3D-printed droplet-based microfluidic sensor based on ion beam-induced graphitic electrodes on diamond for dopamine detection

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ABSTRACT

Dopamine (DA) is an important neurotransmitter in the human body, mainly in the brain. It plays a pivotal role in regulating various physiological functions such as mood regulations and it is crucial for reinforcing behaviors linked to pleasure and survival. Variation of its concentration is a reason for numerous neurological diseases such as Parkinson's disease which makes its detection vital for early diagnosis and drug screening. Detecting dopamine, however, comes with its own set of challenges, particularly due to the necessity for methods that are highly sensitive and specific. This paper reports an improved method for the detection of exocytotic events from dopaminergic neurons by merging the electrochemical properties of the ion beam induced graphitic electrodes on diamond and the capabilities of a stereolithography (SLA) 3D printer to produce a droplet-based microfluidic biosensor.

Keywords: Ion beam lithography, Electrochemical sensors, Dopamine detection, Droplet-based microfluidic.

1. INTRODUCTION

Dopamine (DA) is a neurotransmitter that regulates diverse physiological operations in the brain, whereas malfunctions of the dopaminergic system are associated with the initiation and progression of multiple diseases of the nervous system[1,2]. Therefore, detecting the concentration of DA *in vitro* from the neuroendocrine cells is relevant for drug screening investigations and early diagnosis of sickness[3]. Several techniques are proposed to detect DA, but most of them are labor-intensive, slow, and require expensive diagnostic reagents[4]**.** Electrochemical approaches have shown potential advantages ranging from specificity and sensitivity and they are simple and convenient[5,6]**.** One of the promising DA detection methods is the one highlighted by Tomagra et al[7] where they exploited the capabilities of hybrid graphite/diamond sensors for multi-parametric sensing of chemical and electrical signals. However, the success of this measure requires the presence of the cell on the top of the electrodes to measure the release of the neurotransmitter. This work reports an improved method for the detection of DA by merging the electrochemical properties of the ion beam induced graphitic electrodes on diamond and the capabilities of a stereolithography (SLA) 3D printer to produce a droplet-based microfluidic biosensor**.** Our strategy is based on the development of a versatile method that ensures a time-effective and easy fabrication procedure with reasonable sensitivity for real-time single-cell analysis. This paper outlines the development and testing of our novel droplet-based microfluidic biosensor, demonstrating its potential to facilitate dopamine related research.

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2. METHODOLOGY

Fabrication of hybrid diamond /graphite sensor

The core of this device lies in the use of a hybrid diamond/graphite as the sensing part of our device. The fabrication begins with the selection of a high purity artificial mono-crystalline diamond subtract produced by ElementSix (UK) cut along the (100) crystalline direction. MeV ion beam lithography, a process that induces structural defects in the crystal lattice due to the nuclear interaction between carbon atoms of the diamond and the implanted ions, was employed to create graphitic microchannels embedded in the diamond. This process involves irradiating the diamond surface areas that we want to act as electrodes with a high energy Helium (He) ion beam (1.3 MeV) with a current density of 1.5 $\mu A.cm^{-2}$. This procedure was carried out at the AN2000 accelerator of the Legnaro National Laboratories of the Italian Institute of Nuclear Physics (LNL-INFN)[8]**.** The key to ensuring the fabrication of these electrically conductive electrodes is to promote graphitization, i.e., a phase change from diamond to graphite. This is achievable by performing a high temperature annealing of the diamond regions that were irradiated by the ion beam in which the density of the induced defects crosses a critical limit, the graphitization threshold[9,10]**.** This step is crucial to promote the formation of the graphitic electrodes and ensure their optimal performance for dopamine detection[11]**.**

Desing and fabrication of the droplet-based microfluidic device

To ensure an accurate placement of sample under analysis on the top of the measuring electrode, we opted for the use of droplet-based microfluidics. This technique permits the generation and manipulation of small volumes of fluid droplets through immiscible phases, usually water and oil, flowing inside microchannels[12]**.** It offers various advantages, mainly for the encapsulation of single cells for biological related applications[13]**.** We fabricated this device using a stereolithography (SLA) 3D printer (Formlabs 3B) allowing for fast production, high-precision, and customizable designs. For this work, we used flow-focusing geometry, illustrated in Figure 1.a, in which the dispersed phase (solution to be encapsulated) is injected into the continuous phase fluid (an immiscible oil) within a microfluidic channel system. This geometry is known for offering good precision, reproducibility, and a lower variation rate in droplet size[14]**.** Each of the produced droplets can be considered as an isolated microenvironment, which is for example ideal for single-cell analysis. Figure 1.b. shows the design of the droplet-based microfluidic device we have used in which we modified the angle between the dispersed and continuous phase channels to ensure the production of small droplets at low generation frequencies. This ensures the possibility of small droplets at very low rates, allowing the measure of one droplet before the next one is produced.

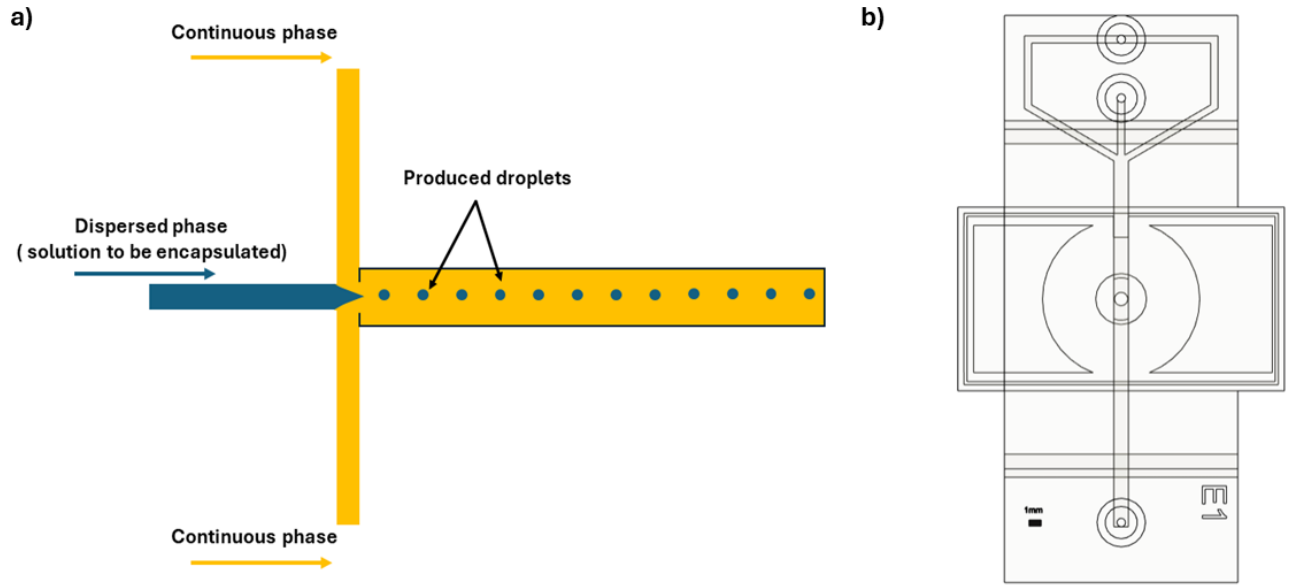

Figure 1 a. Illustration of droplet formation using flow-focusing geometry. b. CAD illustration of the microfluidic device utilized for this work.

Integration of microfluidic device and electrochemical sensor

The assembly of the droplet-based microfluidic device with the diamond electrochemical sensor is a critical step in the success of this work. This process involves aligning the microfluidic device on a printed circuit board (PCB) chip in which we have fixed the diamond biosensor on the bottom as illustrated in Figure 2. This assembly was selected as it permits to easy the microfluidic device from the PCB chip to clean it before each experiment, permitting multiple uses for this device. It permits to perform all the experiments with a low possibility of leakage which represents one of the major problems in the field of microfluidics[15]**.** Also, in this configuration the electrical connection between the diamond biosensor, PCB chip, and detection electronic chain is made far from any contact with the solutions in the microfluidic device, reducing the risk of short circuit.

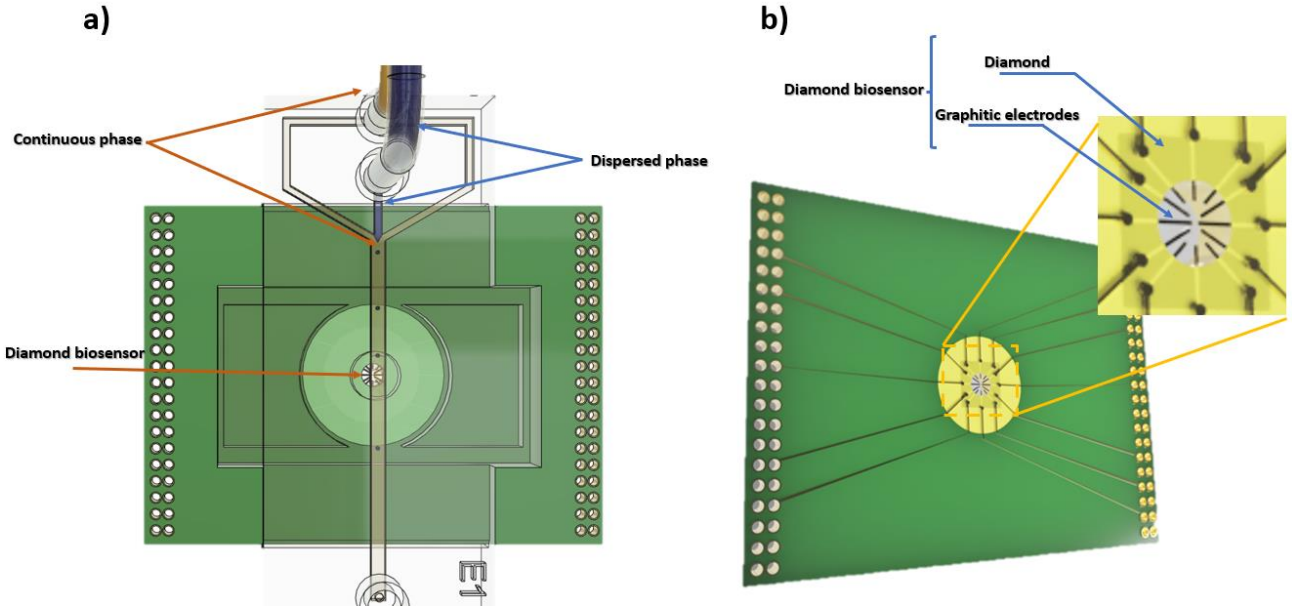

Figure 2 a. Top view of the device assembly. b. Bottom view of the device assembly.

3. RESULTS AND DISCUSSION

The detection process relies on the proportionality between the DA concentration and the generated amperometric current intensity. The higher the concentration of DA, the higher the intensity of the current recorded, providing a precise and reliable means of analysis. The first tests comprise using this device to understand its sensitivity, specifically its capability to accurately identify the current-voltage curve of dopamine and distinguish it from the ones of the other aqueous solution. For this, we performed cyclic voltammetry for a dopamine solution and an extracellular solution (Tyrode's solution) contained (mM): 130 NaCl, 4KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose and 10 HEPES; pH 7.4 as shown in Figure 3. By cyclic voltammetry we refer to an electrochemical technique where the voltage is cycled between two values, in our case between -1.3 V and 1.3 V, while measuring the resulting current. As shown in Figure 3, we see a difference between the recorded response of dopamine and salt solution, mainly the peak appearing at 0.5V which is characteristic of dopamine[7]**.** Then, we moved to the second test in which we tried to measure the presence of dopamine in the encapsulated solution. We used as a continuous phase heavy mineral oil, while for the continuous phase, we used a solution of dopamine. The amperometric detection of dopamine requires that the sensing electrode is properly polarized, have a voltage of 0.5 V, to induce the oxidation of the molecules in the solution on the graphitic electrode. To test the sensitivity of this device, we sent three dopamine droplets generated with a frequency of one droplet each three seconds. We fixed the voltage and recorded the response of the device over time as shown in Figure 4. We observed that the device was able to recognize the presence of each of the three droplets by showing a peak of intensity each time a droplet passes by the working electrode of the device.

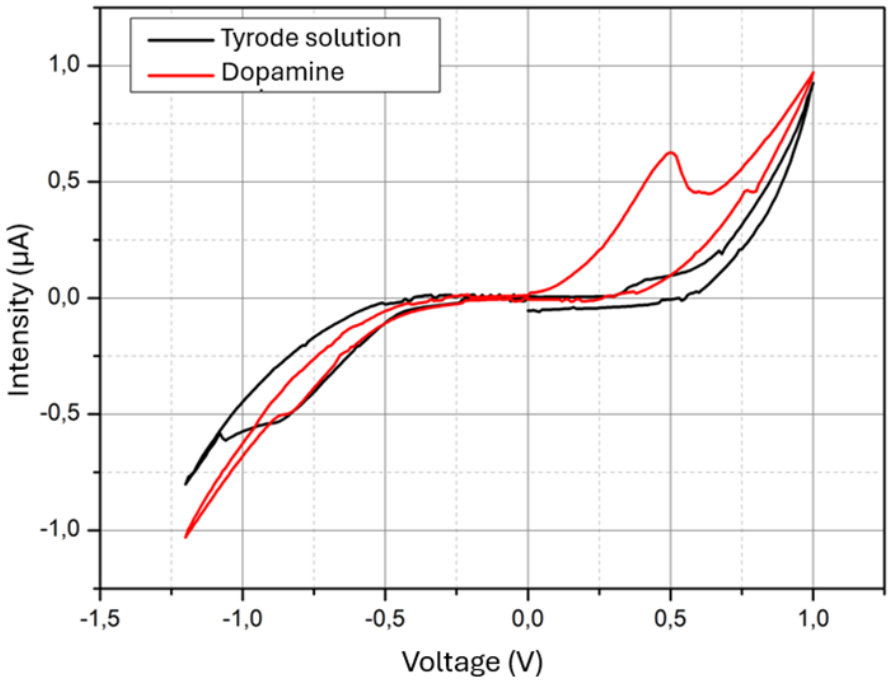

Figure 3 Current voltage curves of a dopamine and salt solution recorded using the device reported in this work.

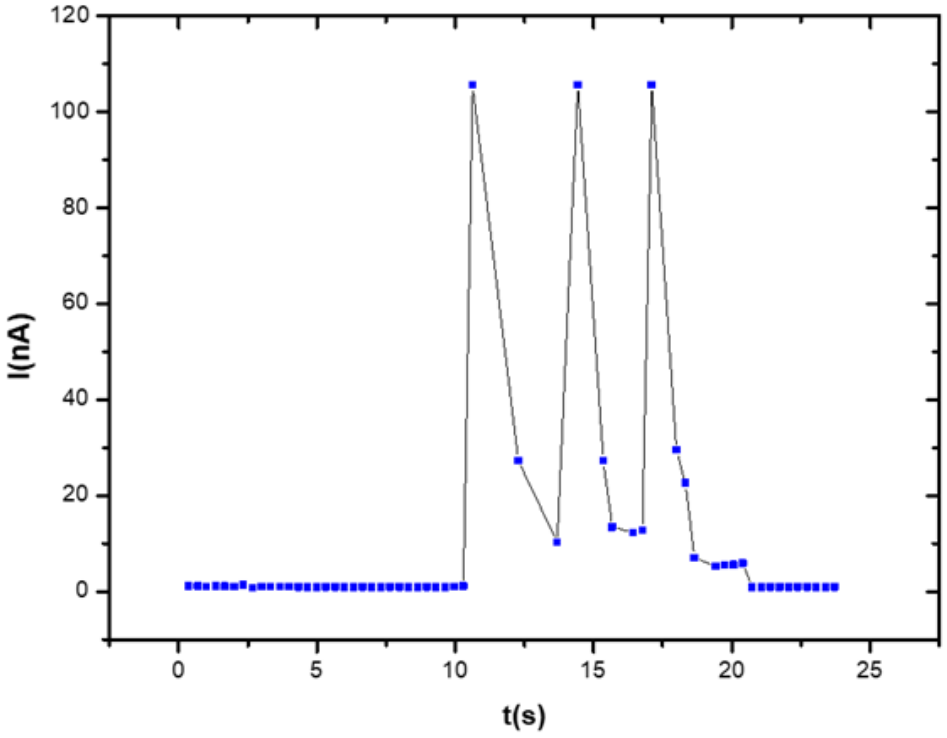

Figure 4 Current measurement over time highlighting of the capabilities of device assembly in detecting the passage of the dopamine microdroplets over the diamond biosensor electrodes.

4. CONCLUSIONS

In this work, we present an improved method to detect dopamine offering a novel approach that combines the precision of ion beam induced graphitic electrodes on diamond with the use of SLA 3D printed droplet-based microfluidics. The preliminary results demonstrate a fast and easy detection of dopamine with the possibility of measuring directly from single cells. Also, the use of 3D printing for the fabrication of droplet based microfluidic devices, offers the possibility to test various geometries and perform multiple electrochemical tests. Future research on this work can open the doors to extend the application of this technology to broader fields within neuroscience diagnostics.

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