Pyrolytic Carbon Nanograss Electrodes for Electrochemical Detection of Dopamine

Asif, A.; Heiskanen, A.; Emnéus, J.; Keller, S. S.

Published in:
Electrochimica Acta

Link to article, DOI:
10.1016/j.electacta.2021.138122

Publication date:
2021

Document Version
Peer reviewed version

Link back to DTU Orbit

Citation (APA):
Pyrolytic Carbon Nanograss Electrodes for Electrochemical Detection of Dopamine

Afia Asif, Arto Heiskanen, Jenny Emnéus, Stephan Sylvest Keller

PII: S0013-4686(21)00412-6
DOI: https://doi.org/10.1016/j.electacta.2021.138122
Reference: EA 138122

To appear in: Electrochimica Acta

Received date: 7 December 2020
Revised date: 11 February 2021
Accepted date: 7 March 2021

Please cite this article as: Afia Asif, Arto Heiskanen, Jenny Emnéus, Stephan Sylvest Keller, Pyrolytic Carbon Nanograss Electrodes for Electrochemical Detection of Dopamine, Electrochimica Acta (2021), doi: https://doi.org/10.1016/j.electacta.2021.138122

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier Ltd.
Pyrolytic Carbon Nanograss Electrodes for Electrochemical Detection of Dopamine

Afia Asif,1,2 Arto Heiskanen,1 Jenny Emnéus,1* Stephan Sylvest Keller2*

1Department of Biotechnology and Biomedicine, DTU Bioengineering, Technical University of Denmark, Produktionstorvet, Kgs. Lyngby, 2800, Denmark.
2National Centre for Nano Fabrication and Characterization, DTU Nanolab, Technical University of Denmark, Ørsteds Plads, Kgs. Lyngby, 2800, Denmark.
*Email: suke@dtu.dk; jenn@dtu.dk

KEYWORDS: carbon, pyrolytic, carbon nanograss, nanoelectrodes, electrochemistry, dopamine.

Abstract

Carbon microelectrodes are being used extensively in numerous applications due to their intriguing and promising material properties. Here, we present the optimized fabrication of carbon nanograss (CNG) electrodes with a single-step UV lithography with SU-8 photoresist followed by maskless reactive-ion etching and pyrolysis. This simple method provides nanostructured carbon electrodes with high surface area suitable for electrochemical applications such as biosensing or electrochemical monitoring of cells. The effects of the initial SU-8 film thickness and the etching time on the electrochemical response of the electrodes was investigated using cyclic voltammetry and electrochemical impedance spectroscopy. Finally, the CNG electrodes were tested for electrochemical detection of dopamine and compared with 2D pyrolytic carbon electrodes without nanograss. The carbon electrodes with the highest CNG displayed an approximately 2-fold increase in electrochemical signals compared to the 2D electrodes due to the increase in electrode surface area.

1. Introduction

Carbon can interact with almost all the elements to form organic molecules and compounds with vast physical and chemical properties1. As a single element it is present in different allotropes, including diamond, graphene, graphite, fullerene, carbon nanotubes, amorphous and glassy carbon.2 Carbon materials
possess several exciting properties relevant for electrochemical applications of advanced microfabricated
electrodes such as excellent electrochemical surface activity, broad potential windows, chemical stability,
and a large number of options for surface modification and functionalization.\textsuperscript{3,4}

Typically, 2D carbon electrodes commonly used as transducers in electrochemical sensing are produced by
simple fabrication processes such as screen printing or inkjet printing.\textsuperscript{5–8} These methods are associated with
relatively low cost and the flexibility to print a large number of different materials. However, these processes
might lead to issues such as low-quality surfaces, presence of organic residues, and less control over intricate
features. Alternatively, the carbon microelectromechanical systems (CMEMS) process provides the
opportunity to develop conductive carbon-based micro- and nano patterns from polymer precursor templates
using pyrolysis as a reproducible, cost-effective, and readily available method first pioneered in the 1970s.\textsuperscript{9,10}

In the typical pyrolysis process, the polymer template is gradually heated and carbonized at high
temperatures (i.e., 900\textdegree C and above) in nitrogen or argon inert atmosphere to achieve unique carbon
microelectrodes.\textsuperscript{11,12} The 2D pyrolytic carbon electrodes fabricated with the typical CMEMS process based
on pyrolysis of photoresists are extremely flat and non-porous. Therefore, the surface area available for
electrochemistry can be considered as equal to the projected area of the electrode.\textsuperscript{13}

A large number of studies have proposed the fabrication of 3D pyrolytic carbon microelectrodes, potentially
providing higher sensitivity due to their larger surface area available for electrochemical detection.\textsuperscript{14–16}

However, fabrication of actual 3D microelectrodes requires multiple steps of photolithography and
expensive microfabrication equipment, thereby severely limiting the simplicity and ease of the
microfabrication process.\textsuperscript{17,18}

Another approach to increase the surface area compared to 2D electrodes is the introduction of
nanostructures. The optimal nanostructured carbon electrode can provide ample surface area, porosity, high
conductivity, electroactive sites, low material costs, good reproducibility, and excellent mechanical, thermal
and chemical stability.\textsuperscript{19} Furthermore, for cell monitoring and characterization, random micro- and nano-
roughness of substrates is of great interest to promote cell adhesion and differentiation.\textsuperscript{20–24}

Recent advancement in fabrication of pyrolytic carbon nanostructures with the CMEMS process
demonstrated sub-10 nm carbon nanogaps in suspended glassy carbon nanofibers (GCNFs), a process in
which GCNFs are thinned using electrical heating of fibers up to their breaking point.\textsuperscript{25} The fabrication of
micro- and nanopillars from pyrolytic carbon, and tetrahedral amorphous carbon (ta-C) based materials has
recently been achieved using UV-embossing and etching followed by thin film deposition processes,
respectively. These structures were investigated as substrates for growth of mouse neural stem cells.\textsuperscript{26} The
electrospinning process of multi-walled carbon nanotubes (MWCNTs) combined with Au nanoparticles, and
synthesis of MWCNTs on Si/SiO\textsubscript{2} micro- and nanopatterned pillars has been evaluated for measurement of
dopamine neurotransmitter release, and guided growth of neural stem cells, respectively.\textsuperscript{27,28} In addition to
this, super-elastic graphene aerogel-based nanostructures have been utilized for high-performance
supercapacitors.\textsuperscript{29} Despite the promising potential of these new fabrication methods for carbon nanostructures, most of them are multistep techniques and predominantly include a complex interplay of several materials. Carbon nanomaterials such as carbon nanodots (CNDs), carbon quantum dots (CQDs), graphene quantum dots (GQDs), CNTs and CNF composites have also been explored for electrochemical and biosensing applications.\textsuperscript{30-32} However, these materials are mainly suitable for surface modification and functionalization techniques for already developed nanostructured carbon electrodes.

Highly aligned pyrolytic carbon nanowires have been fabricated using plasma-assisted etching and subsequent chemically functionalized to bind DNA.\textsuperscript{33} Recently, we have demonstrated that these pyrolytic CNG topographies significantly enhance neurogenesis and dopaminergic differentiation of human midbrain neural stem cells.\textsuperscript{34} Here, we present the role of optimized CNGs as electrodes for applications in electrochemistry particularly for electrochemical detection of dopamine. Herein, we adapted the simple fabrication process to integrate the high aspect ratio pyrolytic CNGs on electrodes for electrochemical sensing applications. The CNG electrodes were fabricated using a one-step photolithography with negative epoxy photoresist SU-8 followed by maskless reactive ion etching (RIE) and pyrolysis. Four types of CNG topographies were optimized based on electrochemical characterization using cyclic voltammetry and electrochemical impedance spectroscopy (EIS). It was found that an identical thickness of the bulk electrode electrically connecting the CNGs was essential for appropriate electrochemical behavior. Finally, the CNG electrodes were applied for electrochemical detection of dopamine, and the results are compared to the ones obtained with 2D pyrolytic carbon electrodes. The highly aligned, branched, and hierarchical nanostructured pyrolytic carbon electrodes provide a straightforward and scalable alternative to complex microfabricated 3D electrodes, displaying a low production cost and high electroactive surface area for electrochemical sensing applications.

2. Experimental Section

2.1. Carbon nanograss electrode chips

CNG electrode chips (10 mm × 30 mm) containing a pyrolytic carbon electrode with CNG structures on its top surface were designed and fabricated as shown in Fig.1. The central circular area of the electrode chip with a diameter of 4 mm served as a working electrode (WE) for electrochemical sensing. The fabrication process of CNG structures mainly involved UV photolithography using SU-8 photoresist, maskless reactive-ion etching (RIE) of the patterned SU-8, and pyrolysis of the etched SU-8 grass. Once the CNG structures were formed after pyrolysis, a 200 nm thick Au metal film was deposited defining the lead and contact pad of the WE. Finally, the entire chip except the sensing area and the contact pads was covered with a 5 \( \mu \text{m} \) thick SU-8 passivation layer patterned by a second step of UV photolithography.
Fig. 1. Design and fabrication process flow for the CNG electrodes. A) dispensing SU-8 2035; B) SU-8 spin coating with soft bake; C) UV exposure and soft bake; D) development to obtain a patterned planar SU-8 layer; E) maskless RIE of patterned SU-8 film to fabricate SU-8 nanograss; F) pyrolysis to convert SU-8 nanograss into CNG; G) e-beam Au deposition using a shadow mask; H) second photolithography step to define passivation layer of SU-8 2005.

2.2. Fabrication of pyrolytic carbon nanograss electrodes

The entire fabrication process flow is illustrated in a cross-sectional view in Figure 1A-H and top-view in Figure 1E-H. The UV lithography process starts with the growth of a 0.6 µm thick insulation layer of SiO₂ by low-pressure chemical vapor deposition (LPCVD) on a 4-inch silicon wafer. Each wafer contained 14 CNG electrode chips. Approximately 5 ml of negative epoxy photoresist SU-8 (SU-8 2035, MicroChem, USA) were manually dispensed and spin-coated on the SiO₂ substrate using an RCD8 T spinner (Süss Micro-Tec, Germany), Fig. 1A-B. A two-step process consisting of a spread cycle at 1000 rpm for 10 s with an acceleration of 200 rpm/s, followed by a thinning cycle at maximum speed of 5000 rpm for 120 s with an acceleration of 1000 rpm/s was used to deposit a uniform SU-8 layer with a thickness $t_{SU8} = 15.5$ µm. The soft bake process of 30 minutes was performed at 50°C with a temperature ramping rate of 2°C/min using a programmable hotplate (Harry Gerstigkeit GmbH, Germany). The soft baked SU-8 layer was then exposed to UV light with an energy dose of 2x250 mJ/cm² with a MA6/BA6 aligner (MA6-001320, Süss Micro-Tec, Germany), followed by 2 hours of post-exposure bake at 50°C with a ramping rate of 2°C/min (Fig. 1C). The uncrosslinked SU-8 was developed in PGMEA for 5 min (Fig. 1D). An additional step of UV flood-exposure
with an energy dose of 2x250 mJ/cm² followed by a long hard bake of 15 hours at 90°C on a hotplate was performed to completely crosslink the SU-8 layer.

For fabrication of the nanograss patterns, the crosslinked SU-8 layer was etched by a high power RIE method using oxygen plasma in an Advanced Silicon Etcher (ASE, STS MESC Multiplex. ICP serial no. 30343). The ASE was operated with parameter settings of 2000 W coil power, 30 W platen power, and 20 mTorr process pressure with oxygen gas at a flow rate of 95 sccm. The etching time was varied systematically with values of 5, 10, 15, and 20 mins to investigate four different types of SU-8 nanogras in terms of height and interspacing.

Next, the hairlike nanograss structures in SU-8 precursor material were carbonized in an inert nitrogen atmosphere at 900°C in a PEO604 annealing furnace from ATV Technologies GmbH (Vaterstetten, Germany). The pyrolysis process included mainly three steps: (i) temperature ramping to 200°C with a rate of 10°C and a hold time of 30 min, (ii) temperature ramping with 10°C/min from 200°C to 900°C for thermal decomposition and pyrolysis of the polymer, and (iii) a dwelling time of 1 hour at 900°C for further annealing of the carbon followed by cool down to room temperature with a rate of 10°C/min. After pyrolysis, the contact leads were fabricated using a 200 nm layer of Au on top of a 20 nm Ti layer using e-beam evaporation through a shadow mask. This was followed by a second UV lithography step for deposition and patterning of a passivation layer. A single spin coating step of 2000 rpm for 30 s with an acceleration of 500 rpm/s was used to spin coat a 5 µm thin SU-8 layer followed by a soft bake process of 2 hours at 50°C. The passivation layer was UV exposed with an energy dose of 2x250 mJ/cm² with a MA6/BA6 UV mask aligner and was baked for 2 hours at 50°C with a ramping rate of 2°C/min, followed by the development of SU-8 layer in PGMEA for 5 min. A flood exposure and a hard bake process was performed using the same parameters as described above. Finally, the CNG electrode chips were diced from the backside of the wafers with a 355 nm laser on a laser micromachining tool (microSTRUCT vario from 3D-Micromac AG) to a trench depth of 300 µm in order to protect the CNG structures on the frontside.

2.3. Structural characterization

The CNGs are high aspect-ratio random nanostructures that were easily destroyed during measurements using characterization methods such as atomic force microscopy (AFM) and stylus profilometry. Therefore, to carry out height analysis of CNG, scanning electron microscopy (SEM) on cross-sections was performed after laser-dicing of the wafers from backside with a 355 nm laser on a laser micromachining tool (microSTRUCT vario from 3D-Micromac AG) to a trench depth of 300 µm. The structural interspaces were measured by analyzing top-view SEM images, and determining the distance between CNG clusters.
appearing as bright spots. The dimensions for height and interspace are presented as mean value with ± standard deviation for n=15.

2.4. Optimization of CNG electrodes

First, SU-8 films with a thickness of $t_{SU8} = 15.5 \, \mu m$ without nanograss were prepared and pyrolyzed, serving as flat carbon (FC) 2D control resulting in a carbon film thickness $t_c = 2.2 \, \mu m$. These values were used to estimate the shrinkage of the film thickness during pyrolysis, which here is expressed as a proportional shrinkage factor $s$:

$$s = \frac{t_{SU8}}{t_c} = 7.0$$

In an initial series of CNG fabrication, the etching time of SU-8 films with the same thickness of $t_{SU8} = 15.5 \, \mu m$ was varied with values of 5, 10, 15, and 20 min to investigate the influence of this parameter on the height and interspacing of the nanograss. Accordingly, four different types of CNGs were defined as CNG5, CNG10, CNG15, and CNG20. The cross-sectional SEM images acquired as described above were used to measure the thickness of the residual bulk carbon layer $t_{Creel}$ for each of the four types of CNG after completed etching of the SU-8 film and pyrolysis. In a next series of experiments, the thickness of the SU-8 films before etching and pyrolysis was adjusted for the fabrication of each of the four types of CNG electrodes to achieve a thickness of the residual bulk carbon layer identical to the one of the 2D FC control according to:

$$t_{SU82} = t_{SU81} + s(t_c - t_{Creel})$$

This estimation is done with the assumption that shrinkage of bulk and residual SU-8 films during pyrolysis is independent of thickness in the investigated range of thin films. To achieve the calculated thicknesses $t_{SU82}$ of the SU-8 layer before etching, the spin coating process was optimized by tuning the spin speed during the thinning cycle. First, spin coating with predefined values was performed and the thickness values were interpolated to obtain the spin curve for SU-8 2035 (Fig. S1. Supporting Information). Based on this, the final spin coating speed was adjusted to be 3500 rpm, 2800 rpm, 2550 rpm, and 2200 rpm for CNG5, CNG10, CNG15, and CNG20, respectively. The other processing parameters were identical as described above. The thickness of the residual bulk carbon layer $t_{Creel}$ was again measured using cross-sectional SEM.

2.5. Electrochemical characterization
All reagents for electrochemical characterization were purchased from Sigma Aldrich/ThermoFischer. Prior to electrochemical characterization, all the electrodes were treated with oxygen plasma (Diener plasma surface technology, Zepto, Germany) with 50 W power at a 0.6 mbar pressure for 60 s. A custom-made electrode chip holder was fabricated using micromilling to carry out electrochemical measurements in a controlled environment (Fig. S2. Supporting Information). The electrochemical measurements were performed on 2D and all four types of CNG electrode chips using 10 mM potassium hexacyanoferrate (III/II) (K₃[Fe (CN)₆]/ K₄[Fe (CN)₆]) redox couple. The cyclic voltammograms (CVs) were acquired using a CHI 1010 potentiostat (CH Instruments, Austin TX), while the EIS spectra were acquired using a Multi PalmSense4 potentiostat (PalmSense; compact electrochemical interfaces, the Netherlands). The fabricated CNG electrode chips were employed as a working electrode (WE), a standard 500 µm diameter platinum wire (Advent Research materials Ltd., Oxford, England) as a counter electrode (CE), and a DRIREF-L Ag|AgCl with saturated KCl (WPI, Sarasota, FL) as the reference electrode (RE). After the fabrication of CNG electrodes was optimized, CVs were acquired for 2D and CNG electrodes using 300 µL volume of 10 mM and 250 µM concentrations of [Fe (CN)₆]³⁻⁴⁻ at a scan rate of 50 mVs⁻¹. The CNG and 2D electrodes were also characterized with EIS using 10 mM [Fe (CN)₆]³⁻⁴⁻, and by scanning the frequency range of 100 mHz to 1 MHz with 50 mV sinusoidal AC signal. The PSTrace 5.8 software was used to devise a modified Randles equivalent circuit and for extracting the fitted EIS parameters.

2.6. Dopamine detection

The 2D and CNG electrodes were evaluated for dopamine oxidation based on CVs acquired at a scan rate of 50 mVs⁻¹. Dopamine hydrochloride solutions with concentrations ranging from 1.25 µM to 250 µM were prepared in phosphate buffered saline (PBS, pH 7.4) under constant purging with nitrogen gas. The electrochemical setup used for these measurements was the same as described above. The solutions were utilized immediately after preparation to prevent uncontrolled oxidation of dopamine.

3. Results and Discussion

3.1. Optimization of CNG fabrication

The CNG structures were obtained by the three main process steps of i) UV photolithography with SU-8 photoresist, ii) maskless RIE of the SU-8 layer in oxygen plasma to form SU-8 nangrass structures, and iii) pyrolysis of the SU-8 nangrass in inert nitrogen atmosphere at 900°C converting it into pyrolytic carbon nangrass. The reason for the formation of these random high aspect ratio hairlike nangrass structures upon etching of the SU-8 layer is an accumulation of antimony present in the photoinitiator of SU-8 acting as a
micro-masking component during the etching process in oxygen plasma.\textsuperscript{33,35} Four different types of CNGs were fabricated by varying the etching time systematically from 5 to 20 min (CNG5, CNG10, CNG15, and CNG20) to achieve four variants of heights and interspaces. Fig. 2A-H shows cross-sectional SEM images of these four CNGs. The results showed that longer etching times led to larger CNG heights and interspaces between CNG clusters (Fig. 2. and Fig. 3A; values are tabulated in Table S1. Supporting Information).

To be able to explore these carbon nanostructures in electrochemical applications, the CNGs were then patterned on an electrode chip as shown in Fig. 1A-I. Fig.2I-L shows an edge of the electrode pattern with CNG. Here it is important, that the CNG structures are electrically connected and can be fully explored as integral part of the working electrode.

After the RIE etching process and pyrolysis, in addition to the formation of CNG, there was a residual bulk carbon layer left below the CNG. When the etching time was varied to achieve different heights of CNGs, the thickness of the residual bulk carbon layer in the initial experiments $t_{\text{Cres1}}$ also changed in an inversely proportional relation to the etching time, as shown in Fig. 2A-D and summarized in Fig. 3C. Hence, it was observed that CNG5 with the lowest nanograss displayed the highest $t_{\text{Cres1}} = 1.53 \pm 0.02$. This value decreased significantly for longer etching times to a minimum of $0.14 \pm 0.10 \mu\text{m}$ for CNG20. For the application of the CNG as electrodes, the residual bulk carbon layer has the important function of a current collector, electrically connecting the carbon nanostructures together and allowing interfacing with the external measurement setup. Therefore, the significant differences in thicknesses of the carbon bulk layers for different CNGs affected the electrochemical behavior. Thus, a specific optimization of the fabrication process was required to achieve an identical thickness of the residual carbon bulk layer for all CNG electrodes.

For the optimization of the thickness of the residual carbon layer, the SU-8 film thickness $t_{\text{SU8}}$ prior to etching and pyrolysis was adjusted for all CNG electrodes using Equation 2. The adjusted values of $t_{\text{SU8}}$ and experimentally measured values of $t_{\text{Cres2}}$ are shown in Fig. 3B-C and summarized in Table S1 (Supporting Information). After the optimized fabrication process, an identical thickness of the residual bulk carbon layer $t_{\text{Cres2}}$, was obtained for all four types of CNGs, as shown in Fig. 2E-H.
Fig. 2. (A-H) Representative cross-sectional SEM images of the four types of CNG structures on electrodes fabricated with 5, 10, 15 and 20 minutes of etch time from left to right respectively; (A-D) initial unoptimized CNG electrodes obtained from 15.5 µm thin SU-8 film resulting in variable thickness of the residual bulk carbon layer \( t_{Cres1} \) connecting the CNG (red-arrows); (E-H) final optimized fabrication with adjusted SU-8 film thicknesses resulting in CNG electrodes with uniform residual bulk carbon layer \( t_{Cres2} \) connecting the CNG, (red-arrows); (I-L) sideview of optimized CNG at the edge of an electrode area (Scale bars 5 µm).

Fig. 3. A) Dimensional analysis of the four types of CNG electrodes in terms of CNG height and CNG cluster interspace. B) Initial SU-8 thickness \( t_{SU81} \) and adjusted SU-8 thickness \( t_{SU82} \) before etching. C) Initial variable residual carbon thickness \( t_{Cres1} \) and final uniform residual carbon layer thickness \( t_{Cres2} \) after etching and pyrolysis

3.2. Electrochemical characterization with cyclic voltammetry
All four types of CNG electrodes were characterized as working electrodes (WE) in a three-electrode setup with cyclic voltammetry using [Fe(CN)₆]³⁻/⁴⁻ redox couple. The primary aim of this characterization was to evaluate the electrochemical performance of the four CNG structures on the WE and to compare them with the planar 2D carbon electrodes. Two main parameters were considered to identify the best electrochemical behavior of the electrodes: (i) higher peak currents \( i_p/i_{pc} \) demonstrate a higher electron-transfer electrode surface area, and (ii) a lower peak separation \( \Delta E_p \) indicates favorable electron transfer kinetics. Initially, 2D and the unoptimized CNG electrodes (Fig. 2A-D) were characterized with 10mM [Fe (CN)₆]³⁻/⁴⁻ solution by acquiring CVs without O₂ plasma treatment. However, it was observed that O₂ plasma treatment is crucial to obtain a hydrophilic CNG surface (Fig. S3A. Supporting information). Therefore, 2D and CNG electrodes were treated with 60 s of O₂ plasma before cyclic voltammetry characterization. Although, this improved the electrochemical behavior of the CNG electrodes significantly, the electrochemical behavior of the four unoptimized CNG electrodes was inconclusive (Fig. S3B. Supporting information). This was attributed to the decreasing thickness of the residual bulk carbon layer \( t_{Cres1} \) for increasing etching times, resulting in partially discontinuous CNG electrodes (Fig.2A-D). The fabrication of the CNG electrodes was then optimized as described above to provide the same thickness of the residual bulk carbon layer \( t_{Cres2} \) for all four types of CNG. The electrochemical response of CNGs was clearly improved compared to the unoptimized CNG electrodes (Fig. S3C. Supporting information). The optimized CNG electrodes were characterized by acquiring CVs using 250 μM [Fe (CN)₆]³⁻/⁴⁻ at 50 mVs⁻¹ as shown in Fig. 4A. All the CNGs exhibit approximately 2 folds increase in both anodic and cathodic peak currents compared to the flat 2D electrode (Figure 4B, Table S2. Supporting information) and for all cases the \( i_p/i_{pc} \) ratio was close to 1, which typically is one of the criteria for a reversible electrochemical process. Furthermore, it was observed that the peak currents increased with increasing height of the CNGs, which indicates a higher surface area for electrodes etched for a longer time. Moreover, the \( \Delta E_p \) for the CNG electrodes is considerably lower than for the 2D electrode and gradually approaches the value of 59 mV for longer etching times, which is the second criteria for reversibility (\( \Delta E_{p2D} = 250.3±0.6 \) mV, \( \Delta E_{pCNG5} = 138.7±2.1 \) mV, \( \Delta E_{pCNG10} = 128.0±1.0 \) mV, \( \Delta E_{pCNG15} = 114.0±1.7 \) mV, \( \Delta E_{pCNG20} = 111.7±1.5 \) mV).
Fig. 4. A) Characteristic CVs acquired in 250 μM [Fe (CN)₆]³⁻/⁴⁻ solution with the optimized CNG electrodes compared to the flat 2D electrode at a scan rate of 50 mVs⁻¹. B) Measured oxidation (black bars) and reduction (red bars) peak currents for the CNG electrodes compared to 2D; C) Typical EIS Nyquist plots acquired in 10 mM [Fe (CN)₆]³⁻/⁴⁻ solution for all four CNG electrodes compared with 2D electrode (the symbols show the experimental data points of EIS spectra and the solid lines indicate the fit of the data) with the equivalent modified Randles circuit (inset); D) Extracted charge transfer resistance $R_{ct}$ of the CNG electrodes and 2D electrode.

3.3. Electrochemical characterization with electrochemical impedance spectroscopy

EIS characterization with 10 mM [Fe (CN)₆]³⁻/⁴⁻ was performed to analyze the differences in charge transfer resistance among the four types of CNG electrodes and to further investigate the previous observation with respect to the electrode surface area. In EIS characterization, all the CNG electrodes and the 2D control electrode exhibited a similar general behavior with impedance spectra showing a small capacitive semi-circle
in the high-frequency region and a large semi-circle leading to a straight line at lower frequencies (Fig. 4C). To analyze the EIS behavior of the CNG electrodes, an equivalent circuit was devised (Fig. 4C, inset) based on the Randles model, where \( R_s \) is the electrolyte solution resistance, \( R_{ct} \) is the charge transfer resistance, and \( W \) is the Warburg impedance. To account for the double layer capacitor, the Randles model was modified using a constant phase element \( \text{CPE}_{dl} \), represented by \( Q \) (magnitude of \( 1/|Z_{\text{CPE}}| \) at \( \omega = 1 \text{ rad s}^{-1} \), and \( n \) (exponent with a value between 0 and 1). The equivalent circuit model was then used to fit impedance spectra for all four CNG electrode designs and the 2D electrode serving as a control. The extracted parameters of the equivalent circuit model are summarized in Table 2.

The high frequency part of the impedance spectra is modeled by resistance \( R_B \), and capacitance \( C_B \) which is related to the properties of bulk pyrolytic carbon material and additional contributions from the electrochemical setup such as parasitic capacitances. This distributed resistance and capacitance are mainly due to the \( sp^2 \) and \( sp^3 \) regions inside the material of the pyrolytic carbon that act as conducting and insulating areas, respectively. The large semi-circle in the intermediate frequency region represents the electron charge transfer kinetics of the redox couple at the electrode surface, while the straight line at low frequencies is the result of a diffusion-limited mass transfer process. For the intermediate frequency region, it was observed that the large semi-circles were recessed (i.e., the center of the circle was below the x-axis). This response typically means that the modelling of the electrode interface requires a constant phase element (CPE) to accommodate double-layer capacitance and probable surface inhomogeneities. For the CPE, the extracted values of \( Q \) increased and \( n \) decreased mainly due to the overall increase in the roughness and inhomogeneous electron transfer resulting from the increased surface areas for higher CNG on the electrodes.

The most relevant output value of EIS characterization was the charge transfer resistance \( R_{ct} \), which was observed to decrease gradually from CNG5 to CNG20 (Fig. 4D). \( R_{ct} \) is typically inversely proportional to the electrode area, and the results confirm a significant increase in the area of the CNG electrodes in comparison with the 2D electrode. Furthermore, the extracted \( R_{ct} \) values for the different CNG electrodes indicate that longer etching times lead to larger electrode surface areas, which is an advantage for many electrochemical applications.

### Table 2. Parameters of equivalent circuit model fitted to EIS spectra acquired in 10 mM [Fe (CN)₆]³⁻/⁴⁻ solution with pyrolytic CNG electrodes compared to the control 2D electrode, presented as average ± standard deviation for \( n = 3 \).

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>( R_B ) (Ω)</th>
<th>( C_B ) (nF)</th>
<th>( R_s ) (Ω)</th>
<th>( R_{ct} ) (Ω)</th>
<th>( Q ) (µmho)</th>
<th>( n )</th>
<th>( W ) (S s(^{1/2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D</td>
<td>51.4 ± 1.0</td>
<td>3.2 ± 0.3</td>
<td>72.1 ± 0.7</td>
<td>582.5 ± 9.2</td>
<td>2.6 ± 0.2</td>
<td>0.88 ± 0.02</td>
<td>216.7 ± 0.7</td>
</tr>
<tr>
<td>CNG5</td>
<td>53.3 ± 0.7</td>
<td>4.5 ± 0.5</td>
<td>65.1 ± 0.5</td>
<td>291.4 ± 9.4</td>
<td>7.1 ± 0.1</td>
<td>0.85 ± 0.02</td>
<td>205.7 ± 0.7</td>
</tr>
</tbody>
</table>
3.4. Electrochemical detection of dopamine

Dopamine (DA), a neurotransmitter in the central nervous system, is among others responsible for modulating the functions of motor neurons, and regulating many critical functions such as emotions and reward. The real time electrochemical detection of DA released by exocytosis from neural stem cells has been introduced as promising method to identify dopaminergic phenotypes in stem cell research. Therefore, the fabricated and characterized electrodes were applied for the electrochemical detection of DA. More specifically, the oxidation of DA was investigated using cyclic voltammetry. The CVs acquired in 250 μM DA solution showed a significant increase in the anodic peak current \( i_{pa} \) for the CNG electrodes compared to the 2D control (Fig. 5A). The increase in the anodic peak currents was larger for higher CNGs and was almost 2 folds for CNG15 and CNG20 (Fig. 5A-B, and Table S2. (Supporting Information)). This is explained by an increase in the surface area of CNGs as their height increases, confirming the previous observations for cyclic voltammetry and EIS with \([\text{Fe(CN)}_6]^{3-/4-}\). However, when comparing CNG15 and CNG20 electrodes, the increase becomes only marginal (Fig. 5A and B). The increase in cathodic peak currents was also observed to be more pronounced for electrodes with higher CNGs and currents were larger for CNG electrodes compared to the 2D control (Fig. 5A and B).

Thus, the CNG15 was selected for DA detection in a more detailed comparison with the 2D electrodes. First, CNG15 electrodes were characterized in a wider range of scan rates by using 250 μM DA solution (Fig. 5C). A linear relationship of the anodic peak current with the square-root of the scan rate (up to 500 mVs\(^{-1}\)) for both the CNG15 and the 2D electrodes was observed. Moreover, the \( \Delta E_p \) was observed to decrease from 2D to the CNG15 electrode with a value of \( 69.2 \pm 0.8 \) mV for 2D, and \( 49.9 \pm 0.8 \) mV for CNG15, (Table S3. Supporting Information). Overall, this demonstrates the excellent electrochemical behavior of the pyrolytic carbon material even at higher scan rates (Fig. 5D).

The sensitivity of the CNG15 electrode for DA detection was determined by acquiring CVs in solutions with a range of DA concentrations (Fig. 5E). The measured values were compared with those acquired on 2D electrodes (Fig. 5F). The results in Figure 5F show a linear increase in the anodic peak currents with increasing concentrations. For CNG15 the peak currents were almost twice the ones obtained with 2D electrodes throughout the complete concentration range. The slope of the fitted linear function in \( i_{pa} \) vs. concentration graphs was extracted to determine the sensitivity of CNG15 electrodes compared to 2D and was calculated to be 96.7 μA mM\(^{-1}\) and 46.5 μA mM\(^{-1}\) for CNG15 and 2D electrodes, respectively. Overall,
the results demonstrate excellent electrochemical properties of the CNG15 electrodes and show that the etching process improves the electrochemical behavior.
Fig. 5. A) Characteristic CVs of 250 μM DA solution acquired at 50 mVs⁻¹ using the optimized CNG electrodes and a control 2D electrode. B) \( i_{pa} \) and \( i_{pc} \) of the four types of CNG electrodes compared to the 2D electrode for 250 μM DA. C) Multiple CVs of the CNG15 electrode with 250 μM DA solution acquired at different scan rates. D) Relation of \( i_{pa} \) vs. the square root of scan rate for the CNG15 and 2D electrodes. E) Multiple CVs acquired using different concentrations of the DA at 50 mVs⁻¹. F) Relation of \( i_{pa} \) vs. the different concentrations of DA for the CNG15 and 2D electrodes.
4. Conclusions

We have optimized the fabrication process for CNG electrodes with a single step UV lithography process followed by maskless etching and pyrolysis. The required identical thickness of the bulk carbon layer for all four types of CNGs was achieved by estimating the shrinkage of the SU-8 photoresist during the pyrolysis process and adjusting the parameters of the spin-coating step. The CVs for the optimized CNG electrodes were acquired with \([\text{Fe (CN)}_6]^{3−/4−}\) redox couple. The obtained results showed an almost 2 folds increase in peak currents for the highest CNGs fabricated with the longest etching times (i.e., CNG 15 and CNG 20) compared to a 2D electrode with the same geometrical area. The optimization resulted in an evident increase of peak currents from lower to higher CNG electrodes due to an increase in electrode surface area. The EIS analysis of the CNG electrodes demonstrated a significant decrease in charge transfer resistance with the increase in the height of the CNGs. The combined results of the electrochemical characterization are excellent agreement with the observations during SEM inspection indicating that the electrode surface area increases with an increase in the etching time of SU-8.

Finally, the CNG electrodes were applied for electrochemical detection of DA using cyclic voltammetry. The CNG15 and CNG20 electrodes displayed twice the peak current for DA detection compared to the 2D electrode. This also resulted in an almost 2 folds increase in sensitivity of the CNG electrodes compared to the 2D control. The CVs of DA in a wide range of scan rates measured using the CNG15 electrode confirmed the excellent electrochemical properties of the pyrolytic carbon nanostructures. The optimized fabrication of CNG electrodes is a simple approach to provide nanostructured carbon electrodes with a high surface area, which is of high interest for a large number of electrochemical applications such as biosensing or electrochemical monitoring of cell cultures.

Acknowledgements

The authors are thankful to Marie-Skłodowska-Curie Horizon-2020 Innovative Training Network (H2020-MSCA-ITN-2016) to support this work with the financial grant; agreement number 722779.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit author statement

Afia Asif: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Original Draft, Visualization Arto Heiskanen: Conceptualization, Methodology, Validation, Supervision, Review & Editing Jenny Emnéus: Conceptualization, Methodology, Validation, Supervision, Review & Editing, Funding acquisition Stephan Sylvest Keller: Conceptualization, Methodology, Formal analysis, Review & Editing, Supervision
References


