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Biotin grafting on boron-doped diamond

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Grafting of biotin on top of a polycrystalline boron-doped diamond layer was achieved by surface oxidation followed by an esterification reaction and revealed by fluorescently labelled streptavidin.

The combination of electrical conductivity, mechanical stability and specific surface properties makes boron-doped diamond a promising material for numerous applications. The non-adsorbing feature of the H-terminated substrate induces an original electrochemical behaviour, namely a very low background current in water and a particular sensitivity for outer sphere redox systems.[1] Anodic polarisation of such a diamond electrode in water is highly effective in oxidising any soluble or deposited organic species.[2] At the same time this process leads to an oxygenated diamond surface exhibiting modified properties, especially a different adsorbing behaviour[3] and a larger potential window in aqueous electrolytes.[4] The carbon nature of this material is also of prime interest in the design of biologically active functionalised surfaces. As a matter of fact, DNA-modified diamond thin films showing good stability under successive hybridization–denaturation cycles have been described recently.[5] The authors grafted DNA on the diamond substrate using aminoalkyl linkers which were covalently attached by the UV assisted reaction of ω-unsaturated alkylamines on the H-terminated surface.

Anodic polarization of the boron-doped diamond in aqueous media is expected to produce a mostly hydroxylated surface but only few examples of diamond functionalisation by means of these chemical groups have been described up to now. The most illustrative could be the silanisation reaction performed by Fujishima *et al*.[3] We propose here a straightforward esterification reaction, based on a peptide synthesis method, to graft biotin on a boron-doped diamond surface. In order to obtain versatile biological surfaces, the great affinity between avidin and biotin is extensively used for biochemical micro-engineering. Then we checked our surface biotinylation with fluorescent labelled streptavidin. This method appears quite effective for investigating the hydroxylation of the diamond surface. It also opens exciting perspectives in the bioengineering of diamond materials.

Polycrystalline diamond layers were synthesised on a silicon high purity p-type wafer by microwave plasma enhanced chemical vapour deposition in a conventional reactor.[6] The growth conditions used were as follows: substrate temperature 700–900 °C; total gas flow of a mixture of 0.7% methane in hydrogen 100 sccm; total pressure in the reactor 20 torr (30 mbar); microwave power 700 W. The dopant source was boron oxide set in a Pt crucible placed on the substrate holder near the silicon substrate. Before commencing the diamond growth, the silicon substrates were ultrasonically damaged with diamond powder in ethanol in order to improve the nucleation density.[7] After deposition, the methane flow was stopped and the films were kept under hydrogen plasma for an additional 30 min. After 24 hours of deposition, the film thickness averaged 8 μm. Dopant concentration in the diamond layers, as estimated from Raman spectroscopy measurements,[8] was in the range 10^{19} to 10^{20} cm^{-3}. The film resistivity was $\leqslant 0.1$ Ω.cm as measured with a four-point probe. The polycrystalline diamond film consists of randomly oriented crystallites of few microns size and with predominantly cubic (100) and triangular (111) faces. No element traces other than carbon or oxygen were found by XPS on the diamond surface.

The H-terminated diamond layer was then oxidised in a classical three-electrode cell. The silicon wafer (1 cm diameter) covered by the diamond layer was set into a home-made Kel-F® sample-holder fitted with an electrical back connection. A platinum plate (1.8 cm^2) was used as counter electrode, a saturated calomel electrode (SCE) as potential reference and a 0.2 M aqueous sodium sulfate solution as electrolyte. A 2.2 V potential against SCE was then applied to the diamond electrode for 200 seconds. Typically the current rose rapidly to roughly 1 mA and then decreased steadily on 100 s to reach a background value of around 0.1 mA. The charge consumption during this polarization time was around 20 mC. We observed no oxygen bubble on the diamond surface. The material sample was then copiously washed with de-ionised water. Interestingly the central area, which was in contact with the electrolyte, was more easily wetted by water than the peripheral layer edge, which was masked by the sample-holder. The samples were then allowed to dry in ambient atmosphere.

For chemical grafting we firstly dissolved 0.3 g of dicyclohexylcarbodiimide (DCCI) in 2 cm^3 of dichloromethane and then added 0.1 g of biotin. The studied diamond layers on silicon wafers were immediately immersed into this suspension in a capped flask for one day at room temperature. The substrate was then washed alternately with acetone and water several times and then placed in a pH 7 phosphate buffer solution (PBS) for one day to remove any remaining free biotin molecules from the surface.

To reveal the biotin grafting we used the well-known very strong affinity of this chemical group for streptavidin. The surface was first blocked with a bovine serum albumin (BSA) solution (1% BSA in PBS) for 5 minutes in order to reduce the non-specific protein adsorption sites. A solution of streptavidin–R-phycoerythrin (Molecular Probes 100 μg cm^{-3}) was then deposited on top of the diamond layers and the so conditioned samples were set in dark for 15 minutes. The substrates were then thoroughly rinsed and observed by fluorescence microscopy, using the green radiation of a mercury lamp and recording the red filtered images with a CCD camera. Fig. 1 displays characteristic sample images obtained for an electrochemically oxidised diamond layer that was submitted to biotin grafting (Fig. 1(a)) and also for a sample that was equally treated with the grafting suspension but without first oxidation stage (Fig. 1(c)). The phycoerythrin fluorescence was visible for the former while the second remained completely dark. Another equivalent negative test was also obtained when oxidised diamond layer was directly subjected to streptavidin adsorption. A magnifica-

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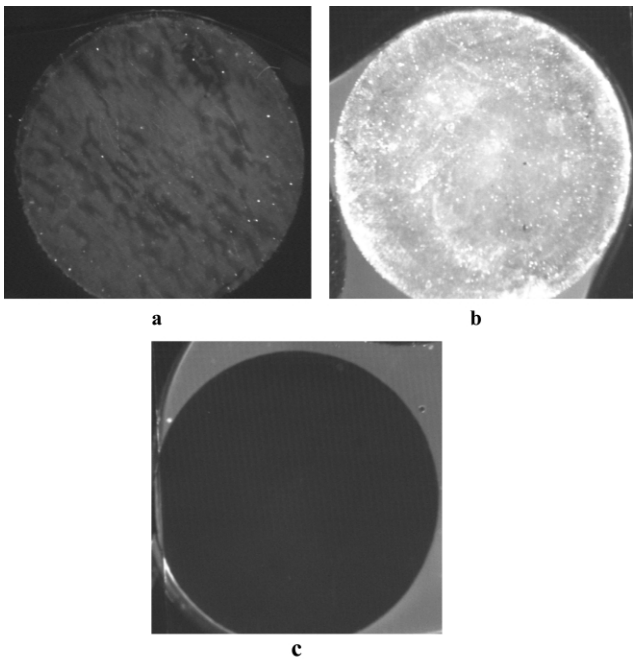

Fig. 1 Fluorescence microscopy imaging of a boron-doped diamond polycrystalline layer surface oxidised by electrochemical means (**a**) or singlet oxygen (**b**) and a H-terminated (**c**) after treatment by a mixture of DCCI and biotin and then by streptavidin labelled by phycoerythrin (see text).

tion of the Fig. 1(a) revealed a homogeneous lighting on the entire surface that did not seem to depend on the crystals face or size.

Grafting of biotin on the diamond surface was so demonstrated thanks to its strong affinity for streptavidin. Moreover previous oxidation is a prerequisite for the biotin chemical bonding which is in accordance with an esterification reaction as schematised in Fig. 2. We can also conclude that diamond oxidation indeed generates hydroxy groups on the surface. The nature and amount of the chemical functions generated by oxidation of the diamond surface are known to depend on the technique used and on the experimental conditions. Oxygen plasma for instance would produce fewer hydroxy groups than the electrochemical method.[3] The relation between electrical charge consumption during electrochemical oxidation and amount of grafted biotin remains to be precisely determined. As a matter of fact, drastic oxidation conditions at high potential led to a lesser number of hydroxy groups as revealed by our derivatisation method. We obtained much better results by using singlet oxygen produced by classical means. Submitting the diamond surface to oxygen produced this way led to the brightest samples following identical biotin grafting, bovine serum albumin adsorption and then phycoerythrin-labelled streptavidin adsorption (Fig. 1(b)).

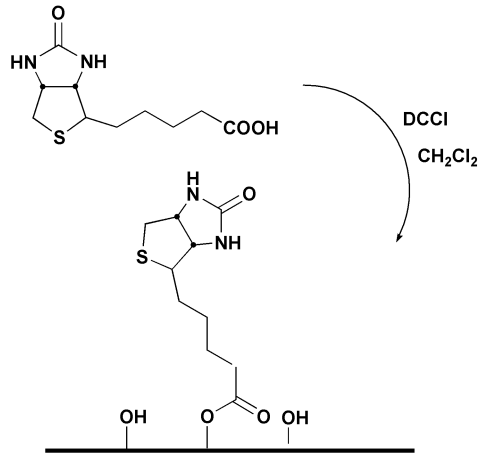

Fig. 2 Scheme of the proposed esterification reaction at the oxidised diamond surface.

The streptavidin can be removed from the diamond by a denaturing sodium dodecylsulfate solution (2% in water at 37 °C for 1 h), to restore the original biotinylated surface. Further streptavidin recognition tests were performed on these restored surfaces and proved the stability of the ester linkage in biological buffers.

Modification of the diamond surface by esterification reaction opens numerous future prospects. Indeed many chemical groups can be grafted by this method. The density of the hydroxy groups on the diamond surface depends on the oxidation technique. We found singlet oxygen the most powerful reagent for this purpose. It could open the way to selective activation of diamond electrodes in order to produce micropatterned surfaces. Our results reinforce the interest in boron-doped diamond as a substrate material for biosensing.

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