Solution-Processed Graphene Films for Electrochemical Monitoring of Extracellular RNS

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Abstract: Reactive oxygen and nitrogen species (RONS) make up a family of oxygen-containing radicals (e.g. $OH^{\cdot}$, $O2^{-\cdot}$), ions (e.g. $ONOO^{-}$), and other molecules (e.g. H_2O_2) involved in numerous biological processes, including cell signaling and immune function.[1] Excessive RONS, however, can induce a state of oxidative/nitrosative stress, which can have deleterious effects.[1,2] Thus, developing sensors that can monitor extracellular RONS may provide new insight into the mechanisms of cellular proliferation, communication, and death. Electrochemical sensors are well-suited for this role as they can detect changes in the concentration of a target molecule in real time.

To this end, we are exploring solution-processable graphene films as an electrochemical sensing platform for real-time monitoring of extracellular RNS levels in human breast cancer cells grown directly on the graphene surface. The graphene is deposited onto silicon substrates *via* a two-step spin-coating and annealing process. A polydimethylsiloxane (PDMS) chamber is attached to the sensor to allow for the direct culturing of cells on the graphene surface. This is crucial given the short half-life and limited diffusion length of NO. The graphene is functionalized with a fibronectin layer to improve cellular adhesion, which is confirmed by changes in the interfacial electrochemical properties after functionalization. Optical microscopy images show that MDA-MB-231 cells attach and spread on the graphene surface after 72 h. L-arginine is added to the culture to promote NO generation *via* nitric oxide synthase (NOS). The increased level of L-arginine and NO causes a shift in the morphology of the cells to a more spherical shape. However, this effect can be mediated by also adding a NOS inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME), in addition to the L-Arginine. The generation of NO is confirmed electrochemically using chronoamperometry measurements. While the cells exposed to a mixture of L-arginine (stimulant) and L-NAME (inhibitor) show an initial spike in current followed by a rapid drop back to baseline, the cells exposed to L-arginine alone maintain an elevated current level after addition. This points to a prolonged generation of extracellular NO at the graphene-cellular interface. Although this work focused on monitoring RNS generation, the biocompatibility and electrochemical properties afforded by our graphene films make them a suitable platform for other cell model systems, such as monitoring neurotransmitter release from neurons.

(1) Weidinger, A., Kozlov, A. V., *Biomolecules* **2015**, *5*, 472–484.
(2) Pourova, J., Kottova, M., Voprsalova, M., Pour, M., *Acta Physiol.* **2010**, *198*, 15–35.