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3D Neuronal Monitoring Platforms for Electrochemical Sensing Of Neurotransmitters

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Extended Abstract

Nowadays, most of the neurological and neurodegenerative diseases are still incurable. At the base of several neurological diseases (e.g., Alzheimer's disease, Parkinson's disease) there are neurotransmitters dysfunctions. Thus, determination of neurotransmission dynamics is a compelling phenotype to judge drug-induced neuroprotection. [1] This project aims to develop an electrochemical biosensor of 3D hybrid hydrogel based on extracellular matrix and functionalized carbon nanotubes (f-CNTs) that quantifies neurotransmitters concentration in 3D neuronal cell cultures (Figure 1).

As first step, this study presents a comprehensive investigation into the development of neuronal cultures with distinct neurotransmission phenotypes. Models were developed from induced pluripotent stem cells (iPSC) derived neurons [2] resulting in mature dopaminergic, cholinergic, and glutamatergic neurons with observable morphological traits and functional properties. Generation of the differentiation protocol was based on the inducible over-expression of Ngn2 and Ascl1 neuronal genes through pUNA plasmid. Characterization included immunochemistry to evaluate the dregree of maturacion, multi-electrode array recordings to record the electrical activity, gene expression analysis (rt-PCR), and calcium imaging to analyze the spontaneous beating behavior, demonstrating the maturation and phenotypic characteristics of these neurons. Additionally, the impact of carbon nanotubes (CNTs) on neuronal differentiation was examined and characterized, revealing improved cell adhesion and gene expression in the presence of CNTs, as well as an increase in the frequency of spontaneous transient activity of the neurons.

In parallel, an electrochemical sensor platform was designed using Indium-Tin-Oxide (ITO)-coated coverslips with f-CNTs serving as working electrode. In particular, CNT modified electrodes are revolutionizing this field, due to their large active surface area, high conductivity, and fast electron transfer kinetics, resulting in improved sensitivity and reduced limits of detection. [4,5] For this purpose, CNTs were functionalized with gold nanoclusters (AuNCs) or cobalt phthalocyanine (CoPC), followed by the inmobilization of glutamate oxidase for dopamine and glutamate detection, respectively. The f-CNTs underwent thorough characterization utilizing various techniques including Inductively coupled plasma mass spectrometry (ICP-MS), TGA, TEM, and Fourier-transform infrared spectroscopy (FT-IR), as needed.The CNT-Au sensor's [3] electrochemical performance was characterized by differential pulse voltammetry (DPV), demonstrating a noticeable limit of detection (LOD) of 8,2nM and a sensitivity of 3,98E-04 A cm^{-2} μM^{-1}. Afterwards, iPSCs were derived into dopaminergic neurons in the CNT-Au sensor to prove excelent biocompatibility of the material. Moreover, our sensor was able to record and detect dopamine release of the iPSC derived dopaminergic neurons through electrochemical detection using both DPV and chronoamperometry (CA). As proof of concept benzotropine mesylate, an inhibitor of the recaptaton of dopamine was added to confirm the increase of dopamine of the neurons.

Overall, this study lays the foundation for the development of a 3D neuronal culture monitoring platform coupled to the iPSC derived neurons with potential applications in neuroscience research and drug discovery. In conclusion, this study serves as a pivotal step toward establishing a monitoring platform for 3D neuronal cultures coupled with an advanced electrochemical biosensor. The incorporation of functionalized carbon nanotubes enhances both the biocompatibility and sensitivity of the sensor, laying a solid foundation for future research aimed at unraveling the complexities of neurotransmission dynamics in neurological diseases.

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