Mild and versatile (bio-)functionalization of glass surfaces via thiol-ene photochemistry

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Materials

All solvents and common chemical reagents were purchased as high-purity reagent-grade materials from Sigma-Aldrich and Fluka and were used as received. 1-Allyl α-D-glucopyranoside (>99%) and 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranose (>99%) were purchased from Glycon Biochem. GmbH (Luckenwalde, Germany). (3-Mercaptopropyl)-trimethoxysilane (MPTMS, 95%), methacrylic acid (MAA, 99%; stabilized with 250 ppm of p-methoxyphenol), 1H,1H,2H-perfluoro-1-decene (>99%), polybutadiene (~62 mol % 1,2-units), Concanavalin A from Canavalia ensiformis (Jack bean) FITC conjugate (Type IV, lyophilized powder), were purchased from Sigma-Aldrich. Fluorescein Ricinus communis Agglutinin I (98%, solution in 10 mM HEPES, 0.15 M NaCl, pH 7.5, 0.08% NaN₃) was purchased from Vector Laboratories (Burlingame CA, USA). Solvents were purified using conventional methods reported elsewhere in the literature. Glass slides were purchased from Marienfeld Laboratory Glassware (Lauda-Königshofen, Germany).

Light source

The light source was an UV-immersion lamp Heraeus TQ 150 (Hg medium-pressure, 150 W). Irradiation occurred through the borosilicate glass (Duran) of the reaction vessel, filtering off the high-energy UV light with $\lambda < \sim 300$ nm.

Experimental procedures

Activation of glass slides. In order to regenerate the surface silanol groups of the commercial glass slides, Si–OH, an activation process was carried out (checked by FT-IR). This process consists in dipping the non-coated glass slides into a piranha solution (H₂SO₄:H₂O₂ 2:1 v/v) for 1 h at room temperature. Glass slides were thoroughly rinsed in distilled water and dried at 75 °C overnight.

Sulphydrylation of glass slides with MPTMS. To a 50 vol % solution of MPTMS in THF (freshly distilled) was added 0.4 vol% HCl (37 %). The solution was spin coated on the glass slide with a speed of 500 rpm during 30 s. The slides were “baked” at 150°C for 3 h, and then submitted to a sequential washing with CH₂Cl₂, toluene, hexane, acetone, and again CH₂Cl₂. Finally, the Glass-SH slide was dried at 75 °C for 1 h.
**Determination of [SH].** The degree of modification was determined for Glass-SH following the method described by Kast and Bernkop-Schnürch (*Biomaterials* 2001, 22, 2345), which is a direct titration of the thiol content with iodine and starch as colorimetric indicator: Glass-SH (~500 mm$^2$) was immersed in 10 ml of water, and the pH was adjusted to pH 2 with 1N HCl. After the addition of 5 mg of starch, the sample was titrated with a 1 mM aqueous iodine solution until a permanent light blue color was maintained; $V_{eq} = 0.6 \pm 0.1$ ml. Thus, $[SH] = [I_2] * V_{eq} / 500 = 1.2 \pm 0.2 \mu$mol/mm$^2$.

**Surface initiated photopolymerization of MAA.** A ~4 wt % solution of MAA in water was added to the sulphydrylated glass slide. The mixture was degassed twice, put under an argon atmosphere, and exposed to UV light for 24 h at room temperature. The MAA-modified slide Glass-PMAA was subjected to washing with water and were then dried at 65 °C for 3 h.

**Grafting of olefins.** (1) A solution of 1H,1H,2H-perfluoro-1-decene (1.3 wt%) or 1,2-PB (4 wt%) in dry THF was added to the sulphydrylated glass slides. The mixture was degassed twice, put under an argon atmosphere, and exposed to UV light for 24 h at room temperature. The glass slides Glass-F and Glass-PB, respectively, were subjected to washing with THF and were dried at 65 °C for 3 h.
(2) A ~0.9 wt % solution of allyl-α-β-glucopyranoside in THF/MeOH 1:1 v/v was added to the sulphydrylated glass slide. The mixture was degassed twice, put under an argon atmosphere, and exposed to UV light for 24 h at room temperature. The glass slide Glass-Glc was subjected to washing with MeOH and was dried at 65 °C for 3 h.

**Grafting of glucose on 1,2-PB coated glass slides.** First, 1,2-PB was grafted under UV irradiation to the sulphydrylated glass slide following the procedure for the grafting of olefins; reaction time was 1 h. Tetra-O-acetyl-1-thio-β-d-glucopyranose was then grafted onto 1,2-PB-coated glass with UV light for 24 h. The acetate protecting groups are removed by dipping the sugar-coated glass slides in a solution of 1 mL MeOH + 1 mL 1M NaOH for 1 h at room temperature. The glass slide Glass-PB-Glc was then rinsed with water and MeOH and dried.

**Labeling with Con A.** Glucose-grafted glass slides, Glass-Glc or Glass-PB-Glc, were incubated with FITC-labelled Concanavalin A (ConA) (1 mg FITC-ConA in 1 mL PBS solution). After incubation of 1 h at room temperature, unbound protein was washed away by rinsing with PBS and washing with MilliQ water and the surface was dried.
Labeling with RCA I (control). Glucose-grafted glass slides, Glass-Glc or Glass-PB-Glc, were incubated with FITC-labelled *Ricinus communis Agglutinin I* (RCA I) (a solution of FITC-RCA I in HEPES at a concentration of 5mg/mL is diluted 10 times: final concentration = 0.5 mg/mL). After incubation of 1 h at room temperature, unbound protein was washed away by rinsing with MilliQ water and the surface was dried.

**Analytical instrumentation**

**Raman spectroscopy.** Spectra were acquired using a confocal Raman microscope (CRM300, WITEC, Germany) equipped with an objective from Nikon (100x, N_A = 0.26) and a linear polarized laser (diode pumped Green laser, λ = 532 nm, CrystaLaser). The Raman light was detected by a CCD camera (DV401-BV, Andor), behind a grating (600 g/mm) spectrometer (UHTS 300, WITEC).

**Fourier-transform infrared (FT-IR) spectroscopy.** Spectra were acquired using Varian resolutions 4.1.0.101 on Varian Scimitar 1000 FT-IR with ATR golden gate from Pike (diamond crystal).

**Contact angle measurements.** Contact angles were measured by using a contact angle measuring system G10 from Kruess. Contact angles were determined by placing a drop of water from a syringe, advancing the periphery of the drop by adding more liquid, withdrawing the syringe, and measuring the contact angle within <10 s of application of the drop. Measurements were done at room temperature. Reported values are averages of three measurements taken at different points on the surface.

**Scanning force microscopy (SFM).** Images were collected in tapping mode using an AFM-Dimension 3100 produced by Veeco. The tips are used with a frequency of ~285 kHz and a spring constant of 42 N/m.

**Fluorescence microscopy (FM).** A microscope Olympus IX71 with an objective epiplan Neofluar (20x/0.50) was used to characterize the fluorescent glass slides. A camera Hamamatsu C5810 (color chilled 3CCD, 0.63x) was used to take the pictures.
Figure S1. FT-IR spectra of the (a) piranha-treated glass slide and (b) sulfhydrylated glass slide Glass-SH.

Figure S2. Raman spectra (top) and chemical image (confocal Raman microscopy, 10x10 µm², integration of SH absorption from 2545 to 2586 cm⁻¹, 50 points/line, 50 lines/image, retrace: 0.05 s, integration time: 1s) (bottom) of (a) the piranha-treated glass slide and (b) the sulfhydrylated glass slide, Glass-SH.

Figure S3. Photographs and contact angles (θ) of water droplets on the (a) piranha-treated glass slide and (b) sulfhydrylated glass slide Glass-SH before and after washing.
Figure S4. Representative SFM (tapping mode) height images of the (a) pirhana-treated glass slide and (b) sulfhydrylated glass slide Glass-SH. Section analysis shows a region of Glass-SH where the MPTMS layer was scratched off with the tip.

Figure S5. Raman spectra of the (a) glucosylated glass slide Glass-Glc and b) polybutadiene-coated glass slide Glass-PB.
Figure S6. FT-IR spectra of the (a) PMAA-grafted glass slide Glass-PMAA (b) fluorinated glass slide Glass-F, (c) glucosylated glass slide Glass-Glc, and (d) PB-coated glass slide Glass-PB.

Figure S7. Photographs and contact angles (θ) of water droplets on the (a) PMAA-grafted glass slide Glass-PMAA, (b) perfluorinated glass slide Glass-F, (c) PB-coated glass slide Glass-PB, (d, e) glucosylated glass slides Glass-Glc and Glass-PB-Glc.
Figure S8. SFM (tapping mode) height image of the scratched PMAA-grafted glass slide Glass-PMAA; height difference = thickness(PMAA) + thickness(MPTMS).

Figure S9. SFM (tapping mode) height images of the scratched PB-coated glass slide Glass-PB; height difference = thickness(PB) + thickness(MPTMS).
Sulfhydrylation of glass slides with MPTMS (alternate procedure). Slides were placed in inert flasks, followed by the addition of distilled THF and MPTMS (to create solutions containing 0.1 vol% to 5 vol% MPTMS) and then 0.4 vol% HCl (37 %). Total solution volume was always 10 mL. Solutions were kept for 24 h at room temperature. Slides were then removed from the solutions and subjected to a sequential washing regime consisting of water, dichloromethane, toluene, hexane, acetone, and again dichloromethane. Slides were then dried at 70°C for 3 hours.

**Figure S10.** Representative SFM (tapping mode) height images of glass slides prepared sulfhydrylation with MPTMS at different concentrations (alternate procedure); $R_g$: root-mean-square roughness.