly exposed to the analyte, have exceedingly high sensitivity, whereas electrochemical microelectrode arrays (EMAs) have all the advantages of single microelectrodes (higher current density, faster mass transport and lower detection limits), while providing amplification of the signal by the array. In this work we fabricate both ISFET and EMA devices. Using EMAs for direct detection of DNA hybridization based on cyclic voltammetry and electrochemical impedance spectroscopy, we achieved detection of a 25 nucleotide long target DNA in the range 5 pM to 50 nM, with single nucleotide polymorphism (SNP) sensitivity.

Graphene immuno-ISFETs with a receded, integrated gate architecture, are fabricated at the 200 mm wafer scale, for detection of a panel of biomarkers of the hemorrhagic transformation of ischemic stroke. Specific target biorecognition requires surface functionalization and so we immobilize probe molecules on graphene, using a pyrenic linker (PBSE) that binds to graphene through π-π interactions, and reacts with a primary amine from the antibody protein at the other end of the molecule. The device is able to detect MMP-9 in concentrations down to 0.01 ng/mL, in a range up to 10 ng/mL. Compared to existing MMP-9 immunoassays, it has a shorter time to diagnostic since it is based on a simpler label-free protocol.

For use of the ISFET as genosensor we functionalized graphene with the same 25 nucleotide DNA sequence used before, with a C7-amino modification on the 3'-end that binds to the ester group of the PBSE linker. Fully complementary target DNA is detected in a linear range between 1 aM and 100 fM, with SNP sensitivity down to 10 aM. The results are normalized for different initial probe surface densities, estimated by fitting the transistor transfer curves to an electrostatic model relating the observed shift in the charge neutrality point to local gating induced by the negatively charged DNA. The results of this work pave the way for a wide range of application of graphene devices in analyte detection for the health and food industries.

References

[1] N. C. S. Vieira et al., J. Phys.: Condens. Matter, 28 (2016) 085302
[2] A. Bonanni, M. Pumera, ACS Nano, 5 (2011) 2356
[3] Y. Ohno, K. Maehashi, K. Matsumoto, J. Am. Chem. Soc., 132 (2010) 18012

Figures

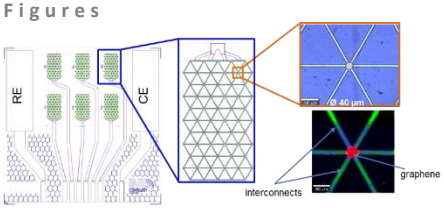

Figure 1: Layout of the EMA and Raman map (bottom right) of one microelectrode.

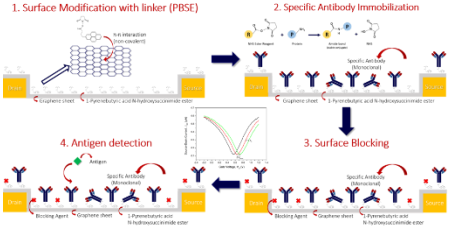

Figure 2: Graphene ISFET channel functionalization steps for biorecognition.