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Rapid Grafting of Azido-labeled Oligo(ethylene glycol)s onto an Alkynyl-terminated Monolayer on Non-oxidized Silicon via Microwave-assisted "Click" Reaction

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Abstract

Microwave (MW) irradiation was used for the grafting of azido-labeled oligo(ethylene oxide) (OEG) on alkynyl-terminated non-oxidized silicon substrates via copper-catalyzed "click" reaction. The "clickable" monolayers were prepared by photografting of an α,ω-alkynene, where the alkynyl terminus was protected by a trimethylgermanyl (TMG) group, onto hydrogen-terminated Si(111) surfaces. X-ray photoelectron spectroscopy (XPS) was primarily employed to characterize the monolayers, and the data obtained were utilized to calculate the surface density of the TMG-alkynyl-functionalized substrate. MW-assisted one-pot deprotection/click reaction was optimized on the surfaces using azido-tagged OEG derivatives. Using MW instead of conventional heating led to a substantial improvement on the rate of the reaction while suppressing the oxidation of the silicon interface and OEG degradation. The antifouling property of the resulting substrates was evaluated using fibrinogen as a model protein. Results show that the OEG-modification reduced the protein adsorption by >90%.

Keywords

Click chemistry; Silicon substrates; Monolayers; Protein resistance; Oligo(ethylene glycol)

1. Introduction

Since the pioneering work of Linford and Chidsey,[1] interest in functionalization of non-oxidized silicon substrates with organic monolayers has grown rapidly, which is relevant to the development of silicon-based molecular electronics and biodevices.[2-4] In most reported systems, closely-packed alkyl chains are needed to stabilize the silicon interface against oxidation.[5,6] Recently, a variety of functional groups have been incorporated on the alkyl monolayers to achieve specific properties.[4,7,8] For many biological applications and studies, the ability to suppress non-specific binding of cells and proteins on the surfaces is critical.[9-11] For this purpose, the silicon substrates are commonly modified with oligo- or poly(ethylene glycol) (OEG or PEG), which are well-known antifouling and biocompatible materials.[12-14]

Photo- or thermal-assisted grafting of OEG-terminated alkenes onto hydrogen-terminated silicon surfaces via Si-C bond formation has been reported by us and others.[15-17] It was found that the OEG-terminated monolayers are highly protein-resistant and stable in

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Supporting Information Available: Synthetic schemes for the preparation of **TMG-C10**, **EG5N3** and **EG11N3**; angle-resolved XPS study for **TMG-C10** functionalized Si(111) surfaces. This material is available free of charge via the Internet at http://pubs.acs.org.

phosphate-buffered saline (PBS).[18] However, the OEG-alkenes (e.g. **EG7** in Scheme 1) are hygroscopic and likely to trap trace amount of water that is reactive to the H-Si surface under UV. Therefore, the photo-assisted grafting must be carried out in a strictly anhydrous and oxygen-free environment.[15] Additionally, compared to the deposition of common alkyl chains, direct grafting of OEG derived alkenes typically results in a lower density of molecules on the surface and hence poorer protection of the underlying silicon from oxidation.[19]

A useful alternative to the above approach is to first establish a well characterized monolayer platform containing handles on the silicon substrate, and then attaching the OEG and other functional components to this platform.[19-22] If the chemistry is properly chosen, this "graft-from" approach should greatly expands the range of functional moieties that can be incorporated on the platform. Over the past few years, copper-catalyzed alkyne-azide cycloaddtion (CuAAC) reaction, a well-recognized "click" reaction, has been widely used for surface modification due to its specificity and versatility.[23,24] Very recently, we have developed a novel platform presenting OEG-alkynyl groups.[25] The monolayer platform was prepared by photo-assisted grafting of an alkynene (**TMG-EG10,** Scheme 1**)** in which the terminal alkynyl group was protected with a trimethylgermanyl (TMG) group. We demonstrated that various azido-labeled biomolecules, such as OEG, biotin and carbohydrates could be directly grafted onto the TMG-alkynyl surface via a one-pot deprotection/CuAAC reaction.[25] Although using CuAAC reactions to graft OEG-azide onto surfaces presenting terminal alkynes were reported, the yields were generally not satisfactory (<50%) for surfaces with a high alkynyl density, probably due to steric hindrance and/or side reactions.[24,26,27] We demonstrated that the direct CuAAC coupling of OEG-azides onto the TMG-alkynyl surfaces generally gave higher yields (~70%), probably because the surface density of the free terminal alkynes is minimized, thereby may limit the potential competing side reactions and facilitate the click coupling.

Despite the simplicity and reliability of click coupling, the heterogeneous reaction with OEG-azide suffers from lower yield and slower reaction rate compared to the reaction with simple alkyl azido derivatives.[20] As part of our ongoing optimization of the click immobilization process, we report here a convenient and fast procedure using microwave (MW) irradiation for immobilization of OEG on TMG-alkyneterminated surfaces.

2. Experimental Section

Materials

Single-side polished silicon (111) wafers were purchased from University Wafer. For rinsing and contact angle measurements, Ultrapure water of 18.3 MΩcm resistivity obtained from a Milli-Q water purification system (Millipore) was used. 40% ammonium fluoride (NH_4F) solution (Sigma/Honeywell) was used as received. Other reagents and solvents were purchased from commercial suppliers (Aldrich, Fluka, or Alfa Aesar) and were used without further purification. The synthesis of **L1** was described previously,[28] and the protocols for the preparation of **TMG-C10, EG5N3** and **EG11N3** were described in the Supporting Information.

Formation of TMG-terminated monolayer on Si(111)

H-terminated silicon (111) surfaces were prepared similar to the procedures described by Chidsey et al.[1] Briefly, one-side polished silicon (111) wafers were cut into pieces of ca. 2 × 2 cm^2, cleaned with piranha solution H_2SO_4/H_2O_2 (v/v 3.5:1) at 80 °C for 20 min. Then the wafer was thoroughly washed with Millipore water, etched in 40% NH_4F solution for 10 min under N_2 purge, and dried with a flow of nitrogen. The freshly prepared H-Si (111)

substrate was placed inside a N_2 chamber, facing a droplet (~1 mg) of the alkene **TMG-C10** that was placed on freshly cleaned and dried quartz. After the system was degassed at vacuum for 10 min, the wafer was allowed to contact with the droplet, sandwiching a thin and homogeneous layer of the alkene between the substrate and the quartz wall. The substrate was then illuminated with a hand-held 254 nm UV-lamp (Model UVLS-28, UVP, 9.0 W) for 1.5 h, followed by washing sequentially with CH_2Cl_2 and ethanol, and finally dried with a stream of N_2. **Warning**: Piranha solution reacts violently, even explosively, with organic materials. It should be used with extremely care and should not be stored.

Microwave-assisted one-pot activation/click coupling reaction

The click coupling between the OEG derivatives and the **TMG-C10** derived monolayer on Si(111) surfaces was carried out in a sealed vial using a microwave reactor (Biotage Initiator 2.0, Figure S1 in the Supporting Information).

In a nitrogen glove box, a stock solution of ligand **L1** in H_2O (100 mM, 0.1 mL), OEG-azide in EtOH (50 mM, 0.1 mL) were combined in a reaction vial containing the TMG-terminated silicon surface ($0.5\times1 cm^2$) and a mixture of 2:1 degassed EtOH/H_2O (0.9 mL). Subsequently, sodium ascorbate (2.64 mg, 15.0 μmol) followed by $Cu(MeCN)_4PF_6$ (0.37 mg, 2.5 μmol) were added to the reaction mixture as the catalytic system. The reaction vial was sealed in the N_2 glove box, and then placed in the microwave reactor. The power of microwave reactor was in the range from ~30 W at 60 °C to ~40 W at 70 °C. After cooling to room temperature, the wafer was rinsed with EtOH, sonicated in 10 mM EDTA for 15 min, dried with a stream of N_2, and stored in PBS buffer (pH=7.4) before analysis. The sonication cleaning must be carried out to remove absorbed copper residue from the surface. The modified silicon wafers were stored in PBS buffer, which has been proved to suppress the autoxidation of OEG.[18]

X-ray Photoelectron Spectroscopy (XPS)

XPS data was obtained by a PHI 5700 X-ray photoelectron spectrometer equipped with a monochromatic Al Kα X-ray source ($h\nu$=1486.7 eV) incident at 90° relative to the axis of a hemispherical energy analyzer. XPS data was obtained at a photoelectron take off angle of 45 ° from the surface, and an analyzer spot diameter of 1.1 mm. The survey spectra were collected from 0 to 1400 eV, and the high resolution spectrum were obtained for photoelectrons emitted from C 1s, O 1s, Si 2p, N 1s and Ge 3d. All spectra were collected at room temperature with a base pressure of less than 1×10^{-8} torr. Electron binding energies were calibrated with respect to the C 1s line at 284.8 eV for C-C bond. A free software XPSpeak41, developed by Dr. Raymond Kwok (The Chinese University of Hong Kong), was used for all data processing. The high resolution data were analyzed first by background subtraction using the Shirley routine and a subsequent non-linear fitting by mixed Gaussian-Lorentzian functions. The sensitivity-corrected peak areas were used to estimate the atomic ratios.

For the angle-resolved studies, we determine the surface density of the monolayer by carefully measuring the XPS spectra of an H-terminated sample and a sample with grafted organic monolayer. The two Si samples (grafted and H-terminated) were placed side by side on a single sample holder to ensure identical alignment. The high-resolution spectra of Si 2p signal for these two silicon substrates were collected at 20°, 30°, 45°, 60° and 70° respectively.

Ellipsometry Measurement

The ellipsometer (Rudolph Research, Auto EL III) was employed for thickness measurement. It was operated with a 632.8 nm He-Ne laser at an incident angle of 70°. At least three measurements were taken for each sample.

Contact Angle Measurements

Static water contact angle was measured at room temperature and ambient relative humidity using a KSV CAM 200 instrument (KSV ltd.). The data were collected and averaged over the results from at least three separate slides.

Protein-resistance Measurements

The silicon substrates were immersed in a solution of 1 mg fibrinogen in 1 mL of 0.01 M phosphate buffered saline (PBS) at 20-25 °C for 1 h with slight shaking, and then rinsed with Millipore water to remove the non-adsorbed protein and salts, followed by drying with a stream of Ar. The sample obtained was immediately measured by XPS, and the increase of the N 1s signal intensity compared to the one measured on a monolayer of the protein and expressed as (% monolayer). The monolayer of fibrinogen was prepared under the above condition on a hydrogen-terminated silicon substrate and its monolayer thickness (64 Å) was confirmed by ellipsometry measurement.[15]

3. Results and Discussion

3.1 Assembly of TMG-terminated Monolayer on Si(111) (surface A)

As depicted in Scheme 1, the TMG-alkynyl-terminated monolayers were photografted on hydrogen-terminated Si(111) surfaces. The H-Si(111) surfaces were readily prepared by etching of the oxidized silicon (111) substrates with NH_4F solution. The photografting was performed in a N_2 glove box instead of in high vacuum as previously described for the OEG-alkenes (**EG7** and **TMG-EG10**),[15,25] since **TMG-C10** has a low boiling point and is non-hygroscopic. Compared to thermal-assisted grafting, the photochemical grafting is safer and cleaner, which proceeds rapidly (<2 h) at room temperature. Moreover, since only the irradiated surface needs to be covered with a thin film containing the molecule attached, it strongly reduces the required amount of material for monolayer formation, thus more suitable for the costly alkenes.

The formed TMG-terminated surface was characterized by XPS. Selected spectra acquired are depicted in Figure 1. The survey spectrum shows the presence of Ge, Si, C, and adventitious O, which is in good agreement with the presence of a TMG-terminated organic monolayer on the silicon substrate. In a narrow scan of Si 2p (Figure 1b), no appreciable features were observed at 101-104 eV, indicating that the surface oxidation is negligible after the UV irradiation. Figure 1c is the narrow scan of the C 1s region. The broad signal (1.52 eV fwhm) centered at 284.8 eV was arising from contributions of carbon atoms being either sp or sp^3 hybridized.[19] The emission of the carbon bound to the silicon (C-Si) and germanium (C-Ge) lay very close to the binding energy of the aliphatic carbons, and therefore, no effort for further deconvolution of the C 1s signal was made. Figure 1d shows the Ge 3d peak at about 32 eV, which is a characteristic signal from the TMG group. For the freshly prepared surface **A**, the atomic ratio of Ge/C was about 0.072. This value is slightly higher than the stoichiometric ratio (0.067), likely because the attenuation of C atoms is higher than that of the Ge atoms that are only present on the top of the monolayer.

Contact angle of 97±2° was obtained from surface **A**, which was consistent with the presence of hydrophobic TMG group on the top of the monolayer. As expected, this contact angle is higher (~15°) than those reported for the monolayers derived from non-1-yne-8-ene.

[29] Thus, both the results of XPS and contact angle measurements suggest that the TMG-protected alkyne did not react with the underlying hydrogen-terminated surface.

3.2 Quantitative XPS analysis of molecular coverage for surface A

The molecular coverage for the surface **A** was derived from XPS. Characterization of molecular coverage by XPS needs careful consideration of the attenuation of photoelectrons by the overlayer.[30] However, the previous "clickable" monolayer was generated by an alkene bearing ten EG units (**TMG-EG10,** Scheme 1). The long and flexible OEG chain, which helps to ensure the high protein resistant property, makes the surface coverage obtained from XPS characterization unreliable, largely due to the uncertainty of the attenuation length for Si 2p and C 1s photoelectrons through the OEG layer. In this study, the absence of the OEG layer in the film derived from **TMG-C10**, (surface **A**, Scheme 1) should allow more reliable characterization of density by XPS.

In order to derive the surface density of **TMG-C10** on Si(111), we use the following equation (Eq.1), which is similar to that reported by Hamers and coworkers:[31,32]

$$D=\frac{N_c L}{n_c}=\frac{C_{1s}\rho_{Si}}{Si_{2p}\rho_c}\left(\frac{N_{Si}}{n_C}\right)\left(\frac{\lambda_{SiC}}{\lambda_{CC}}\right)\left(\frac{L}{\lambda_{SiC}}\right)\lambda_{SiSi}e^{-(L/\lambda_{SiC})Sec\theta}\left[1-\frac{1}{1-e^{(\lambda_{SiC}/\lambda_{CC})(L/\lambda_{SiC})Sec\theta}}\right] \quad (1)$$

where D is the number of chains per unit area; N_c is the number density of carbon atoms; L is the thickness; n_c is the number of carbon atoms per molecular chain; N_{Si} is the number density of silicon atoms in Si substrate; ρ_{Si} and ρ_C are the sensitivity factors of Si 2p and C 1s photoelectrons; λ_{SiC} and λ_{CC} are the inelastic mean free path of C 1s and Si 2p photoelectrons in the alkyl monolayer; λ_{SiSi} is the inelastic mean free path of Si 2p photoelectrons in silicon; θ is the take-off angle between the surface and analyzer. The above parameters used for the density calculation were mainly referred to published literatures[31-34] or our instrument settings, which was summarized in Table 1.

In equation 1, there are two experimental values need to be determined: L/λ_{SiC} and C_{1s}/Si_{2p}. To obtain the value of L/λ_{SiC}, we used the following equation (eq. 2), which is applicable to an organic layer on non-oxidized silicon.[33,34]

$$\mathrm{Ln}\left(Si_{2\mathrm{pGrafted}/Si_{2\mathrm{pH}}}\right)=-L/\lambda_{\mathrm{SiC}}\,(1/\cos\theta) \quad (2)$$

where $Si_{2\mathrm{pGrafted}}$ and $Si_{2\mathrm{pH}}$ are the integrated area of Si 2p measured while the film is present ($Si_{2\mathrm{pGrafted}}$) and the Si 2p area of a freshly produced H-Si substrates ($Si_{2\mathrm{pH}}$), respectively. Thus, plotting the data points of -Ln($Si_{2\mathrm{pGrafted}}/Si_{2\mathrm{pH}}$) as a function of (1/cosθ) resulted in a straight line with a slope of 0.359 as L/λ_{SiC}. The detailed approach and corresponding XPS data is described in the Supporting Information (Table S1, Figure S1 and S2).

After determining L/λ_{SiC}, the other experimental value C_{1s}/Si_{2p} can be substituted into equation 1. C_{1s} and Si_{2p} is the integrated area of C 1s and Si 2p emission in XPS narrow scan, respectively. For H-Si (111) reacted with compound **TMG-C10** under 254 nm UV irradiation for 1.5 h, the ratio of the C 1s and Si 2p signals (C_{1s}/Si_{2p}) in equation 1 was measured to be 1.1 (before the correction with sensitive factors). Then a surface coverage of 3.3 (± 0.2) ×10^{14} molecules/cm^2 can be derived from Eq.1. Since the number of silicon atoms on Si (111) surface is 7.8 × 10^{14} atoms/cm^2, (42 ± 2) % of the silicon atoms on the Si(111) surface were bounded to the TMG-alkynyl-terminated molecules. Despite the presence of bulky TMG group at the distal end, the value was close to the coverage of monolayers derived from unsubstituted alkenes (43-49%) on Si (111). [33-35]

3.3 MW-assisted surface click reaction with OEG derivatives

In this work, microwave (MW) irradiation was used to accelerate the surface click reaction on surface **A**. Our initial inspiration of using MW irradiation as the heating source was motivated by the high efficiency of MW-assisted one-pot activation/Sonogashira coupling scheme for trimethylsilyl protected alkyne.[36] Very recently, similar palladium-catalysed alkynylation has also been developed on non-oxidized silicon surface in short reaction time via MW irradiation.[37]

MW irradiation is known to greatly accelerate the homogenous CuAAC reaction in solution.[38,39] Recently, Schubert and co-workers reported the rapid functionalization of azido-terminated alkylsiloxane nanostructures via MW-assisted click reaction.[40] We expect that MW irradiation may overcome the steric hindrance during the click grafting of the OEG chains, thus increasing the OEG density on the film. However, the increase of reaction temperature may also promote the oxidation of the silicon interface and the degradation of the OEG chains.[41-43] Thus, it was of great interest to test whether the use of MW would balance these two opposing factors.

3.3.1 MW-assisted degermenylation to produce alkyne-terminated monolayer (surface B)—The removal of TMG groups may be needed prior to the CuAAC reaction. We previously demonstrated that the removal of TMG from the alkynyl groups was promoted by Cu(I) and proceeded faster than the CuAAC reaction.[25] The effect of MW irradiation on the degermenylation of surface **A** in the absence of the OEG-azide was investigated with various reaction temperature (55-75°C) and times (10-20 min). The results are summarized in Table 2.

The resulting substrates (surface **B**) were characterized by XPS with the initial TMG-terminated monolayer as a reference, and the distinct Ge 3d signal was used to monitor the deprotection process. As shown in Figure 2, most of the TMG group (~75%) can be removed in 10 min at MW temperature of 60°C. Increasing the temperature and the exposure time of MW irradiation facilitated the degermenylation, but also led to the oxidation of the underlying silicon substrate, as indicated by the appearance of a broad Si 2p peak between 101-103 eV corresponding to silicon oxide or suboxide (SiOx, Figure 3b).

The presence of silicon oxide has detrimental effect on the electrochemical property of the system[44] and the stability of the films against hydrolytic cleavage.[45] To address the issue of oxidation, the solvent was first degassed (through 3 freeze-pump-thaw cycles) in a Schlenk tube to remove most of the dissolved oxygen. Subsequently, the reactants were carefully transferred and sealed in a MW reaction vessel in N_2 glove box. This approach minimized the oxidation of the silicon interface. As shown in Figure 3, when the MW reaction was carried out in aerobic environment, the signal from SiOx in the Si 2p region was significantly reduced and close to the noise level after 20 min at 70 °C. However, noticeable oxidation of the silicon substrate still occurred after exposure to MW for extended period of time (>25 min) or at high temperature (>75°C, data not shown), likely due to the presence of adventurous oxygen.

After the degermenylation with MW irradiation for 20 min at 65 °C, the water contact angle of the film was decreased by 10° to 86 ± 2°, in consistent with the higher polarity of the terminal acetylene relative to TMG groups.

3.3.2 MW-assisted one-pot click coupling of OEG on silicon substrate (surface C and D)—The above results provide a reasonable starting point for the optimization of the combined degermenylation and CuAAC coupling of OEG-azide derivatives on surface **A**. Various catalytic systems for the reaction on the ethynyl-terminated surface were examined,

including $CuSO_4$+sodium ascorbate,[46,47] $Cu(MeCN)_4PF_6$+ligand+sodium ascorbate in aqueous solution,[25,28] and $Cu(MeCN)_4PF_6$ + phosphorous ligands in organic solvents.[47] We found that the use of $Cu(MeCN)_4PF_6$ together with a monotriazole ligand (**L1**) and sodium ascorbate gave superior results in our hand as compared to other catalytic systems, and thus was also employed in this MW-assisted one-pot click coupling.

We then optimized the MW temperature and reaction time using two kinds of azido-terminated OEGs (**EG11N3** and **EG5N3**, Scheme 1). The range of temperatures (55°C-65°C) and the reaction times (10-30 min) were based on the optimization of the degemenylation in which the TMG groups have been largely removed while the oxidation of the silicon substrate was kept at minimum. The results are summarized in Table 3.

The conversion of the TMG protected alkyne to triazole was indicated by XPS spectra. As shown in Figure 4a, after MW irradiation for 20 min at 60 °C in the presence of **EG5N3**, the emergence of N 1s peaks in XPS at around 400.5 eV indicates the OEG derivative was successfully attached on the monolayer via a triazole linker. Deconvolution of the nitrogen emission gives two peaks at 400.3 and 401.4 eV with a 2:1 ratio of the integrated areas, which corresponds to the three nitrogen atoms in triazole.[20,48] Physically absorbed OEG was largely removed from substrate by the thorough wash process, as no apparent peak was observed near 405 eV which is the characteristic signal of azide residue.[49] Based on the ratio of N and C signal, the approximate yields of the coupling reaction was estimated to be 71 % for **EG5N3**, assuming all the TMG groups have been removed.[50]

A parallel approach to estimate the reaction yields relied on the comparison between C-C and C-O/C-N signals in the C 1s region. As shown in Figure 4b, the successful click coupling of **EG5N3** introduces C-O and C-N bonds to the monolayer, which show additional signals at higher binding energy relative to C-C peak in C 1s region. In order to simplify the curve-fitting, the C-O and C-N components were simulated by one peak at 286.5 eV. The ratio of the C-C and C-O/C-N signal intensity reflects the actual amount of OEG attached on the substrate. Compared with the two approaches used to quantify the OEG attachment, good agreement (less than 5% difference) was generally observed in relative short reaction time (<25 min) at low temperature (<70 °C).

The reaction of TMG-alkynyl-presenting surface **A** with the long OEG-azide (**EG11N3**) showed similar appearance of N 1s for the triazole group and C-O signal for EG units in XPS narrow scan (Figure 5), indicating a successful attachment of OEG components. A reaction time of 20 min at 65°C (Entry 7, Table 2) only resulted in modest conversion of the protected alkyne to triazole functionalities. The required increase of the temperature and the decrease of the coupling yield compared to its shorter counterpart **EG5N3** were attributed to the larger steric hindrance of the **EG11N3**. Further extending the irradiation time did not increase the actual amount of OEG immobilized. In fact, the yield based on the C-O/C-C ratio decreased with longer heating times and became significantly lower than the yield calculated from the N/C ratio. This result is likely due to the oxidative degradation of OEG chains during the CuAAC reaction, especially under elevated temperature.[52] The setup of the experiment in N_2 atmosphere is critical to reduce the formation of oxy radicals and electrophiles that are detrimental to OEG component.[53]

Besides XPS characterization, the progress of the reaction can also be monitored *ex situ* by ellipsometer and contact angel measurements. Upon the MW irradiation for 20 min, the ellipsometric thickness was increased by 11.1 ± 1.6 Å after the attachment of **EG5N3**. This thickness change is significantly smaller than the theoretical length of the extended **EG5N3** molecule (18.7 Å) calculated with MM2 (Chem3D Ultra 12.0, CambridgeSoft). Figure 6 shows the dependence of the contact angle upon the irradiation time. The variation in the

reaction times was investigated for temperature at 60°C. The initial TMG-terminated monolayer exhibited a water contact angle of 96 ± 1°. The coupling of **EG5N3** renders the surface hydrophilic, and the decrease of the water contact angles reflects the amount of OEG components on the substrate. After MW irradiation for 20 min, the water contact angle was decreased to 51 ± 3°, which is close to the value observed for the directly-grafted OEG monolayers on silicon.[15]

3.4 Comparison to Reactions under Conventional CuAAC Condition

The efficiency of using MW irradiation for the one-pot deprotection/click coupling was demonstrated by comparison with the reaction run under conventional heating. Without MW irradiation, the reaction of the surface **A** with **EG5N3** was carried out in an oil bath at 60°C under otherwise identical conditions. After reaction for 20 and 45 min, the yields were estimated to be 37% and 66%, respectively. However, these yields estimated from the measured C/N ratio were much lower than those estimated by the C-O/C-C ratio (32% and 42%). The decreasing C-O/C-C ratio over time indicated the degradation of OEG during the CuAAC reaction at elevated temperature. Moreover, significant oxidation of the silicon interface was also observed with prolonged heating time. On the other hand, the reaction performed under MW irradiation for 20 min lead to ~70% yield, with negligible degradation of the OEG chains and oxidation of the silicon interface (Table 2 and Table 3). The enhanced efficiency of using MW for the reaction is probably due to the confined heating in the near vicinity of the interface.[38–40, 54]

3.5 Adsorption of Fibrinogen onto OEG-Grafted Silicon Substrates

To evaluate the protein resistance of the resultant OEG-presenting surfaces, a previous reported method was used to quantify the relative amount of fibrinogen irreversibly adsorbed on the surfaces using XPS.[15,18]

As shown by the large increase of N 1s signal in Figure 7a, fibrinogen readily adsorbed on the freshly prepared H-Si(111) surface. The increase of ellipsometric thickness approximately corresponded to the width of fibrinogen (64 Å).[15,55] This sample with a monolayer (ML) of fibrinogen absorbed on the surface was used as a standard for the measurement of the % ML of fibrinogen onto the other OEG films. As for the **EG11N3** modified substrate (Entry 7, Table 3), weak (but still noticeable) increase of N 1s area was observed by the resulting N 1s area subtracting the initial triazole background. Then, the amount of proteins (%ML) adsorbed onto a sample surface is estimated by:

$$\text{Absorbed protein}\,(\%\text{ML}) = \Delta A_{\text{N}}{}^{\text{m}} / A_{\text{N}}{}^{\text{HSi}} \times 100\% \quad (3)$$

Where $\Delta A_{\text{N}}{}^{\text{m}}$ and $A_{\text{N}}{}^{\text{HSi}}$ is the increase of N 1s integrated areas for the modified substrates and H-Si (111) substrate, respectively. For comparison, experiment was also carried out for a directly-deposited **EG7** derived organic monolayer on Si (111), which shows remarkable antifouling property according to our previous study.[15]

Table 4 shows the results of protein-resistant measurements. Before the modification with OEG, the hydrophobic TMG-terminated monolayer absorbed significant amount of protein (86.7 ± 6.1% ML), compared to that of H-terminated silicon substrate. On the directly deposited **EG7** derived monolayer, however, negligible adsorption of fibrinogen (~2%) was observed, which was in agreement with our earlier work.[15] The adsorption for the substrates with click immobilized OEG is dependent upon the coupling yield and OEG chain density. The well characterized underneath monolayer makes the calculation of EG units on surface possible. Using the yield of the coupling and the density of the underlying monolayer (3.3

chains/nm^2), the click immobilized **EG5N3** was estimated to be about 11 EG units/nm^{-2} on Si(111), which is substantially lower than that of the directly deposited **EG7** monolayer (~21 EG /nm^{-2}). Thus, the lower protein resistant property of click immobilized OEGs compared to the directly deposited **EG7** monolayer can be rationalized.

On the other hand, the use of longer OEG derivative (**EG11N3**) does not help much to improve the OEG density immobilized on the surface in our experiment, which was also confirmed by the similar antifouling effect between **EG11N3** and **EG5N3** modified silicon substrate. During the click coupling, the long and flexible **EG11N3** chain may block many reactive sites due to the steric hindrance. In addition, the higher temperature used to immobilize **EG11N3** is more likely to induce the degradation of OEG through autoxidation.

It is worth mentioning that highly protein-resistant (<2%) substrate was obtained after attaching **EG5N3** to **TMG-EG10** derived monolayer via click chemistry.[25] However, complete protein resistance has not yet achieved when **TMG-C10** was used as a background, even though the yields of click coupling on the two surfaces were similar (~70%, according to the N/C ratio in XPS). This observation suggests that a high density of OEG chains on the previous platform also plays an important role to ensure an inert background against non-specific protein adsorption. In other words, the density of the OEG chains grafted on a neat alkyl monolayer should be further increased in order to nearly completely eliminate the non-specific protein adsorption.

4. Conclusion

In this work, rapid formation of an OEG layer on alkynyl-terminated monolayers on Si (111) surfaces via MW-assisted click reaction to suppress the non-specific adsorption of protein has been achieved, which provides an inert background for specific binding of biological targets. The reaction conditions are optimized to improve the rate while reducing the oxidation and other side reactions.

The alkynyl-terminated (“clickable”) monolayer on silicon was generated by photo-assisted grafting of the H-Si substrate with an α,ω-alkenyne where the alkynyl terminus was protected by a TMG group. The process provided high-quality monolayers presenting TMG-alkynyl groups, as indicated by the XPS and contact angles measurement. The TMG group could be rapidly removed in the presence of Cu(I). The combined degermenylation and click coupling was greatly accelerated by MW irradiation.

Furthermore, since photochemical grafting of organic alkenes can also be performed on other H-Si terminated substrates, such as SiC[18], [56] and silicone[57], applications of this strategy can be well extended to the modification of other semiconductors and biomaterials, especially when terminal alkyne is not compatible with the grafting conditions. Relevant research is currently underway in our lab.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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50. We assume that the yield of CuAAC reaction on the surface is X. Then, assuming that all TMG groups have been removed, there is a mixture of **EG5** and bare alkyne on the substrates where the mole fraction of **EG5** is X and the mole fraction of the alkyne is (1-X). Since there are 12 carbon atoms and 3 nitrogen atoms in **EG5N3**, the yield X can be calculated from the equation 3X/[24X +12(1-X)] = N/C, where N/C is measured by XPS.
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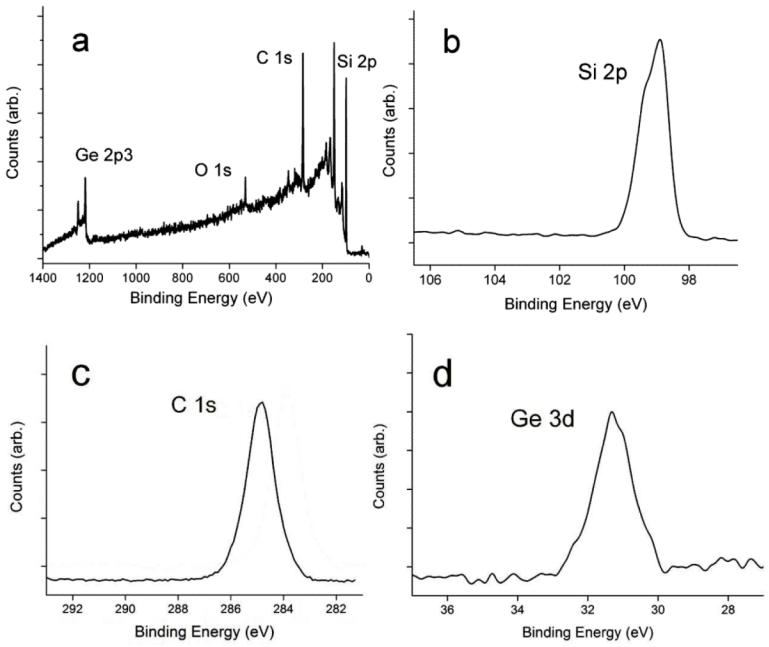


Figure 1.

XPS spectra of the films derived from compound **TMG-C10** on Si(111) (surface **A**, Scheme 1). (a) Survey spectrum; b) high-resolution scan of Si 2p; (c) high-resolution scan of C 1s and (d) high-resolution scan of Ge 3d.

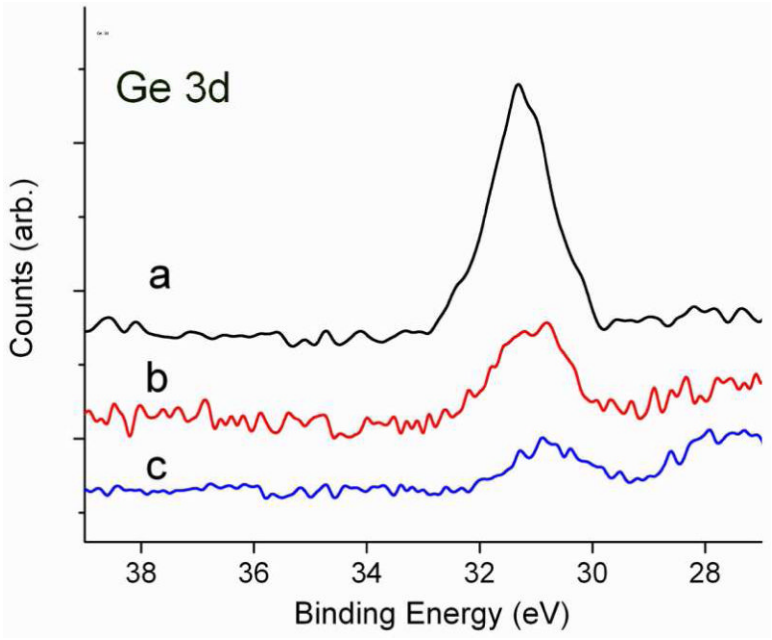


Figure 2.
High-resolution XPS narrow scans for the Ge 3d region of TMG-terminated monolayer: a) before microwave irradiation, b) 10 min MW irradiation at 60°C and c) 10 min MW irradiation at 70°C.

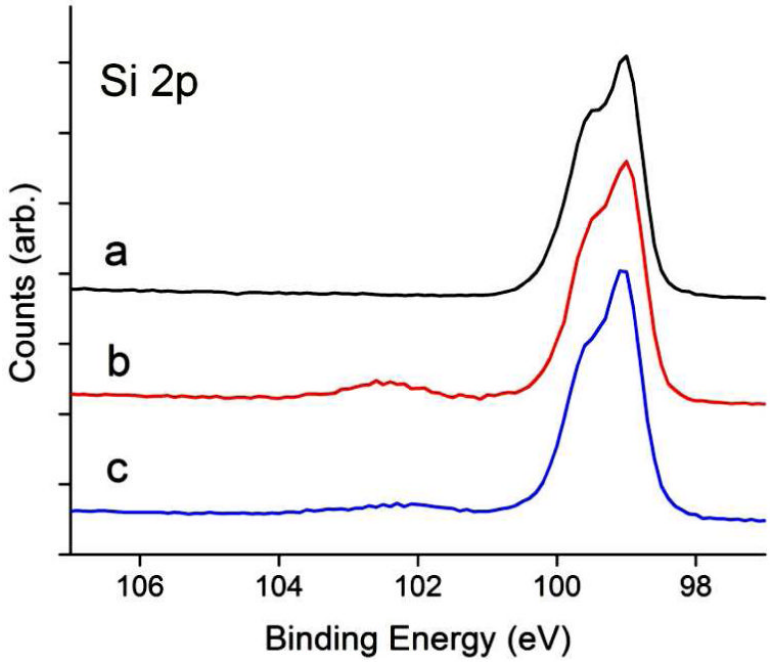


Figure 3.

High-resolution XPS narrow scans for the Si 2p region of TMG-terminated monolayer: a) before microwave irradiation, b) 20 min MW irradiation at 70°C under ambient condition and c) 20 min MW irradiation at 70°C under N_2 atmosphere in degassed solution.

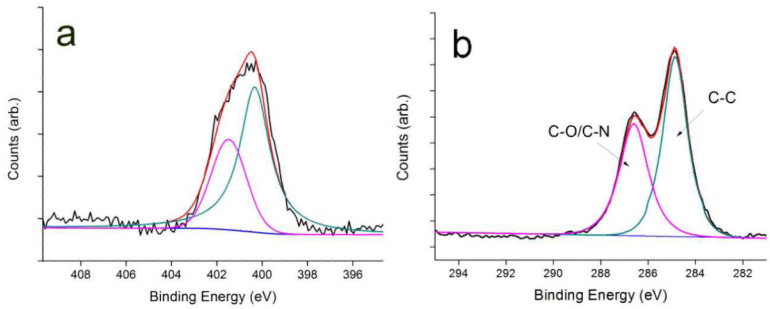


Figure 4.

MW-assisted reaction with **EG5N3** under 60°C for 20 min: narrow scans for the a) N 1s region indicating the formation of the triazole moiety and b) C 1s region showing the deconvoluted peaks for **C**-C at 284.8 eV and **C**-O/**C**-N at 286.5 eV.

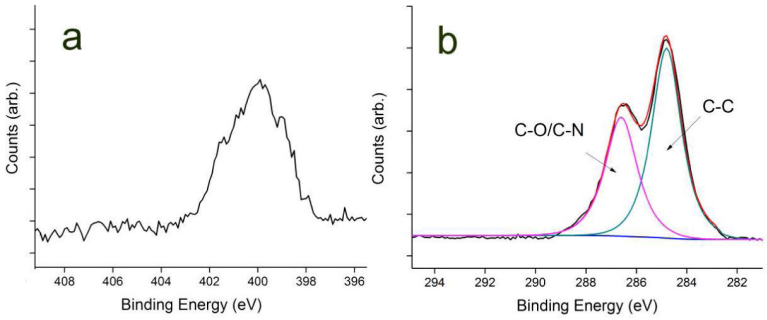


Figure 5.

MW-assisted reaction with **EG11N3** under 65°C for 20 min: narrow scans for the a) N 1s region indicating the formation of the triazole moiety and b) C 1s region showing the deconvoluted peaks for **C**-C at 284.8 eV and **C**-O/**C**-N at 286.5 eV.

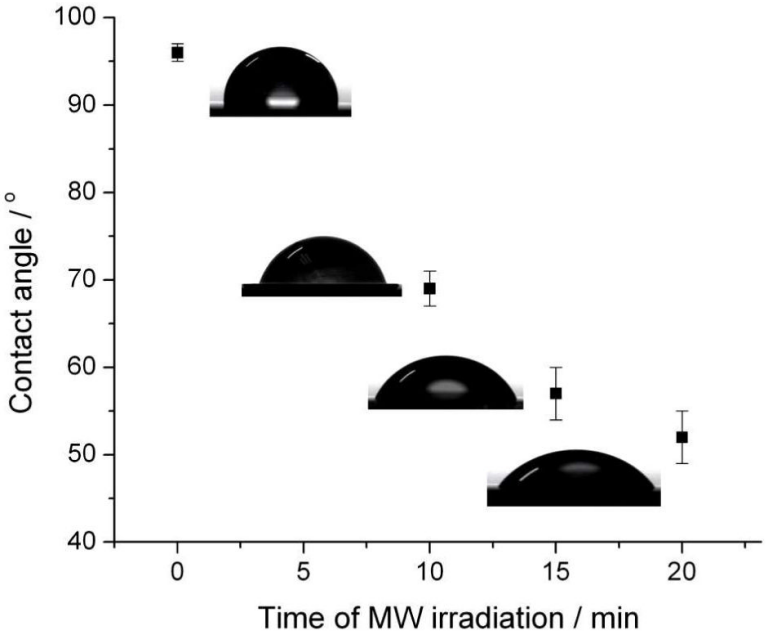


Figure 6.
Contact angle of the modified silicon substrates versus the MW irradiation time for the click modification of the surface **A** with **EG5N3**.

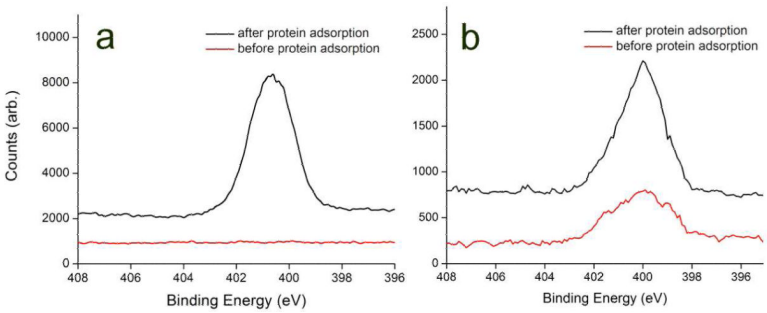
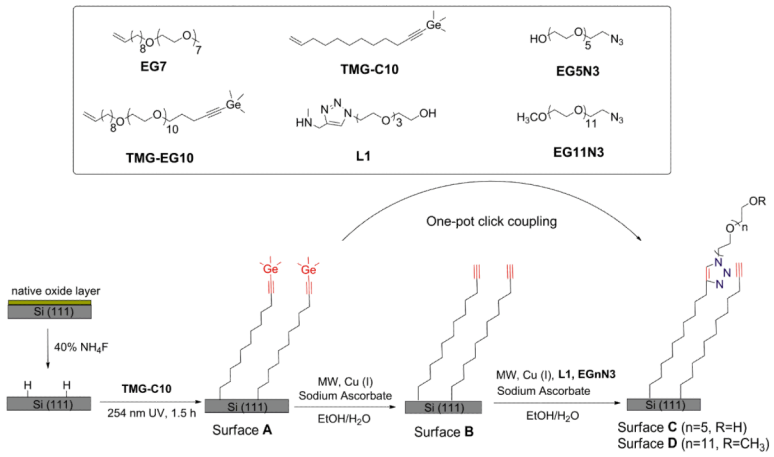


Figure 7.

XPS N 1s narrow scans of a) H-terminated Si substrates and b) **EG11N3** modified silicon substrate after incubation in 1 mg/mL fibrinogen PBS buffer (pH=7.4) for 1 h at room temperature, followed by rinsing with Millipore water for 30 s.



Scheme 1.

Formation of TMG-terminated monolayer on Si(111) and the subsequent attachment of OEG derivatives using MW-assisted click reaction.

Table 1

List of Parameters Used for the Quantitative Analysis of XPS Spectra

	Symbols	Value
Silicon	N_{Si}	5.0 atoms / Å ^{3}
	λ_{SiSi}	19 Å
Organic monolayer	n_C	15
	λ_{CC}	35.4 Å
	λ_{SiC}	39.5 Å
Instrument setup	ρ_{Si}	0.368
	ρ_C	0.314
	θ	45°

Table 2

Evaluation of reaction conditions for removal of TMG groups on surface A[a]

Entry	Reaction temperature (°C)	Reaction time (min)	Percentage of TMG deprotection
1	55	10	63
2	60	10	75
3	65	10	86
4	65	20	>90
5	70	10	>90
6	70	20	>90
7	75	20	>90[b]

[a] All reactions were carried out in degassed solution of EtOH/H_2O=2:1, using $Cu(MeCN)_4PF_6$ (2.5 mM) and sodium ascorbate (15 mM).

[b] Oxidation of silicon substrate occurred.

Table 3

Evaluation of reaction conditions for the MW-assisted one-pot click coupling[a]

Entry	OEG	T/°C	t/min	Yield /%[b]	Yield /%[c]
1	**EG5N3**	55	10	26	30
2	**EG5N3**	60	10	51	47
3	**EG5N3**	60	15	63	61
4	**EG5N3**	60	20	71	68
5	**EG11N3**	60	20	27	23
6	**EG11N3**	65	15	33	30
7	**EG11N3**	65	20	40	36
8	**EG11N3**	65	30	43	31

[a] All reactions were carried out in degassed solution under N_2 atmosphere using $Cu(MeCN)_4PF_6$ (2.5 mM), Sodium Ascorbate (15 mM), azide-OEG (5 mM), ligand **L1** (10 mM), EtOH/H_2O=2:1.

[b] Based on XPS N/C ratio.

[c] Based on C1s signal.

Table 4

Amount of adsorption for fibrinogen (c = 1 mg/mL) on differently modified silicon wafers, measured by the increase of N 1s signal and normalized to the amount of fibrinogen adsorbed on H-Si(111)

Substrate	Yield%[a]	Density of EG (unit/nm^{-2})	Protein adsorption (%ML)
H-Si (111)	-		100
TMG-terminated	-		86.7±6.1
EG5-terminated	47	8	20.9±5.4
EG5-terminated	68	11	9.6±2.5
EG11-terminated	36	13	8.4±2.9
Directly-grafted **EG7**[b]	-	21	2.1±0.7

[a] Based on the XPS narrow scan for C1s spectra.

[b] Preparation of the directly deposited OEG monolayer on Si(111) was described in ref.[15].