FABRICATION OF 3D DIAMOND MEMBRANES FOR MICROFLUIDIC SYSTEMS

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Abstract

Perfusion of cell medium, especially in microfluidic devices, can provide *in-vivo*-like conditions for cell cultures. The most recent demand on such systems is to include electronically active artificial cell support for in-situ monitoring. Diamond thin films exhibit advantageous combination of physical, mechanical, chemical, biocompatible and electronic properties for this purpose. In this work we explore two strategies for fabrication of self-standing three-dimensional nanocrystalline diamond membrane for implementation in microfluidic *in-vivo like* experiments: i) nucleation and chemical vapour deposition (CVD) growth of diamond on porous 3D carbon foam (with 80 pores per inch) and ii) selective diamond growth predefined by photolithographic processing using copper grid mask. The morphology and material quality of the fabricated membranes are characterized by scanning electron microscopy and Raman spectroscopy. We discuss influence of surface pre-treatment (plasma *vs.* wet chemistry), seeding procedure (water *vs.* methanol based diamond nanoparticle suspension), and diamond growth regimes (focused *vs.* linear antenna microwave plasma CVD system). Moreover, we identify crucial factors for fabrication of such membranes.

Keywords: nanocrystalline diamond, membranes, carbon foam, microwave plasma, microfluidics

1. INTRODUCTION

While majority of in-vitro cell growth studies and discoveries from the twentieth century were performed on a flat surface of Petri dishes [1], the use of three-dimensional cell culture continues to become more popular. This is because cells behave differently in three dimensions with structures at or below their own size [1, 2]. To improve cell growth supports, there's a general tendency towards perfusion cell cultures [3], especially in microfluidic devices [4, 5], which can provide in-*vivo*-like conditions for the cell cultures. Such conditions are considered fundamental for obtaining relevant data closer to conditions in living organism and avoiding experiments on animals at the same time.

In addition, there is an increasing variety of biosensors and read-out techniques to facilitate monitoring of cell cultures, including reaction of epithelial tissues and tumours to specific chemical agents. Cell-on-chip can be considered as the state-of-the art concept in this field [6]. It is invaluable for fast and sensitive cytomic and single-cell analyses. Implementation of smart and functional materials is highly demanded for such systems. Such materials are intended not only as passive cell supports or scaffolds, but also as electronically and/or electromechanically active devices (i.e. sensors and actuators). Here, nanocrystalline diamond exhibits a unique combination of physical, mechanical, chemical and semiconducting properties [7]. It is also highly biocompatible and generally well suitable as cell growth support [8-10]. In spite of these beneficial features, diamond devices have not yet been established in complex biotechnological systems such as electrically active lab-on-chip devices or artificial extracellular matrix (ECM) bio-reactors.

The biochips and bioreactors should integrate the ability: i) to create a sufficient support and environment for living cells, ii) to directly or indirectly detect cell responses to stimulation, and iii) to facilitate exchange of fluids. In this work, we focus on the fabrication of the core part in such system, a porous permeable three-dimensional (3D) nanocrystalline diamond membrane. Unlike many other commonly available membranes, the diamond membrane is highly optically transparent, it provides suitable cell support, and it can be electronically active at the same time. To accomplish this aim we applied two different fabrication strategies:

i) the diamond chemical vapour deposition (CVD) growth on porous 3D carbon foam (with 80 pores per inch) and ii) the selective diamond growth [11] predefined by optical lithography. Substrate characteristics and pre-treatment as well as membrane growth in two fundamentally different microwave plasma CVD systems are discussed. We identify crucial factors for optimal diamond membrane fabrication.

2. EXPERIMENTAL

3D nanocrystalline diamond membranes were fabricated by two fabrication strategies. A schematic overview of the fabrication processes is shown in Fig. 1.

Strategy I.: Duocel® carbon foam (15x8x3 mm^3) with 80 pores per inch (PPI) was used as substrate. The foam scaffold was used due to a simple cut into different shapes and sizes as needed for microfluidics. A uniform diamond coating inside the foam was expected as in the case of metallic meshes [12]. Due to a strong hydrophobic property some of the samples were oxidized in inductively coupled oxygen plasma at a radio-frequency power of 280 W for 3 minutes to facilitate aqueous solution permeability. Next, diamond seeding (nucleation) was performed by loading the foam into a closed 2 ml test-tube containing mixture of deionized water or methanol with diamond nanoparticles (DNPs) (powder from Microdiamant AG, Lengwil, Switzerland with median grains size of 18 nm) and ultrasonication for 30 minutes. The seeding was uniform and we did not observe a noticeable difference in seeding quality between aqueous and methanol-based suspensions.

Strategy II.: Silicon-silica substrates (6×6 mm^2 Si wafer with 1.4 µm layer of SiO$_2$) were spin-coated with positive polymer (ma-P1215) and processed by two-step optical lithography process where in the middle of the substrate a polymer grid (3x3 mm^2) was formed by using a grid mask on foil (100x100 µm^2 squares separated by 50 µm wide stripes). Then the samples were nucleated in ultrasonic bath for 40 minutes using the solution of DNPs (powder from NanoAmando, New Metals and Chemicals Corp. Ltd., Kyobashi with a median grain size of 5 nm) dispersed in deionized water. Nucleated substrates were dipped upside down into the acetone so that the rest of the polymer with diamond particles was removed (i.e. modified lift-off process). Thereby, a grid of diamond seeds with 100x100 µm^2 large openings was created.
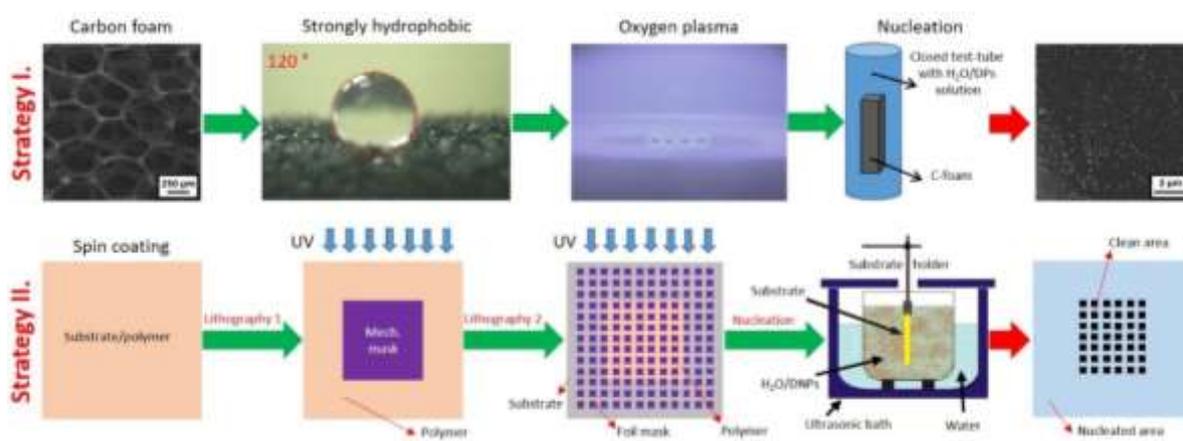

Fig. 1 Schematic view of the pre-treatment steps for the diamond membrane preparation by using porous carbon foam (Strategy I.) and the selective growth by using photo-lithography (Strategy II.).

The diamond CVD growth (Strategy I.) was performed by two systems: i) focused microwave (MW) plasma chemical vapour deposition (CVD) using an ellipsoidal cavity resonator for fast diamond growth [13] and ii) pulsed linear antenna MWCVD system with large spreading of plasma which is beneficial for coating 3D objects [12]. For the Strategy I. we studied the influence of deposition parameters on the diamond growth, namely the process pressure (2÷15kPa), MW power (2÷6kW) substrate temperature (500÷1200°C) and the gas composition (CO$_2$+CH$_4$ in H$_2$).

For Strategy II, when high growth rate is needed, the focused MW CVD system was employed to grow the self-standing diamond membrane. Depositions were done at the MW power of 5 kW, total gas pressure of 15 kPa from gas mixture of CH_4/CO_2/H_2 gases (3% CH_4 +2% CO_2 in H_2) for several hours.

The surface morphology was investigated by scanning electron microscopy (SEM, e_LiNe workstation, Raith, Dortmund, Germany) in standard configuration or by SEM field-emission gun operating in secondary electron mode (FE-SEM Tescan MAIA3). The material quality of the deposited diamond films was determined from the Raman spectra acquired by a Renishaw InVia Reflex Raman spectrometer with an excitation wavelength of 442 nm.

3. RESULTS AND DISCUSSION

3.1 Strategy I. - diamond growth on porous 3D carbon foam

The diamond growth on porous 3D carbon foam (Strategy I.) was done in the focused MWCVD system at varied growth conditions (deposition time, gas pressure, MW power and/or substrate temperature). Always, nucleated as well as not nucleated carbon foams were loaded to the chamber to compare the influence of seeding process. Fig. 2 shows the SEM images of representative samples which were deposited at optimized process conditions, i.e. at the total gas pressure of 3 kPa, MW power of 2.5 kW, 10% of CH_4 in H_2 and temperature 1100 °C. The using of high CH_4 concentration was found as crucial to increase diamond re-nucleation and the diamond growth rate. In other words, high CH_4 is needed to minimize erosion of the carbon foam by hydrogen rich plasma. The time dependence (15-120 min) confirms that increasing of deposition time results in the growth of larger spherical diamond crystals (or more precisely nanocrystals aggregates), Fig. 2a-c. For all deposition times the diamond film is not fully closed and exhibits weak adhesion as indicated in Fig. 2f. Similar limitation and/or troubles of diamond growth on carbon foams were also observed by Kobashi group who used diamond-coated carbon foams as a field emission source [14]. As diamond seeding was rather uniform and the inhomogeneities were independent of sample depth in our case, the limitation is assumed to be in the diamond growth and its adhesion to the carbon foam. Marton et al. have showed that different carbon allotrope forms are formed onto carbon foam substrate during the diamond deposition by modified hot filament CVD system [15, 16]. It should be noted that this limitation is common for other diamond growth techniques. A comparable results on the diamond growth by linear antenna CVD process [17] where the plasma volume is larger and the plasma density and concentration of active gas species in the substrate vicinity is reduced [18, 19].

Thus, it is cleat either chemical modifications of the substrate surface or deposition of adhesion interlayers are required in order to successfully grow diamond thin films. However, implementing and optimizing additional technological steps makes the fabrication process time-consuming and costly.
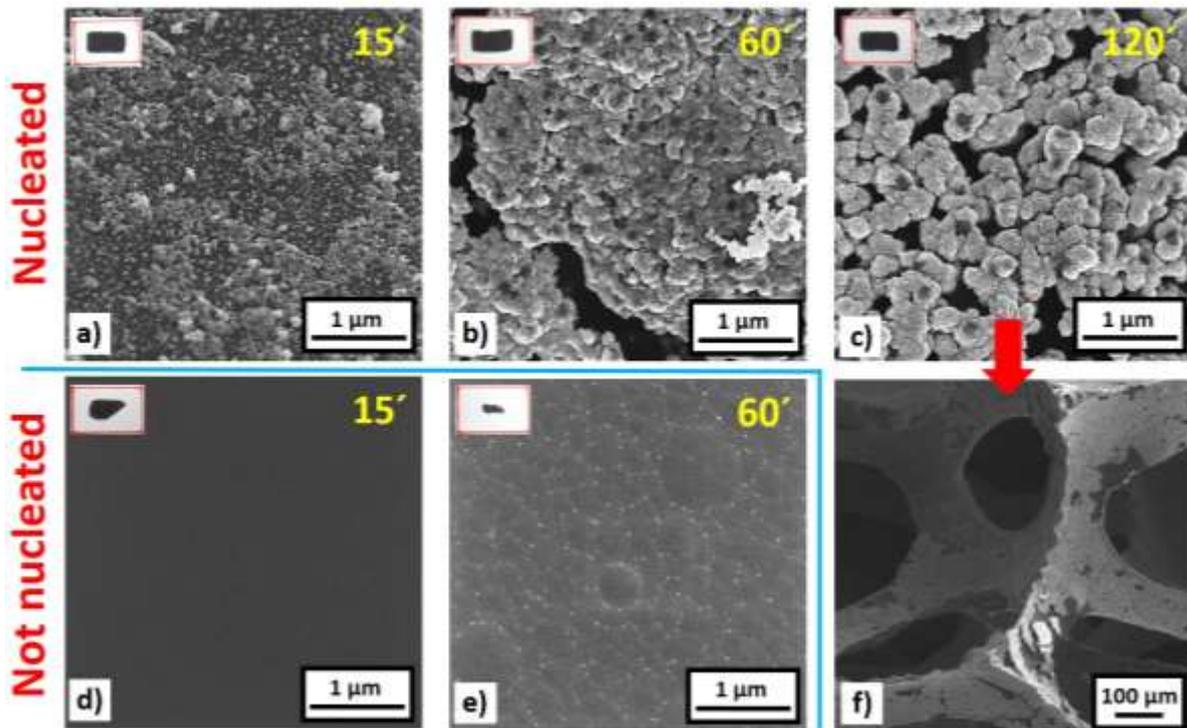
Fig. 2 Influence of the carbon foam pre-treatment (nucleated and not nucleated substrate) and diamond growth duration (15, 60,120 minutes) in focused MWCVD system on C-foam substrates. The insets show optical images of the carbon foam substrates after the process.

For a comparison, Figs. 2d and 2e show surface morphology of not nucleated carbon foam after 15 and 60 min growth process. No diamond film or even single crystals were deposited after the CVD process. We found that the foam volume diminished as shown by the inset images. The foam was fully etched away after 120 minutes. Keeping balance between the two competing processes during the CVD, i.e. growth of diamond particles on carbon materials versus plasma etching of the sp^2 hybridized carbon, is identified as a key and fundamental factor. Of course, the seeding step (i.e. seeding density) is a crucial factor for successful growth of diamond thin films [20].

3.2 Strategy II. - selective growth defined by photolithography

Fig. 3a shows the Si/SiO_2 substrate after the photolithography process. Regularly distributed squares of photoresist polymer are visible on the substrate. After the nucleation and removal of the polymer the DNPs formed a grid pattern on the substrate. In the ideal case, the diamond growth should occur only on the seeded parts, especially for short deposition times and low methane concentrations. However, the fabrication of free-standing membrane requires thickness at least several tens of micrometres, i.e. several hours of diamond growth. Under such conditions, a spontaneous diamond nucleation plays a role even though it is a very slow process in comparison to the standard nucleation methods [21]. Consequently, diamond grows also on non-nucleated parts (albeit with delay) and selective area growth is partially lost by parasitic growth of diamond. Fig. 3b shows the surface morphology of sample grown for 8 hours. Due to lower nucleation density, the parasitically grown grains on non-nucleated parts become larger than grains found on nucleated parts (area I. vs. area II. in the detailed SEM view, respectively)

Fig. 3c confirms that after the first 2 hours, the grown diamond grid was still well-defined and only few parasitically grown diamond grains were observed on non-nucleated parts (area I. in the detailed SEM view). These parasitic diamond grains are preferentially formed near the energetically favourable sites like

substrate defects and/or scratching lines [22]. To avoid a coalescence of the nucleated and not nucleated parts, we implemented an intermediate technological step. This step consists of repeated diamond deposition combined with intermediate wet etching of the substrate which removed the parasitically formed diamond with partial etching of opened substrate (Fig. 3d). One can see that this procedure lead to a successful growth of thick enough diamond membrane.
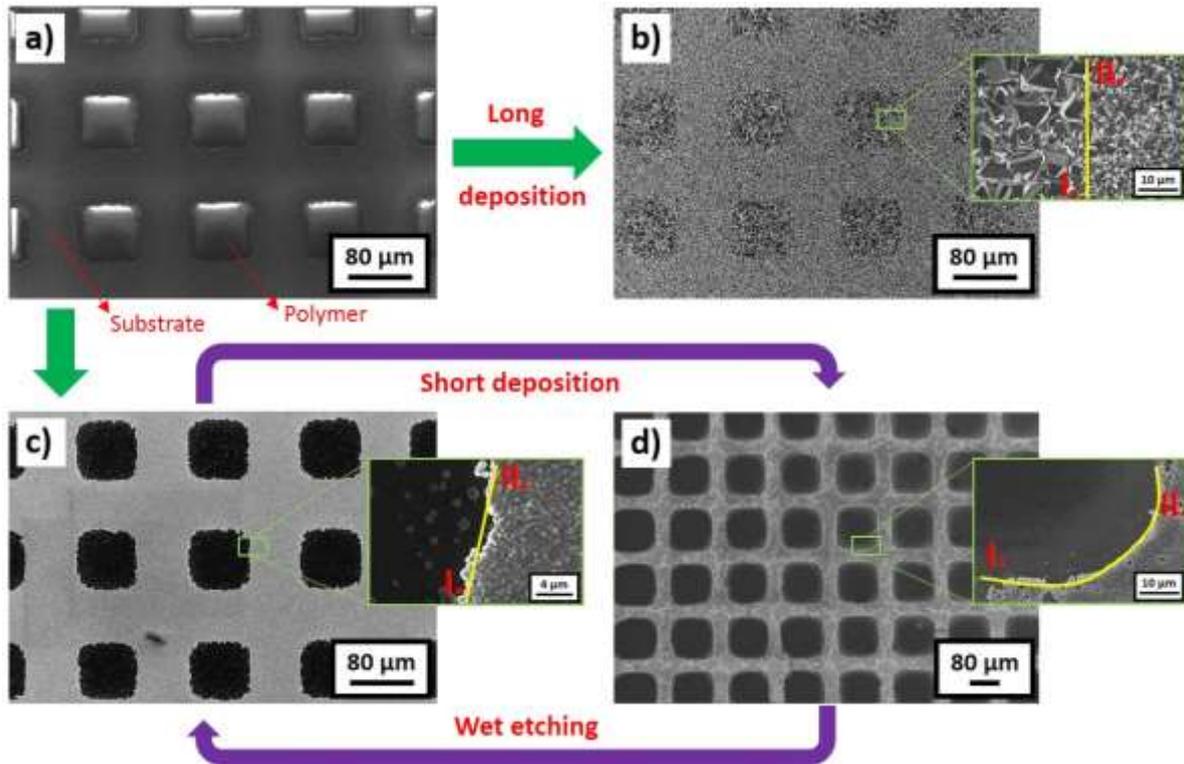

Fig. 3 SEM images of the self-standing diamond membrane during the fabrication process: a) polymer mask, b) diamond coalescence after 8h, c) grid still preserved after 2 h, d) membrane produced with intermediate wet etching steps.

Fig. 4 compares Raman spectra of the carbon foam substrate before and after 2 hours of diamond growth and of the selectively grown diamond membrane. Raman spectra do not reveal any difference between the virgin and diamond-coated carbon foam. Only strong D-band (1 360 cm^{-1}) as a consequence of structural defects and G-band (1 580 cm^{-1}) due to in-plane sp^2 vibrations of hexagonal carbon lattice are present for both samples. In our case the Raman penetration depth is about 1 μm, the spectrum is dominated by volume of the carbon foam compared to a thin diamond coating. Nevertheless, SEM images and change of the foam colour from black to white (due to light scattering) as well as foam resistance to plasma give an indirect evidence of diamond coating on the foam.

On the other hand, the Raman spectrum of the diamond membrane shows a clear sharp peak located at 1 332 cm^{-1} which is attributed to sp^3 hybridized carbon phases (the diamond peak). This is obviously assigned to large thickness of the selectively grown diamond membrane (30 micrometres) and the spectrum confirms high material quality of the membrane.
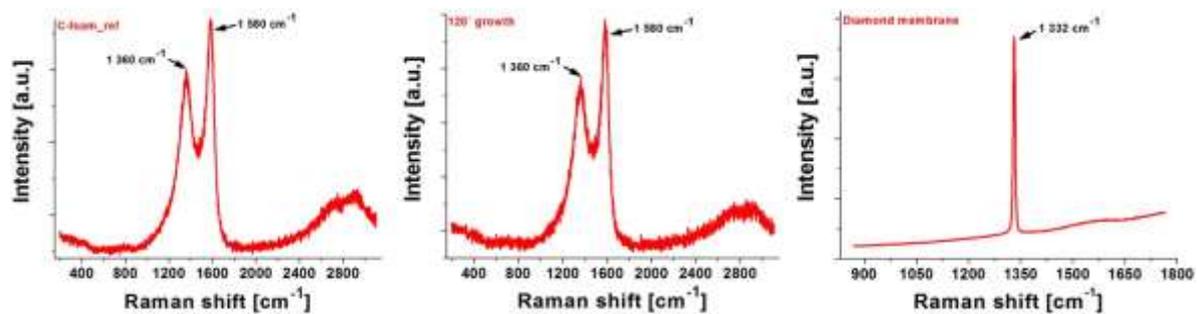
Fig. 4 Raman spectra of the carbon foam a) prior and b) after diamond coating. c) Raman spectra of the self-standing diamond membrane.

CONCLUSION

In this work we presented the fabrication of porous permeable diamond membranes employing two different strategies. At first, we showed the direct diamond growth on solid scaffold, in our case porous carbon foam. However, such promising way to obtain biocompatible diamond membrane met problems with the carbon foam etching and/or diamond film adhesion. The second strategy, based on the selective area diamond growth defined by the photolithography process, was more successful. We omitted the problem of spontaneous nucleation and membrane coalescence by implementing the intermediate wet etching step. Thereby, self-standing diamond membrane was produced after chemical dissolving the SiO_2/Si substrate. Membrane porosity as well as the thickness can be well controlled by the fabrication process, namely photolithography mask dimensions and deposition time. Such thin porous, yet self-standing and high quality membrane is in our opinion highly promising for application as electrically active scaffold in microfluidic perfusion culture systems.

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