SLG Functionalized MEA for Enhanced Detection of Neural Network Development

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Scientific Background
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Microelectrode Array (MEA)

Application of MEAs

- **Electrophysiology** tool
- Extensive *in vitro* studies
- Recording of the **spontaneous activity** of primary neuronal networks
- Recordings used as an **assay** for network performance in applied settings

Problems Faced/Drawbacks

**Low signal-to-noise ratio (SNR)**

Improvements

- Chemical functionalization of the electrodes
- Topographical modification increasing roughness
- Fabrication of porous electrodes
- Graphene MEAs consisting of graphene electrodes

Description

- Consists of a discrete number of metal electrodes integrated on a **solid substrate** (glass or silica)
- Planar gold, titanium and platinum are the most common **electrode** materials
- Encapsulated by a glass ring to perform **cell cultures** on the chips
Objectives

- Exploring the interplay between the carbon based interface and neuronal networks during the complete developmental phase at whole network scale

Role of Single Layer Graphene (SLG) in (MEAs)?

- SLG grown by chemical vapor deposition on Cu foil may be considered extremely favorable in the field of biosensor development
  - transparency
  - scalability
  - convenient transfer onto any substrate, including flexible ones

Large Grain SLG (LG-SLG)
Methodology
Methodology

SLG Transfer to MEA

CVD grown SLG on Cu foil → PMMA spin coating → Stack of PMMA + SLG + Cu → Cu etching → Stack of PMMA + SLG after Cu etching

Raman Characterization

Intensity (a.u.)

Raman shift (cm⁻¹)

PDL coating

SLG on MEA → PMMA removal by acetone → PMMA + SLG on MEA → Annealing at 150°C → Scooping of PMMA + SLG on MEA

PDL = poly-D-lysine

Methodology

Cell Culture

- Dissociated neuronal cultures (hippocampi of 18-day old embryonic rats)

MEA Recordings

- Electrophysiological activity monitored and recorded for 90' at 7 different days in vitro (DIV) (from DIV7 to DIV25)

Spike & Burst Detection

- Spikes: neuronal signals consisting of short electrical pulses (action potentials)
- Bursts: consist of packages of spikes over a few milliseconds

Immunolabeling & Image Analysis

- MEA Recordings
- Spike & Burst Detection
- Immunolabeling & Image Analysis

- Spikes: neuronal signals consisting of short electrical pulses (action potentials)
- Bursts: consist of packages of spikes over a few milliseconds
Results
Confocal Microscopy on Immunolabeled Samples

Control

DIV 7

DIV 13

DIV 25

LG-SLG

MAP2

DAPI
✓ Comparable morphology of healthy cells of the neuronal network on LG-SLG and control substrates
✓ Total cell density ↑ during the development of both LG-SLG and control cultures
✓ Higher number of neurons on LG-SLG
Analysis of Neuronal Networks Activity

SLG-MEA

MEA (Control)

DIV: developmental phase

10-s Raster Plots

A  Control

B  Graphene

DIV 7

DIV 13

DIV 19

DIV 25

Time (s)
Mean firing rate (spikes/s)  
Mean bursting rate (burst/min)

El Merhie A., Ito D. et al., Sensors and Actuators B: Chemical, *submitted*
✓ **Long-term development** of neuronal networks on LG-SLG interface from the first week *in vitro* up to complete network maturation

✓ No major morphological differences with respect to control have been detected (healthy cultures)

✓ The higher survival rate, the higher number of adhered cells and firing activity → LG-SLG devices are compatible with physiological functionality of neuronal network → provide an improved detection capability (due to a better neuron/substrate coupling)

✓ **Neuronal network activity** was detected earlier on LG-SLG and a more synchronous behavior of the network was recorded

→ Results are in agreement with single neuron synaptogenesis study on SLG versus glass substrates by patch clamp (Keshavan et al, *Acta Biomaterialia*, 2017)
THANK YOU for Your Attention!

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