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Attaching molecules to chlorinated and brominated amorphous carbon substrates via Grignard reactions

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Abstract

Amorphous carbon thin films are an attractive material as they provide a chemically robust surface that has been utilized in biomolecule array, biosensor, and bioelectronic applications. These thin films are particularly versatile as they are deposited at room temperature making them readily integrated with other materials and devices. Here we present an alternative means of functionalizing carbon substrates based on Grignard chemistry. First, the amorphous carbon substrates are halogenated with a solution-based, radical-initiated halogenation reaction using PX_5 ($X = Br$ or Cl). Next, the halogenated surfaces are modified via the formation of a carbon-carbon bond between the surface and the Grignard reagents employed. An alkyl-, perfluoroalkyl-, and a poly(ethylene glycol)-Grignard reagent were chosen to show the utility of this reaction scheme in functionalizing carbon surfaces to withstand oxidation and provide a hydrophobic and/or biocompatible substrate.

Introduction

Biomolecular array, bioelectronic, and biosensing applications require materials that are both chemically robust and stable under a variety of reaction conditions. Carbon-based materials have been utilized in such applications and exhibit superior stability over their silicon, glass, and gold analogs.^{1, 2} Amorphous carbon thin films are a particularly attractive material as they can be fabricated at room temperature, as opposed to the high temperatures utilized in glassy carbon and diamond thin film preparation, allowing them to be easily interfaced with a variety of materials and devices. Sputtered amorphous carbon thin films have been applied to electromechanical crystals,³ microelectrode arrays,⁴ and most recently to surface plasmon resonance (SPR)-compatible substrates.⁵

A wide variety of functionalization chemistries for carbon-based materials have been described, utilizing many of the reactions prevalent in organic synthesis. Diamond-related materials have been modified with organic peroxide radical initiators,⁶ gas-phase halogenation reactions,^{7, 8} electrochemical reduction of diazonium salts,⁹ and through the ultraviolet photo-assisted attachment of alkene-containing molecules.¹⁰ Amorphous carbon thin films have predominantly been functionalized by attaching alkene-containing molecules via photochemical and thermal methods.^{11, 12} Here we present a solution-based method of chlorinating and brominating amorphous carbon substrates, which provides an accessible means of halogenating carbon-based materials without need of gas-phase and/or ultrahigh vacuum methods.^{8, 13–16} Once halogenated, the substrates were modified by attaching

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Supporting Information: A detailed description of the fabrication of the amorphous carbon substrates as well as the synthesis of the Grignard reagents can be found in the Supporting Information section. This material is available free of charge via the Internet at <http://pubs.acs.org>.

dodecyl-, 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decyl-, and poly(ethylene glycol) (PEG)-groups to the surface via Grignard reactions (Scheme 1). Grignard reactions are an attractive means of functionalizing a substrate as the reaction proceeds in high yield and forms a carbon-carbon bond between the substrate and the molecule of interest.

Results and Discussion

In this work, amorphous carbon thin films were prepared by DC magnetron sputtering a graphite source (15nm thickness) onto gold-coated, glass slides at a base pressure of at least 6×10^{-6} Torr. The oxygen and nitrogen content of individual amorphous carbon thin films is dependent on the chamber base pressure at the time of deposition. Hydrogen-termination in a RF-generated hydrogen plasma reduces the heterogeneity of individual films by reducing the oxygen content and removing any detectable nitrogen.³ Six different amorphous carbon substrates were analyzed with X-ray photoelectron spectroscopy (XPS) after hydrogen plasma treatment. Each substrate contained peaks at 533.0 electron volts (eV) and 286.0 eV corresponding to O1s and C1s, respectively (Figure 1a). The oxygen-to-carbon ratio for the films was 0.086 ± 0.002 . The substrates were then chlorinated and/or brominated using a radical-initiated halogenation reaction. Each substrate was placed in a solution of 100mM PX₅ (X = Cl, Br) in benzene that contained a catalytic amount of benzoyl peroxide (5mg). The chlorinated substrates were incubated for 1 hour at 100°C and the brominated substrates incubated for 2 hours at 80°C. After the halogenation reaction, substrates were rinsed with benzene, ethanol, and then dried under a stream of nitrogen gas.

It should be noted that several reaction temperatures, times, and PX₅ (X= Cl or Br) concentrations were tested. The reaction conditions above provided the highest coverage of chlorine and/or bromine without rendering visible damage to the substrate such as discoloration and/or delamination of the amorphous carbon thin film. Prolonged exposures to higher concentrations of PX₅ caused the carbon film to delaminate.

After halogenation, each substrate was analyzed with XP survey spectra. In addition to the C1s and O1s peaks, the chlorinated substrates contained peaks at 272.0 and 201.0 eV, corresponding to the Cl2s and Cl2p electrons (Figure 1b).¹⁷ The brominated substrates contained peaks at 258.0, 191.0, 184.0 and 71.0 eV, corresponding to the Br3s, Br3p_{3/2}, Br3p_{1/2}, and Br3d electrons (Figure 1c). High-resolution XP spectra were also taken to investigate carbon-halogen bond formation. Figure 2a is a representative C1s spectrum obtained from a hydrogen-terminated amorphous carbon substrate. The peak contained two components, which are attributed to the presence of carbon-carbon and carbon-hydrogen bonds (285.0 eV) as well as to oxidized carbon species (285.6 eV). Colavita *et al* obtained similar C1s spectra for hydrogen-terminated amorphous carbon substrates.⁹ Figure 2b is the C1s spectrum of a chlorinated amorphous carbon substrate. The increased width of the C1s peak is attributed to the formation of a carbon-chlorine bond, evidenced by the appearance of a third (286.3 eV) component.¹⁸ A high-resolution spectrum revealed the Cl2p peak is shifted to 200.6 eV (Figure 3a), corresponding to previously described results for chlorinated diamond substrates.⁷ The brominated amorphous carbon substrate (Figure 2c) also has a broadened C1s spectrum with an added component at 287.9 eV, which is attributed to a carbon-bromine bond. Figure 3b is the high-resolution spectrum of the Br3d peak.

The survey XP spectra revealed that the chlorination reaction produced a chlorine-to-carbon ratio of 0.205 ± 0.016 while the bromination reaction yielded a higher bromine-to-carbon ratio (0.089 ± 0.006). Both of these values agree with the values obtained from the C1s high-resolution XP spectra. The 286.3 eV component of the chlorinated substrate, corresponding to a carbon-chlorine bond, accounts for $8.59 \pm 0.84\%$ (or 0.094 ± 0.009) of the carbon

components. Likewise, the 287.9 eV component of the brominated substrate, corresponding to the carbon-bromine bond, accounts for $15.93 \pm 0.98\%$ (or 0.189 ± 0.012) of the carbon components. The above values are averages from three amorphous carbon substrates.

The bromine-terminated substrates were modified with a series of molecules via Grignard reactions and then analyzed with polarization modulated Fourier transform infrared spectroscopic (PM-FTIRRAS) methods. The substrate's gold underlayer not only enhanced the overall reflectance but also defined the surface selection rules needed for PM-FTIRRAS measurements.¹⁹ Three molecules were used: dodecylmagnesium bromide, 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decylmagnesium iodide, and methoxy poly(ethylene glycol) magnesium bromide.²⁰ In each reaction, the substrates were immersed in a 1M Grignard reagent solution in diethyl ether and incubated at 80°C for 12 hours. Prior to PM-FTIRRAS analysis, XPS survey spectra were obtained to ensure excess reagent was removed (via the presence of magnesium, bromine, and/or iodine).²¹

The above molecules were chosen to show potential applications of this attachment chemistry. Alkyl-modification reduces the propensity for substrate oxidation in ambient atmosphere as well as in aqueous solutions. Bansal *et al* alkylated silicon surfaces with a variety of alkyl-lithium and alkyl-Grignard reagents and monitored their stability over time.²² Perfluorinated molecules offer a hydrophobic interface that when patterned can be used to sequester individual reactions to specific regions by creating hydrophobic borders. Patterned, perfluorinated self-assembled monolayers on gold have been utilized in this way to study cell adhesion parameters combinatorially.²³ Poly(ethylene glycol) was chosen for its biocompatibility. Several groups have shown that PEG-modified substrates are capable of resisting non-specific absorption of proteins as well as cells.²⁴

In Figure 4 are representative PM-FTIRRAS spectra of the brominated amorphous carbon substrates after the (a) dodecyl, (b) 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decyl, and (c) methoxy poly(ethylene glycol) molecules were attached. The surface infrared spectra were compared to attenuated total reflectance (ATR) infrared spectra of the neat molecules, whose structures were verified with ¹H NMR. To ensure each of the Grignard molecules was covalently attached to the substrate, a series of controls were used. First, hydrogen-terminated substrates were exposed to the Grignard reagents, thoroughly rinsed with ethanol, and their PM-FTIRRAS spectra collected. Second, hydrogen- and bromine-terminated substrates were exposed to the Grignard precursors (dodecane, 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decyl iodide, and methoxy poly(ethylene glycol), thoroughly rinsed with ethanol and PM-FTIRRAS spectra collected.

The PM-FTIRRAS spectra revealed a low level of attachment of the Grignard reagents to the hydrogen-terminated substrates, which is attributed to trace oxygen present on the substrate (Figure 1a). The degree of attachment is minute when compared to the brominated surfaces; after 12 hours of reaction time with the dodecylmagnesium bromide, the integrated intensity of the methylene stretching peaks for the brominated surface was 50 times more intense than for the control hydrogen-terminated substrates. The presence of a halogen is needed for the successful attachment of molecules via Grignard reaction conditions.

The work presented here provides an alternative method for functionalizing carbon-based materials using a two-step reaction scheme: radical-initiated halogenation followed by reaction with Grignard reagents. The solution-based halogenation reactions of amorphous carbon substrates described here, offer an accessible means of terminating carbon-based materials with chlorine and/or bromine atoms. Several Grignard reagents were attached to bromine-modified substrates and analyzed with PM-FTIRRAS methods, and their utility for attaching various molecules was demonstrated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. XP survey spectra of amorphous carbon substrates after (a) hydrogen termination, (b) hydrogen termination followed by chlorination and (c) hydrogen termination followed by bromination. Each spectrum contains peaks corresponding to O1s (533.0 eV) and C1s (286.0 eV). The chlorinated substrates (b) also contain Cl2s (272.0 eV), Cl2p (201.0 eV) peaks and the brominated substrates (c) Br3s (258.0 eV), Br3p (184.0 eV), and Br3d (71.0 eV) peaks.

Figure 2.

High-resolution XP spectra of the C1s region of (a) hydrogenated, (b) chlorinated, and (c) brominated amorphous carbon substrates. The solid black lines correspond to the data obtained from the instrument, the solid gray lines the deconvoluted components, and the dashed gray lines a background reference. (a) The hydrogen-terminated amorphous carbon substrates contain 285.0 and 285.6 eV peak components. The 285.0 eV peak corresponds to carbon-hydrogen/carbon-carbon bonds while the 285.6 eV peak is attributed to residual oxidized carbon species at or near the surface. (b) The C1s spectra for both the (b) chlorinated and (c) brominated amorphous carbon surface have an additional peak at 286.3 and 287.9 eV respectively, which are attributed to the carbon-halogen bond formation.

Figure 3.

High-resolution XP spectra of (a) the Cl2p region of a chlorinated amorphous carbon substrate and (b) the Br3d peak of a brominated amorphous carbon substrate. The solid black lines correspond to the data obtained from the instrument, the solid gray lines the deconvoluted components, and the dashed gray lines a background reference.

Figure 4.

PM-FTIRRAS spectra of the brominated amorphous carbon substrates after reaction with (a) dodecylmagnesium bromide, (b) 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decyl magnesium iodide, and (c) methoxy poly(ethylene glycol) magnesium bromide. (a) The dodecyl-modified substrates contained peaks corresponding to the symmetric and asymmetric methylene stretches (2852 and 2926cm⁻¹) as well as a peak at 2957 cm⁻¹ corresponding to the stretch of the terminal methyl groups. (b) The 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decanyl substrate contained faint methylene stretches (2853 and 2918cm⁻¹) as well as peaks at 1333, 1373, 1224, and 1183cm⁻¹ corresponding to the C–F vibrations. (c) The PEG-modified substrate contains prominent peaks at 2918 and 2853 cm⁻¹, corresponding to asymmetric and symmetric methylene stretches. The terminal methyl group (2954 cm⁻¹) and methylene bending (O – CH₂, 1348 cm⁻¹) are also present in the spectra.

Scheme 1.