

Microwell Array Integrating Ring Nanoelectrodes for The Monitoring of Metabolic Responses at Isolated Mitochondria

V.S.R. Vajrala¹, F. Sekli Belaidi², G. Lemercier², D. Zigah¹, N. Sojic¹, J. Launay², P. Temple-Boyer² and
S. Arbault^{*}

1. University of Bordeaux, ISM, CNRS UMR5255, N²sysA group, F-33400 Talence, France

2. LAAS-CNRS; Université de Toulouse, CNRS, UPS, France

^{*}stephane.arbault@u-bordeaux.fr

Mitochondria are major cell organelles since they are the main source of ATP owing to the oxidative phosphorylation pathway. They also play an important role into several other metabolic pathways (Krebs cycle, lipid synthesis...) and when defective, they are involved into severe pathologies (myopathies, neurological diseases, cancers...). Consequently, new methodological approaches^[1, 2] are required in order to decipher mitochondria metabolisms and to provide efficient tools for diagnosis.

In this context, we have developed microsystems, namely ElecWell platforms, which combine electrochemical^[1] and optical^[2] sensing abilities. These are based on the integration of platinum ring nanoelectrodes (RNE, surface: 10-15 μm^2) into SiO₂-based microwell arrays (well radius: 3 to 4.5 μm , depth: 5 μm , individual volume < 1 pL), as shown on figure 1. Similarly to ultramicroelectrodes, RNE exhibit high current density, fast response time, reduced charging current and high signal-to-noise ratio. These electrochemical devices were characterized by cyclic voltammetry using single well or array configurations, and optimised according to COMSOLTM simulations. In addition, the glass substrate of the microsystems allows the observation by microscopy of the content and reactivity within each well.

Then, a suspension of isolated mitochondria (yeast origin) was deposited on the ElecWell array and allowed to sediment within wells. We monitored by fluorescence the presence of individual mitochondria within wells (Fig. 1b) owing to their NADH content and variations^[2]. Simultaneously, we monitored by cyclic voltammetry the variations of their oxygen consumption rate in response to specific activators and inhibitors of respiratory chain activity (ethanol, ADP, antimycine A). The resolution offered by the ElecWell platform is nearly a few thousands of mitochondria (Fig. 1c), corresponding to the mitochondrial content of a single cell.

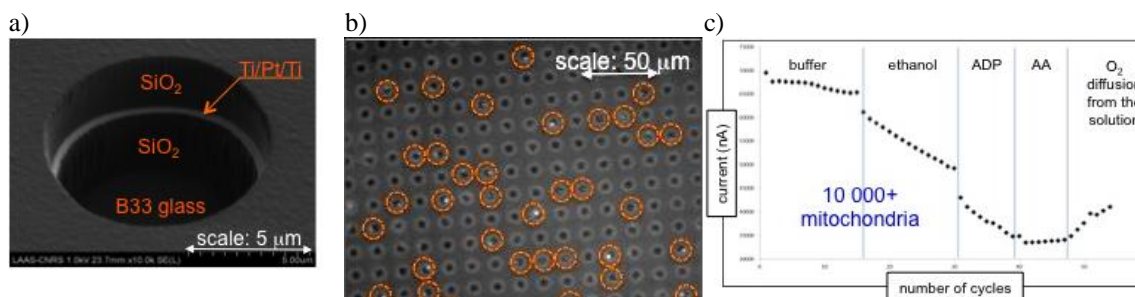


Figure 1. a) Integration of a platinum ring nanoelectrode into a SiO₂-based microwell (radius: 3 μm) within the array (10^6 wells); b) Monitoring by fluorescence (NADH content) of isolated mitochondria deposited within wells. c) Detection by cyclic voltammetry (reduction current sampling) of the oxygen variations during mitochondrial activation (EtOH/ADP) and inhibition (Antimycin A).

References :

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[2] Suraniti E. et al., *Analytical Chemistry*, **2013**, 85, 5146. Vajrala VS et al, *Analytical & Bioanalytical Chemistry*, **2014**, 406, 931