Photografting and patterning of oligonucleotides on benzophenone-modified boron-doped diamond

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Irradiation of a patterned benzophenone-terminated boron-doped diamond (BDD) surface with UV light (λ = 350 nm) in the presence of a 15^{mer} oligonucleotide resulted in the covalent linking of the DNA strand to the BDD interface.

The immobilization of biological molecules, such as oligonucleotides, proteins and enzymes, on surfaces is a critical step in many bioassays, including diagnostic analysis and bioelectronic sensing. Photochemical grafting methods have been widely used next to conventional immobilization methods for the covalent linking of polymers, as well as biomolecules.[1–5] One of the main advantages of using photoimmobilization rather than chemical or mechanical immobilization is almost certainly the possibility of micropatterning the surface with biomolecules using photolithographic approaches. This has important implications for medical diagnostic assays and high-throughput screenings for drug discovery. Benzophenone has been reported to be one of the most efficient photophores as it is stable under ambient light and in protic solvents.[4] It can be activated at wavelengths that cause little or no damage to biomolecules ($\lambda > 340$ nm) and can cross-link with high efficiency to C–H bonds within 3.1 Å of a carbonyl oxygen.[3,6] Thus, benzophenone has been immobilized on interfaces such as glass,[7] Au,[2] Pt,[8] or indium tin oxide[5] using photoreactive silane anchors, amine-functionalized disulfides to which benzophenone was chemically linked, or benzophenone modified polypyrrole. The use of diamond gained remarkable interest in the biomedical field[9–17] due to its excellent mechanical properties, extreme chemical stability and good electrical conductivity.[18] It has been persuasively shown by Hamers and co-workers[19] that the bonding stability of DNA to diamond compared to gold, Si and glassy carbon is significantly better, as no degradation of fluorescence intensity can be detected. Different strategies have been developed for the covalent linking of biomolecules to diamond surfaces.[11,15,20–22] However, there are no examples in the literature of the photochemically-induced immobilization of biomolecules on diamond surfaces.

We report here for the first time the feasibility of performing light-induced reactions on benzophenone-terminated boron-doped diamond (BDD) surfaces. The method relies on DNA immobilization and hybridization on chemically-functionalized and patterned diamond surfaces. The benzophenone termination was obtained *via* an esterification reaction of 3-benzoylbenzoic acid with photochemically oxidized and patterned BDD surfaces using carbodiimide coupling (Fig. 1).† As-prepared polycrystalline BDD substrates are hydrogen-terminated (H-BDD) and are rather chemically inert. However, as recently shown by us, photochemical irradiation of H-BDD using a low pressure mercury arc lamp results in oxygen-terminated diamond (HO-BDD) with a surface composition of ether (C–O–C), carbonyl (C=O) and hydroxyl (–OH) groups. The presence of surface hydroxyl groups was evidenced by the coupling with a perfluorotrichlorosilane.[23] The reactivity of the hydroxyl groups towards benzoylbenzoic acid in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) was employed to modify the HO-BDD with a benzophenone layer (BP-BDD). The resulting surfaces were characterized using contact angle measurements, X-ray photoelectron spectroscopy (XPS) and cyclic voltammetry (CV).

Contact angle measurements were used to follow the changes in the surface wetting properties. The as-deposited diamond surface showed a water contact angle of 93°, as expected for a surface terminated with hydrophobic C–H groups. Photochemical oxidation of the C–H bonds applied to the surface a hydrophilic termination (C–OH groups), with a resulting significant decrease in the water contact angle to θ = 10°, approaching a super-hydrophilic character. The water contact angle increased to 42° after a 12 h reaction of the hydroxylated diamond surface with the benzophenone derivative, and reached a constant value of 56° after 24 h.

XPS was used to analyze the chemical composition of the diamond surface before and after chemical modification, and also the nature of the chemical bonding associated with the transformations that occurred on the surface. The XPS and high-resolution XPS surveys of the as-deposited BDD surface displayed only a signal due to C 1 s at 285.08 eV. After UV/ozone

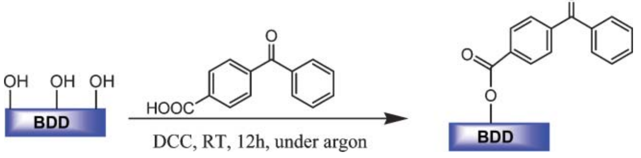

Fig. 1 Schematic illustration of the esterification reaction on HO-BDD linking the benzophenone group.

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irradiation in a vacuum for 30 min using a photo eximer lamp, an additional peak due to O 1 s at 531 eV appeared in the XPS spectrum, consistent with surface oxidation. High-resolution XPS displayed one signal at 288.73 eV corresponding to surface C=O features, a less resolved one at 285.8 eV representing C–O, and a signal at 285.2 eV due to C1 s from the bulk. The signal at 288.73 eV most likely resulted from a contribution of the carbonyl groups on the surface. Based on the diamond structure, it is expected that the sp^3 C–H bonds on the (111) facets will be terminated with hydroxyl groups while the CH_2 groups on the (100) facets will be transformed to carbonyl and ether functional groups. Esterification of HO-BDD with benzoylbenzoic acid did not have any effect on the XPS as only the quantity of carbonyl groups on the surface increased.

Furthermore, we studied the electrochemical properties of the BDD surfaces using $[Fe(CN)_6]^{3-/4-}$ as the redox couple in solution. Fig. 2A shows the CV *i–E* curves for an aqueous solution of 10 mM $Fe(CN)_6^{4-}$ in 0.1 M KCl recorded at as-deposited (hydrogenated), oxidized and benzophenone-terminated BDD electrodes. The *i–E* curve for the H-BDD showed a well-defined wave, with an apparent rate constant of $k^0_{app} = 0.01$ cm s^{-1} (H-BDD), while the photochemical oxidation (HO-BDD) resulted in a significant decrease in the apparent rate constant to $k^0_{app} = 0.0002$ cm s^{-1}. The BP-BDD electrode showed a $k^0_{app} = 0.001$ cm s^{-1}, indicating a less blocking character compared to the HO-BDD.

It is well known that benzophenone can also be reduced in a one-electron process in DMF to its radical anion.[24] From the electrical charge Q_a of the CV of the reduction step, the surface coverage (Γ) of benzophenone can be estimated according to $\Gamma = Q_a/nFA$, where F is the Faraday constant, n the number of electrons exchanged and A the surface area. A surface coverage of $\Gamma = (1.1 \pm 0.3) \times 10^{14}$ molecules cm^{-2} is obtained (Fig. 2B). This is in accordance with the surface densities reported by Hamers *et al.* using diazonium grafting chemistry[25] or photochemical modifications with olefin-containing molecules on H-BDD.[26]

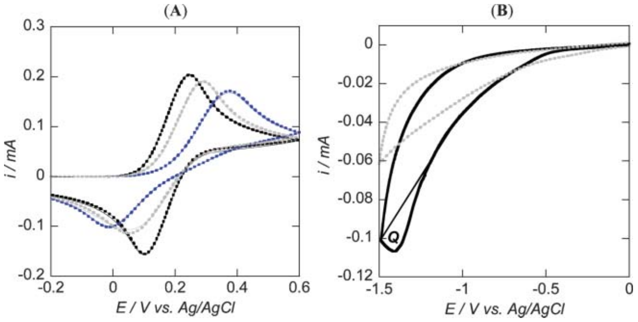

Fig. 2 (A) Experimental cyclic voltammograms of BDD electrodes in an aqueous solution of $Fe(CN)_6^{4-}$ (10 mM) in KCl (0.1 M): Hydrogen-terminated (black line), benzophenone-terminated (grey line) and oxygen-terminated (blue line) electrodes; scan rate = 50 mV s^{-1}, $A = 0.275$ cm^2; experimental data (full lines), DigiSim 3 simulated data (dotted lines). (B) Determination of the surface density of benzophenone by reducing the surface-bound benzophenone (black line) electrochemically in DMF/0.1 M $TBABF_6$ at 50 mV s^{-1}: The grey line corresponds to a control experiment where a HO-BDD surface was left for 12 h in 3-benzoylbenzoic acid in the absence of DCC.

To confirm the covalent attachment of the benzophenone moieties on the BDD surface, a control experiment, where an oxidized BDD surface was left for 12 h in 3-benzoylbenzoic acid in the absence of DCC, was performed. While the *i–E* curve of the resulting surface after rinsing was similar to that of an oxidized surface, a low electrical charge was obtained during the reduction reaction (Fig. 2, grey trace). This clearly suggests that under the experimental conditions, the benzophenone molecules were covalently grafted to the BDD surface *via* an ester linkage.

Photopatterning using a Cu-grid of 50 μm openings provided an approach for creating reactive domains on the surface with micrometer resolution, allowing the site-directed immobilization of biomolecules. The Cu-grid was sealed tightly against the hydrogen-terminated BDD surface and exposed to UV/ozone. Benzophenone moieties were covalently attached to the oxidized areas† to yield an array of benzophenone squares surrounded by hydrogenated areas. DNA arrays were next prepared by illuminating the patterned BDD surface in the presence of a 15^{mer} oligonucleotide (ODN) at $\lambda = 350$ nm for 30 min at a light intensity of 5 mW cm^{-2}.‡ After irradiation, the modified BDD was thoroughly washed with saline sodium buffer (SSC). Contact angle measurements after DNA photolinking showed a significant decrease from $\theta = 56°$ to $\theta = 12°$ on the patterned surface, which is in agreement with the presence of hydrophilic ODN. The hybridization reaction of the immobilized DNA target was performed with its complementary oligonucleotide bearing a Cy3 fluorescent label. After a 45 min reaction at 42 °C, the surface was rinsed with SSC and imaged using fluorescence microscopy. The fluorescence signal was observed in the expected squares (*i.e.* terminated with benzophenone moieties) (Fig. 3), while no signal arose from the H-terminated regions, implying the efficient inhibition of non-specific adsorption.

The results described herein demonstrate for the first time the successful covalent grafting and patterning of benzophenone moieties onto HO-BDD, as well as the subsequent photoinduced immobilization of oligonucleotides onto the surface. We are currently investigating the possibility of linking peptides and proteins to this interface.

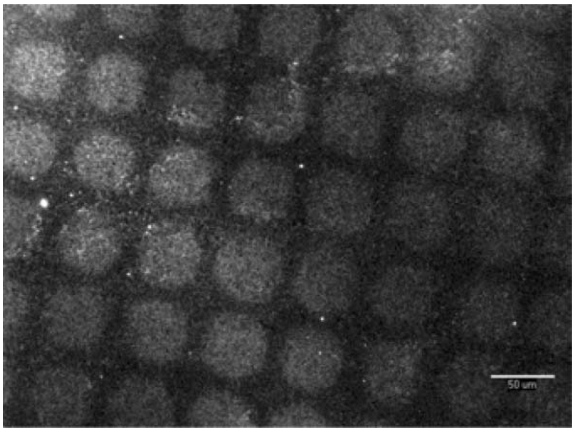

Fig. 3 Fluorescence image of DNA microarrays made from a photo-lithographically-patterned BDD surface. DNA immobilization was achieved in the benzophenone-terminated regions by illumination for 30 min at $\lambda = 350$ nm in the presence of ODN (10 μM) followed by hybridization with Cy-3 complementary ODN.

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Notes and references

† 3-Benzoylbenzoic acid (2 mmol) and *N*,*N*-dicyclohexylcarbodiimide (2.2 mmol) were dissolved in dry DMSO (10 ml). The oxidized BDD was immersed in the solution and left at room temperature for 24 h under a nitrogen atmosphere. The sample was then sonicated for 5 min each in DMSO, chloroform, ethanol and water, and dried under an argon stream.

‡ An ODN solution (10 μM in sodium phosphate buffer) was manually deposited on the BP-BDD surface, and the sample illuminated in air through a filter at a wavelength of $\lambda = 340 \pm 5$ nm for 30 min using a Xenon Lamp (LAX-102, Asahi Spectra, Japan). The resulting BDD sample was then sonicated for 10 min in SSC 2x solution and SSC 0.2x solution, and then dried under a stream of nitrogen.

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