5698 *Chem. Mater.* **2009,** *21,* 5698–5700
DOI:10.1021/cm902176n

CHEMISTRY OF MATERIALS
Communication

Mild and Versatile (Bio-)Functionalization of Glass Surfaces via Thiol−Ene Photochemistry

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Received July 17, 2009
Revised Manuscript Received October 8, 2009

Engineering of the surface of inorganic materials is the key in many applications like materials science and biotechnology.[1−3] A commonly used inorganic substrate for the fabrication of for instance carbohydrate microarrays[2] or biochips[3] is silica or glass. Organosilane chemistry can be applied to introduce amine, carboxyl, vinyl, or thiol groups onto the surface,[4] which can then be used for further functionalization or the immobilization of polymers or biomolecules. Especially for biochemical applications (e.g., high throughput profiling of antigen−antibody interactions, characterization of substrate specificity of enzymes, or drug and toxin proteomics), it is of interest that the second functionalization step occurs efficiently in a mild biological environment.

Candidates suited for this are sulfhydrylated silica or glass surfaces, which can be readily prepared using (3-mercaptopropyl)trimethoxysilane (MPTMS). The surface-bound thiol functions are then usually submitted to further functionalization through thermal radical addition or chain-transfer polymerization processes[5−7] or nucleophilic Michael addition reactions to conjugated carbonyl compounds.[8−10] Thiol−ene step-growth photopolymerization has been applied to produce surface-grafted thin polymer films.[11] Also, thiols could act as nucleophilic initiators of the ring-opening polymerizations of epoxides or lactones.[12]

Thiol−ene photochemistry is now a rather established method for the "click" functionalization of polymers and dendrimers[13−15] and for soft imprint lithography;[16] however, not yet for surfaces (only surface-immobilized dendrimers).[15] Eventually, thiyl radicals can be directly generated using UV−visible light or sunlight;[17] additional radical sources or transition metal catalysts (as for Huisgen-type "click" chemistry)[18] are essentially not needed. Also, the reaction tolerates many functional groups and solvents.[13]

Herein, we wish to demonstrate the applicability of thiol−ene photochemistry to produce glass surfaces with polymeric and bioorganic coatings under mild conditions (Scheme 1). Surface-bound thiyl radicals were used to initiate the polymerization of methacrylic acid as well as to add to α-olefins like allyl-α-D-glucopyranoside, 1*H*,1*H*,2*H*-perfluoro-1-decene, or 1,2-polybutadiene. Surfaces were characterized by means of Fourier transform infrared (FT-IR) and Raman spectroscopy, contact angle measurements, fluorescence microscopy (FM), and scanning force microscopy (SFM).

Commercial glass slides were treated with a piranha solution (H_2SO_4:H_2O_2 2:1 v/v) for 1 h at room temperature to regenerate silanol groups on the surface. Direct sulfhydrylation of the activated glass surface (→ **Glass-SH**) was then done with MPTMS. A solution of MPTMS in tetrahydrofuran (THF) (1:1 v/v), containing 0.4 vol % of HCl conc., was spin-coated at 500 rpm for 30 s onto a glass substrate. Slides were dried at 150 °C for 3 h, promoting condensation and siloxane bond formation with the surface,[19,20] and thoroughly washed with organic solvents (see Supporting Information for details). Raman spectroscopic analysis of **Glass-SH** revealed, apart from Si−O bands, the characteristic stretching vibrations of CH_2 ($\tilde{\nu} \sim 2913\ cm^{-1}$) and SH ($2573\ cm^{-1}$), confirming the successful grafting of MPTMS. The thickness of the MPTMS layer was about 20 nm (SFM, Supporting Information), and the apparent surface coverage with thiol groups was 1.2 ± 0.2 pmol/μm^2 (iodometry, Supporting Information).[21]

Chemical imaging with confocal Raman microscopy reveals that the lateral distribution of SH on the surface

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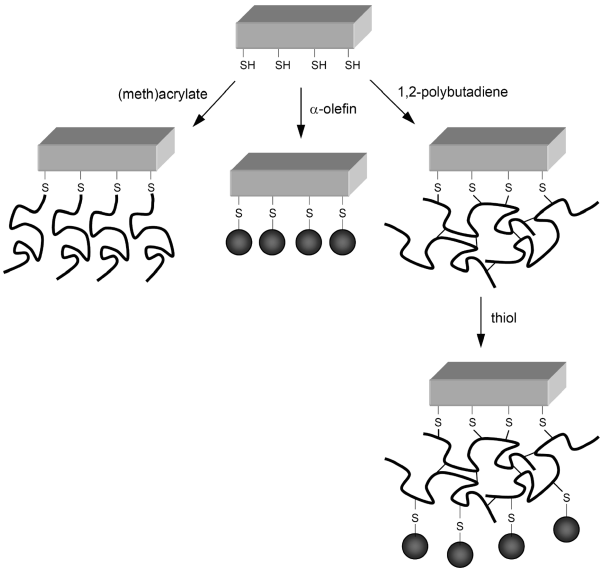
 Published on Web 11/16/2009

Scheme 1. Functionalization of a Sulfhydrylated Glass Slide via Thiol−Ene Photochemistry



may not be perfectly homogeneous (Figure 1a). Further, SFM shows that the surface is not smooth (root-mean-square roughness, R_q = 11.6 nm) but is covered with "spikes" (Figure 1b and Supporting Information). Both the chemical distribution and the surface topography can be explained considering that MPTMS molecules might have polymerized and formed aggregates prior to deposition on the glass surface.[22]

It is worth being mentioned that Raman microscopy failed to give chemical images of surfaces with thinner (and also smoother, R_q < 2.5 nm) coatings of MPTMS (Supporting Information).

Surface-Initiated Photopolymerization. The sulfhydrylated glass slide **Glass-SH** was placed in a ~4 wt % aqueous solution of methacrylic acid (MAA; stabilized with *p*-methoxyphenol) and put under an argon atmosphere. The mixture was exposed to UV−visible light (Heraeus TQ 150, 150 W, λ > 300 nm) for 24 h at room temperature. The glass slide, **Glass-PMAA**, was thoroughly washed with water and dried. The successful grafting of MAA onto the surface was verified by FT-IR spectroscopy, valence vibrations of C−H ($\tilde{\nu}$ ~ 2920 cm^{-1}) and C=O (1698 cm^{-1}), and measurement of the contact angle, θ = 52° (**Glass-SH**: 80°). The thickness of the PMAA brush layer was determined to be ~200 nm (SFM, Figure 2).

Radical Thiol−Ene Photoaddition. Slides of **Glass-SH** were treated with solutions of allyl-α-D-glucopyranoside in THF/methanol 1:1 (v/v) (→ **Glass-Glc**), 1*H*,1*H*,2*H*-perfluoro-1-decene in THF (→ **Glass-F**), and 1,2-polybutadiene (62 mol % 1.2 units, number-average molecular weight, M_n = 2.3 kg/mol) in THF (→ **Glass-PB**). Solutions were exposed to UV−visible light for 24 h at room temperature; the functionalized glass slides were afterward thoroughly washed with methanol (**Glass-Glc**) or THF (**Glass-F** and **Glass-PB**) and dried. Grafting of the vinyl compounds was confirmed by Raman spectroscopy

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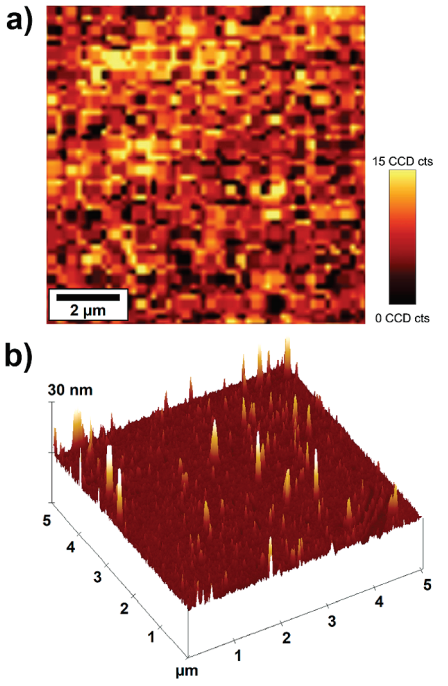

Figure 1. (a) Chemical image of the sulfhydrylated glass slide, **Glass-SH** (confocal Raman microscopy, integration of SH absorption from 2545 to 2586 cm^{-1}, 50 points/line, 50 lines/image, retrace: 0.05 s, integration time: 1s). (b) SFM height image (tapping mode) of the surface of the glass side after treatment with MPTMS.

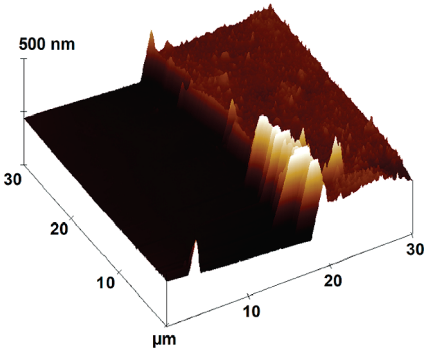

Figure 2. SFM height image (tapping mode) of the surface of **Glass-PMAA**. For determination of the thickness of the PMAA brush, the underlying glass surface (black) was uncovered by scratching off the polymer layer with the tip.

(Supporting Information) and/or by measurements of the contact angles (Figure 3a). Compared to **Glass-SH**, the surface of **Glass-Glc** (θ = 66°) is more hydrophilic and those of **Glass-F** (90°) and **Glass-PB** (87°) are more hydrophobic. Residual SH vibrations in the Raman spectra indicated a less than quantitative conversion of thiol functions (supposedly buried inside the MPTMS layer). The thickness of the polymer layer of **Glass-PB** was ~30 nm (SFM, Supporting Information).

It should be mentioned that a disulfide coupling of thiols onto **Glass-SH** could not be achieved, which is attributed to a low thiyl radical concentration (in the absence of additional radical sources).

Biological Recognition Test. The glucose-coated surface of **Glass-Glc** was incubated with a solution of a legume lectin, *Concanavalin A* (ConA), which is known

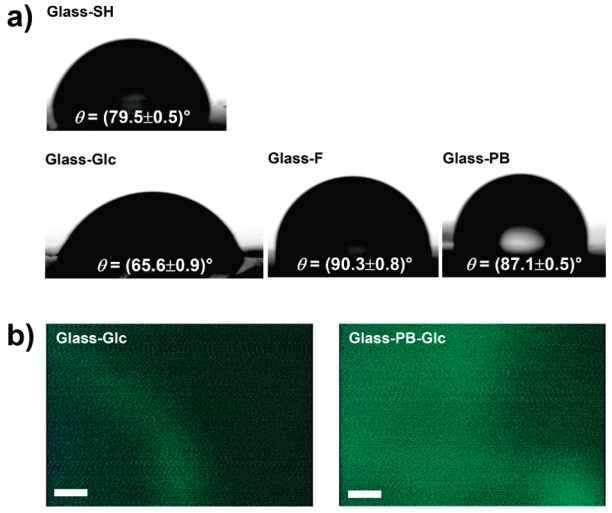

Figure 3. (a) Photographs of water droplets on **Glass-SH** and the thiol−ene functionalized surfaces of **Glass-Glc**, **Glass-F**, and **Glass-PB** (left to right), used for the determination of contact angles (θ). (b) Fluorescence micrographs (740 × 509 pixels, 12.6× magnification) of the surfaces of **Glass-Glc** (left) and **Glass-PB-Glc** (right) after incubation with FITC-ConA in PBS solution. Scale bars = 50 μm.

to specifically bind to glucose and mannose units, in a phosphate buffered saline (PBS) solution (pH 7.2).[23] ConA was labeled with fluorescein isothiocyanate (FITC) for chemical imaging with FM. After incubation and rinsing with PBS and washing with water, the surface displayed a faint green fluorescence as can be seen in the image in Figure 3b, left. The rather low fluorescence intensity indicates a poor binding of the lectin to the surface.

The efficiency of carbohydrate-lectin recognition is known to depend on a number of parameters, among them the length and flexibility of the linker between surface and sugar.[2,18,24] We therefore decided to attach a flexible 1,2-polybutadiene layer onto **Glass-SH** before adding the glucose units. Exposure time to UV−visible light was 1 h (instead of 24 h) to ensure an incomplete conversion of double bonds. Nongrafted polymer chains were removed by washing with THF. The polymer layer was then functionalized with tetra-*O*-acetyl-1-thio-β-D-glucopyranose (UV−visible irradiation for 24 h at room temperature, THF)[17] to yield, after deacetylation, **Glass-PB-Glc**. The contact angle measured for this surface was θ = 65°, similar to that of **Glass-Glc**. The FM image of **Glass-PB-Glc** (Figure 3b, right), which was taken after the incubation with FITC-ConA and washing with PBS and water, displays a much brighter fluorescence, indicating a larger amount of immobilized lectin, than **Glass-Glc**. Also, the distribution of the glucose−lectin complexes on the slide appears to be rather homogeneous.

As control experiments, the glucosylated glass surfaces were incubated with FITC-labeled *Ricinus communis Agglutinin I* (RCA I), a lectin specifically binding to galactose and not glucose residues.[23] Fluorescence could not be observed (images not shown), indicating the absence of unspecific protein adsorption.

In summary, we described a mild and versatile approach for the direct functionalization of glass slides (silica) via thiol−ene photochemistry. Glass surfaces with polymer and/or bioorganic coatings could be produced from readily available starting materials. We further demonstrated that immobilized glucose units retained their function in the recognition of ConA. The efficiency of the interaction between glucose and ConA could be improved considerably by introducing a flexible polymer layer between surface and ligand.

It is envisaged that the process would be well suited to fabricate carbohydrate microarrays, bioassays, or biosensors. Also, there would be the possibility to make patterned surfaces via thiol−ene photochemistry,[15] through photomasks or through "writing" with a suitable laser.

Acknowledgment. Anne Heilig, Notburga Gierlinger, Ingo Burgert, Paulo Fernandes, and Erich C. are thanked for their contributions to this work. Financial support was given by the Max Planck Society and the German Federal Ministry of Education and Research (BMBF) in the framework of "Bionic Initiative" (0313765A).

Supporting Information Available: Descriptions of experimental procedures and analytical instrumentation as well as additional experimental data (Raman and FT-IR spectra, SFM images, and contact angle measurements). This material is available free of charge via the Internet at http://pubs.acs.org.

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