3D shaped mechanically flexible diamond microelectrode arrays for eye implant applications: The MEDINAS project Matrices d'électrodes en diamant pour l'interfaçage neuronal appliqué à la suppléance fonctionnelle (MEDINAS)

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Abstract

The MEDINAS project aims at the fabrication of Micro-Electrode Arrays (MEA) that take advantage of a novel electrode material, namely synthetic conducting diamond, as the bio-interface material. Diamond enables the fabrication of electrodes with high biocompatibility, high electrochemical interfacing performances and long-term stability. The project aims at the development of diamond based MEAs for recording and stimulating of neuron networks for both in vitro applications (rigid substrate) as well as for in vivo retinal prostheses (flexible biocompatible substrate). The performances of this new technology were assessed as compared to standard metallic solutions.

Résumé

Le projet MEDINAS a pour objectif la fabrication de matrices d'électrode (MEA) développés à partir d'un nouveau matériau d'électrodes : le diamant synthétique dopé au bore comme matériau d'interface biologique directe. Le diamant présente en effet une biocompatibilité extrême, des performances électrochimiques remarquables et une excellente stabilité, indispensables pour la fabrication d'électrodes. Le projet a pour but le développement des MEA à base de diamant pour l'enregistrement et la stimulation des réseaux de neurones à la fois pour des applications in vitro (substrat rigide) ainsi que pour les prothèses rétiniennes in vivo (substrat flexible biocompatible). Les performances de cette nouvelle technologie ont été évaluées par rapport aux approches classiques à base d'électrodes métalliques standard.

Keywords

Neurobiological interfaces, CVD diamond, Retina implant, Micro-Electrode Arrays,

Mots clés

Interfaces neurobiologiques, Diamant CVD, Implant rétinien, Micro-électrodes

1. Introduction

The increasing life expectancy is certainly a source of future medical challenges that must be addressed today. The ageing of the population is accompanied with an increasing number of neurological diseases (Alzheimer, Parkinson, dementia, retinal degeneration, deafness, etc.), while other forms of neural trauma are also largely observed (spinal cord lesions, strokes, etc.). To cope with these pathologies, innovative therapeutic solutions are still sought. Recent advances in micro and nanotechnology have opened new routes for the fabrication of Micro-Electrode Arrays (MEAs) [1], paving the way to novel treatment strategies using neural implants. Implants can be used to electrically stimulate neural networks to relay an altered neural network to restore lost functions. For example, current commercial neuronal implants aim at stimulating the thalamus with high frequency for treating the Parkinson disease [2], or the inner ear from deaf people with auditive implants [3]. These successful treatments for everyday life have suggested that other applications could benefit from a similar approach, including treatments of epilepsy [4], dementia, headache [5], or visual disabilities [6].

Today, in the case of retinal degeneration, that affects almost 30 Million people in both EU and US, among the most frequent pathologies, photoreceptor degeneration is causing blindness in both hereditary diseases like retinitis pigmentosa and non-hereditary retinal diseases like age macular degeneration (AMD). Despite a substantial secondary degeneration of inner retinal neurons [7], the neuronal circuit remains active and can still convey information to the higher visual centres. Retinal prostheses propose to restore vision by targeting electrical stimulation of this remaining retinal circuit. The concept has been validated in several clinical trials showing that patients were able to follow moving light targets and were able to identify known contrasted objects [8], [9]. The stimulating MEAs are commonly fabricated using metal electrodes directly in contact with the neurons and electrically stimulating the cells. Such an approach however exhibits several limitations, and particularly since injecting current into the neural tissue requires transforming electronic current (from electronics) into ionic current (within the neural tissue). This transformation is done at the electrode/tissue interface by two phenomena of charge transfer. First, capacitive stimulation occurs as the variations of the electrode potential attract and/or repulse ions on the tissue side. However the amount of charge that can be transferred purely by capacitive effect is small and often not sufficient to stimulate neurons. Most of charge injection is thus achieved by a second phenomenon, charges transfer: When a positive current is injected, the electrode becomes more positive, and the electrode material is then oxidized in order to translate negative ions from the tissue into a flux of electrons. Conversely, a negative current is injected by reduction of the electrode material. In order not to deteriorate the electrode, positive and negative currents injected during a sequence of stimulation should be balanced, and the stimulation current amplitude should be low enough to involve only reversible processes around the electrode material. If the electrode material hinders injection of the desired current, then other, non-reversible reactions occur, including hydrolysis of water contained in the tissue. These reactions deteriorate both the electrode (irreversible change of the material) and the neural tissue (important local changes in pH) [10]. Further, metal electrodes as generally fabricated from Ir, Pt or Au, i.e. passive materials that do not provide an optimal contact to neurons. This non-optimal contact may induce reactive gliosis (Muller cells), in the vicinity of electrical implants that produce an insulating surface between the

implant and the neuron. Such a process could also explain the progressive degradation of the electrodes that has often been observed [10].

2. Diamond electrode MEAs

Within the last two years and in the frame of the MEDINAS[1] project, we demonstrated the interest of a very novel material, namely nanocrystalline diamond (NCD), towards the development of novel MEAs for stimulation and recording, and particularly within the context of retinal diseases [11], [12]. Synthetic diamond can be deposited at low cost from methane and hydrogen in conventional microwave chemical vapour deposition systems and on various substrates from two to four inch in diameter. We demonstrated that NCD offers extreme biocompatibility and stability in physiological media [13]. For example, Fig. 1 shows for retina cells [14] a biocompatibility as high as that of glass for diamond, although it has the overwhelming advantage of being a semiconductor [15].

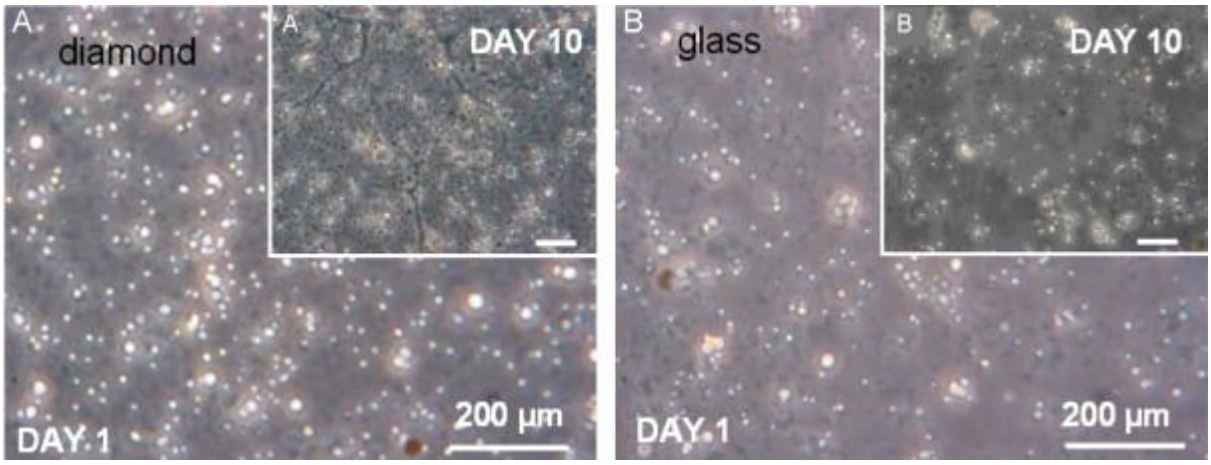

Fig. 1. Retinal cell survival on NCD diamond (left) as compared with glass (right) after one day and ten days (Insets). (Scale bars are 200 µm).

NCD can be processed using standard nanotechnology approaches for the fabrication of pixelized microelectrode arrays using the approach shown on Fig. 2. Based on the patterning of nanodiamond seeds prior to growth, it comes possible to fabricate structured MEAs where the active area is diamond. From the use of 3D shaped moulds performed in a substrate, using e.g. conventional silicon processing approaches, diamond MEAs with 3D geometries can thus be fabricated. Reporting the MEA from a sacrificial layer to a flexible substrate also enables the fabrication of flexible implants for retinal stimulation as shown on Fig. 3. Here this ultimate challenge was to make the fabrication of diamond compatible with a soft substrate material, and it was achieved using a sacrificial substrate lift-off technique, enabling the preparation of such implants on polyimide as well as on parylene. During the MEDINAS project, matrices of 64 independent pixels for the stimulation of tissues were fabricated. Further, the possibility to fabricate electrodes with 3D geometry was demonstrated, and prototypes exhibiting wells with improved performances for cell stimulation were explored (under patent app. [16]).

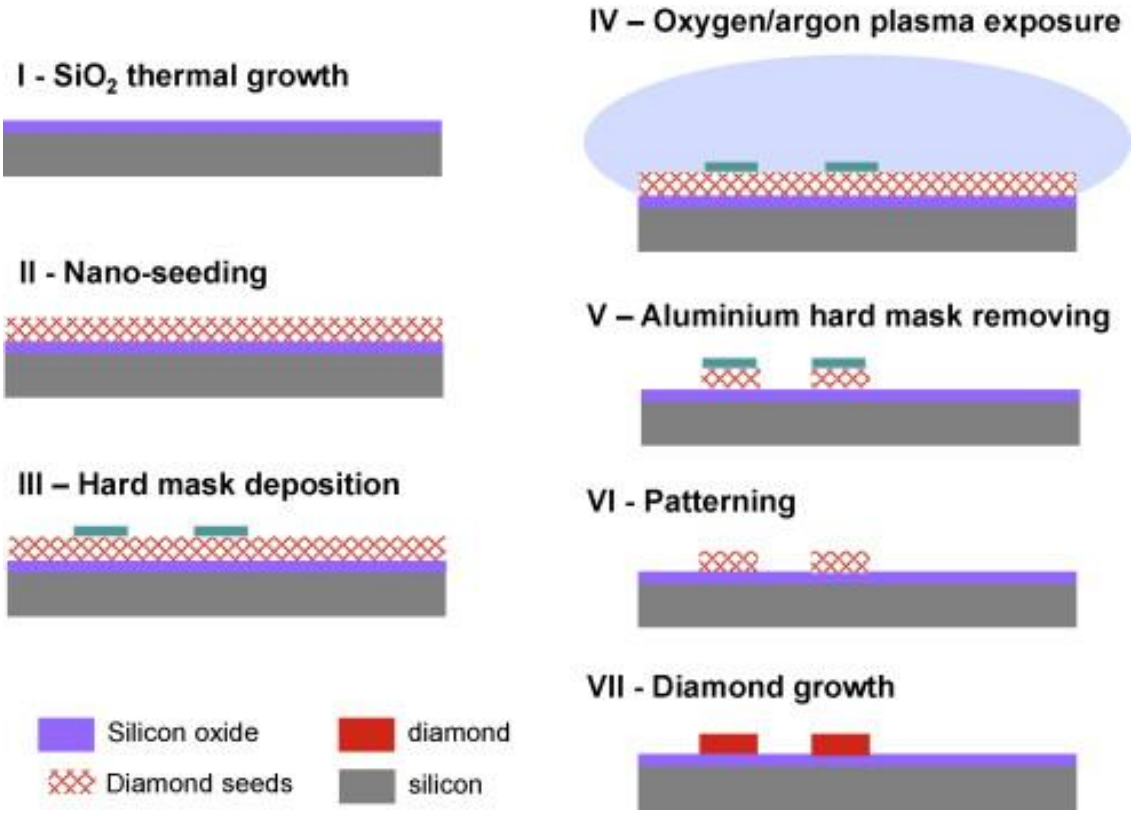

Fig. 2. Schematic of the step flow process to enable patterned diamond growth on varying substrates. Here described on oxidized Si of course processable on glass, quartz, alumina, etc.

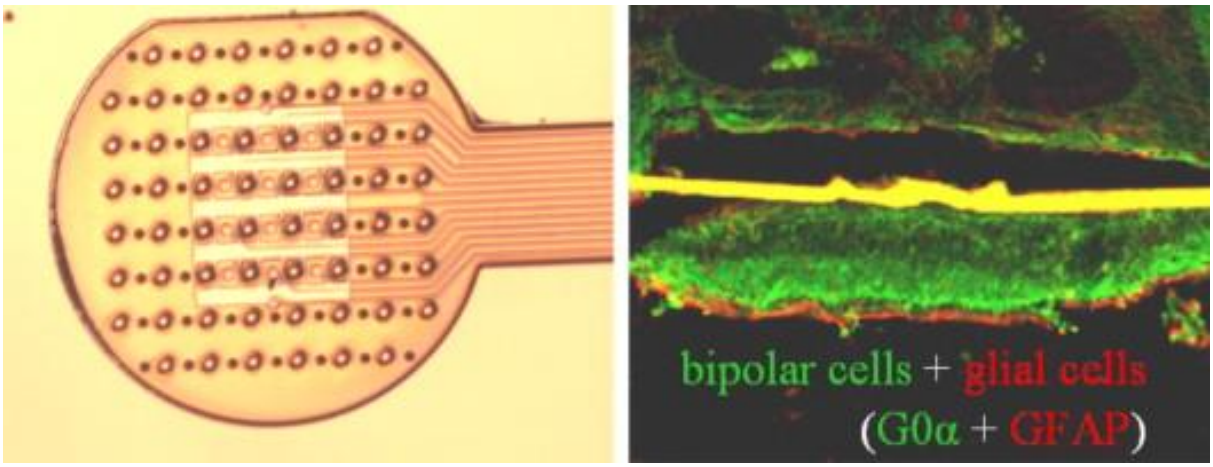

Fig. 3. In vivo implant. Left: view of patterned diamond microelectrode arrays on soft polyimide as fabricated for retinal implant fabrication. Right: section of a rat retina with an implant (yellow).

The electrochemical benefits were evaluated on MEAs where symmetrical current pulses were injected while the local variation of the pH was monitored on the electrodes using the pH sensitive fluorescent dye BCECF [17] in an unbuffered media. While the charge injection limit remained in the 10–100 $\mu C/cm^2$ range with Pt microelectrodes (in agreement with e.g. [18]), values as high as several hundreds of $\mu C/cm^2$ could readily be achieved using diamond microelectrodes [19].

3. Preliminary evaluation of diamond retinal implants

The first prototypes of diamond coated retinal implants were fabricated (Fig. 3) and to test their in vivo stability, we have developed a technique to introduce implant devices in the subretinal space of blind P23H rats. After 14 weeks in vivo, the eye was fixed and histological sections of the eyes were produced to visualize the retinal tissue with respect to the implant. Fig. 3 (right) illustrates the immunostaining of the section to visualize neurons (red) to be stimulated, the bipolar cells, and glial cells (green) isolating the implant from the neurons. The large red band represents the bipolar cells neurites, their cell bodies are visualized in blue with a very small red rim in between the 3D structures. These preliminary results are very encouraging because no major reactive gliosis (red) is detected in contact with the implant.

Conflict of interest statement

There is no conflict of interest.

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