Electronic Supporting Information for the following manuscript submitted to RSC Journal of Materials Chemistry B

Transfer of Functional Thermoresponsive Poly(glycidyl ether) Coatings for Cell Sheet Fabrication from Gold to Glass Surfaces

*Silke Heinen[1], Simon Rackow[1], Jose Luis Cuellar-Camacho[1], Ievgen S. Donskyi[1,2], Wolfgang E. S. Unger[2], Marie Weinhart[1]**

[1]Institute of Chemistry and Biochemistry, Freie Universitaet Berlin, Takustr. 3, 14195 Berlin, Germany.

[2]BAM – Federal Institute for Material Science and Testing, Division of Surface Analysis and Interfacial Chemistry, Unter den Eichen 44-46, 12205 Berlin, Germany

* Corresponding author: email marie.weinhart@fu-berlin.de, phone: +49 30 838 75050

Materials

All chemicals and solvents were purchased from Sigma-Aldrich (Steinheim, Germany) and used without further purification unless stated otherwise. Methyl glycidyl ether (GME) and ethyl glycidyl ether (EGE) were purchased from TCI GmbH (Eschborn, Germany). The monomers GME, EGE and allyl glycidyl ether were dried over CaH_2, distilled and stored over 3 Å molecular sieve purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Triisobutylaluminum (1.1 M in toluene) and benzophenone (99 %) were purchased from Acros Organics (Geel, Belgium). Toluene, predried via the solvent system MB SPS-800 from MBraun GmbH (Garching, Germany), was refluxed with elemental sodium and traces of benzophenone until a persistent dark blue color indicated the absence of water. Afterwards, toluene was distilled on 3 Å molecular sieve prior to use. Diethyl ether was supplied by VWR Chemicals (Fontenay-sous-Bois, France or Leuven, Belgium) and was distilled prior to use in order to remove the stabilizer. Magnesium sulfate (99 %) was supplied by Grüssing GmbH (Filsum, Germany). Pre-wetted regenerated cellulose ultrafiltration discs (molecular weight cut-off 10000 g mol^{-1}, Millipore® Ultracel™ PLC membranes) were purchased from Merck Chemical GmbH (Darmstadt, Germany). For the preparation of coatings, distilled ethanol und deionized water with a minimum resistivity of 18.0 MΩ cm (Millipore™) was used. A 0.1 M solution of 3-(*N*-Morpholino)propanesulfonic acid (MOPS) buffer was prepared with an adjusted pH 8.5. Deuterated solvents were purchased from Deutero GmbH (Kastellaun, Germany). Ellipsometry measurements were performed with coatings on silicon wafers supplied by Silchem Handelsgesellschaft mbH (Freiberg, Germany). Atomic force microscopy (AFM) measurements and cell culture experiments, respectively, were performed with coatings on borosilicate precision cover slips with a diameter of 12 and 25 mm which were purchased from Carl Roth GmbH + Co. KG.

Methods

^{1}H and ^{13}C NMR spectra were recorded on a Jeol ECX at 500 MHz, measured at concentrations of 100 mg mL^{-1} in deuterated solvents. Chemical shifts are reported in δ (ppm) and referenced to the respective solvent. Gel permeation chromatography was conducted on an Agilent 1100 Series instrument in tetrahydrofuran as the eluent solvent with concentrations of 3.5 mg mL^{-1} and a flow rate of 1 mL min^{-1} at 25 °C. Three columns PLgel mixed C with the dimensions: 7.5 x 300 mm, particle size: 5 µm from Agilent (Waldbronn, Germany) were used in-line with a refractive index detector. Calibration was performed with polystyrene standards from PSS (Mainz, Germany). The thiol-ene click reaction was performed under UV-irradiation with a mercury-vapor lamp from LOT-QuantumDesign GmbH (Darmstadt, Germany) which was operated at 85 W with an emission spectra of λ = 250-365 nm. Ultrafiltration was performed in methanol at 5 bar in a solvent-resistant stirred cell (max. volume 300 mL) equipped with a regenerated cellulose membrane. Solvent was refilled at least 3 times. Glass surfaces and silicon wafer were cleaned for 15 min in a UV/Ozone Pro Cleaner from BioForce Nanoscience, Inc. (Ames, USA). Multi-angle spectroscopic ellipsometry at angles of 50 ° and 70 ° in a spectral range of 370-1050 nm was performed on a SENpro ellipsometer and evaluated with the software package SpectraRay 3 Version 5.3.2.1853 from SENTECH Instruments GmbH (Berlin, Germany). Static water contact angles were determined via the sessile drop method on a DataPhysics Contact Angle System OCA from DataPhysics Instruments (Filderstadt, Germany) and fitted with the software package SCA202 version 3.12.11. DLS measurements were performed on a Malvern Zetasizer Nano-ZS analyzer (Malvern Instruments) equipped with a 4 mW He-Ne laser (λ = 633 nm), at concentrations of 10 mg mL^{-1} in ethanol and mixtures of ethanol and MOPS buffer. Measurements were performed in PS-latex cuvettes with a refractive index of 1.590 and low absorption of 0.01 and each probe was equilibrated for 180 s prior to the measurement. X-ray photoelectron

spectroscopy (XPS) spectra were recorded on a Kratos Axis Ultra DLD spectrometer equipped with a monochromated Al Kα X-ray source using an analyzer pass energy of 80 eV for survey spectra and 20 eV for the core level spectra. The electron emission angle was 60° and the source-to-analyzer angle was 60°. The binding energy scale of the instrument was calibrated following a Kratos Analytical procedure which uses ISO 15472 binding energy data. Spectra were recorded by setting the instrument to the hybrid lens mode and the slot mode providing approximately a 300 x 700 μm^2 analysis area using charge neutralization. All XPS spectra were processed with the UNIFIT program (version 2017). A Gaussian/Lorentzian sum function peak shape model GL (30) was used in combination with a Shirley background. If not otherwise denoted the L-G mixing for component peaks in all spectra were constrained to be identical. Peak fitting of C 1s spectra was performed by fitting of all peaks to remove residual structures. After peak fitting of the C 1s spectra, all spectra were calibrated in reference to aliphatic C–C bond C 1s component at a binding energy of 285.0 eV. High-resolution core-level spectra were recorded in FAT (fixed analyser transmission) mode at a pass energy of 20 eV using the following excitation energy 1486.69 eV for all elements: O 1s, N 1s, C 1s.

Cell culture

Dermal fibroblasts from human foreskin biopsies were isolated and cultured as described previously, after ethical approval and with informed parental consent.[1] In brief, cells were cultured in Dulbecco's modified Eagle medium (DMEM, #31966-021, #21885108) purchased from Thermo Fisher Scientific (Darmstadt, Germany) that had been supplemented with 10% fetal bovine serum (#S0115) from Biochrom (Berlin, Germany). Cells were expanded in tissue culture polystyrene flasks at 37 °C with 5% CO_2 in high glucose [4.5 g L^{-1}] DMEM. For passaging, cells were trypsinized (0.05% trypsin-EDTA, #15400054, Thermo Fisher Scientific) for 2 min at 37 °C followed by trypsin inhibition with cell culture medium. After

centrifugation of cells for 4 min at 140 × g the supernatant was removed. The cell pellet was resuspended in DMEM, and the cells were used for studies or further culture. Dermal fibroblasts in passage 3-7 were used for cell culture studies on thermoresponsive poly(GME-*ran*-EGE) equipped with an amine-containing anchor block (PGE-AA) coatings on borosilicate precision cover slips with a diameter of 25 mm as substrates. Phosphate buffered saline (pH 7.4, #14190-094, Gibco Life Technologies, Paisley, UK) without calcium and magnesium ions was used for cell sheet detachment. All expendable materials for cell culture were purchased from Sarstedt (Nümbrecht, Germany).

Interaction of thermoresponsive PGE and PGE-AA with bare and polydopamine (PDA)-precoated glass

In order to investigate the interaction of the thermoresponsive PGE-AA block copolymer with the two hydrophilic surfaces (bare and PDA-precoated glass), we performed quartz crystal microbalance measurements with dissipation (QCM-D) measurements and tracked the self-assembly on the two surfaces in real-time for 2 h under full solubility grafting (FSG) conditions. As a control we also investigated the interaction of a thermoresponsive PGE copolymer without the anchor block. No stable adhesion on bare glass chips (SiO_2-coated QCM-D chips) nor on PDA-precoated chips was observed with PGE without the amine-containing anchor block (AA). As compiled in Figure S1 hardly any change in frequency (Δf) before and after exposure of the surface to the PGE polymer solution under static conditions was observed. In contrast, in the presence of the anchor block with PGE-AA a significant change in frequency (Δf) was detected on both surfaces (Figure S1). Thus, we concluded, that no attractive interactions between the thermoresponsive poly(GME-*ran*-EGE) and the substrate materials existed and affinity of the polymer to the surfaces was solely induced by

the AA-anchor block. Hence, the block copolymer design fulfilled the prerequisites to form self-assembled brushes with PGE-AA on both substrates.

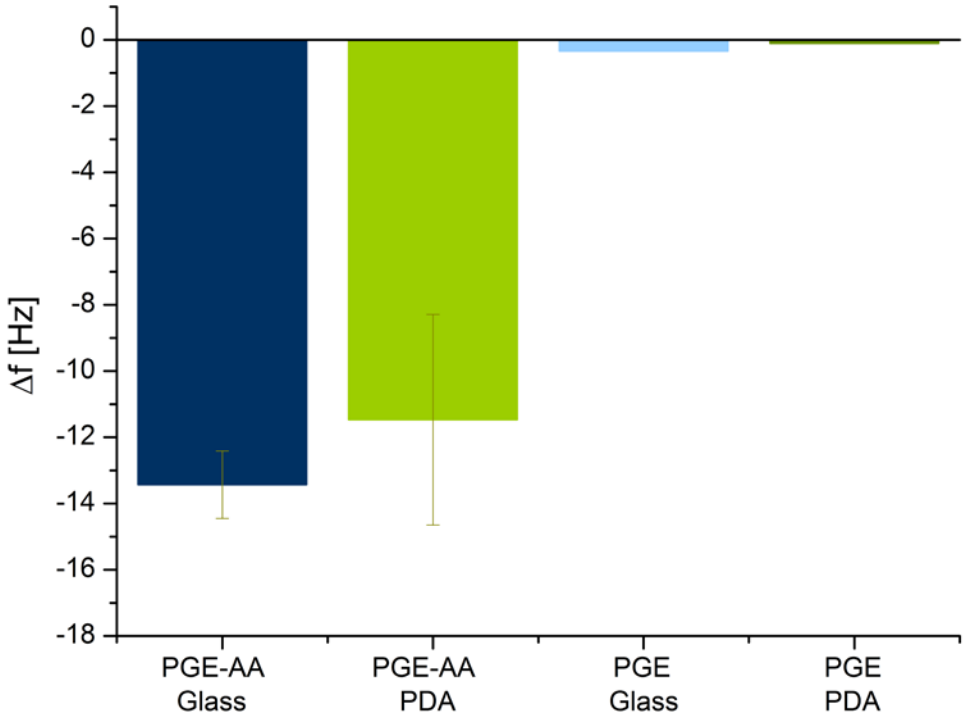

Figure S1. Compilation of the change in frequency values (Δf) extracted from representative QCM-D curves of thermoresponsive coatings on bare and PDA-precoated glass substrates, respectively. Measurements were performed under FSG conditions applying copolymers with (PGE-AA) and without (PGE) a surface reactive anchor group. The baseline acquisition and the final washing step was performed in ethanol.

The deposited mass of PGE-AA on bare and PDA-precoated glass by FSG appeared to be similar, as the changes in frequency, were the same within the range of error. However, due to the vastly different roughness of the two surfaces, bare and PDA-precoated SiO_2 sensors, and the resulting differences in friction during QCM-D measurements the obtained Δf values are not necessarily comparable. They should thus not be interpreted as an equal amount of

deposited PGE-AA on bare and PDA-coated SiO_2 chips. Furthermore, meaningful layer thicknesses cannot be extracted from measurements on rough surfaces as the Voigt model is not valid.

Dynamic light scattering (DLS) measurement of PGE-AA in ethanol and mixtures of ethanol/MOPS buffer

In order to investigate the size of PGE-AA under FSG and cloud point grafting (CPG) conditions we performed DLS measurements. Representative size distribution curves are shown in Figure S2. PGE-AA is fully soluble in ethanol but not in MOPS buffer at high concentrations of 10 mg ml^{-2} at 20 °C. Therefore, PGE-AA was dissolved in ethanol and subsequently mixed with iterative amounts of MOPS buffer until the polymer collapsed in order to obtain CPG conditions. Measurements were thus performed at a concentration of 10 mg mL^{-1} PGE-AA in ethanol and in mixtures of ethanol/MOPS buffer of 4:1 (FSG conditions) and 1:1.4 (CPG conditions).

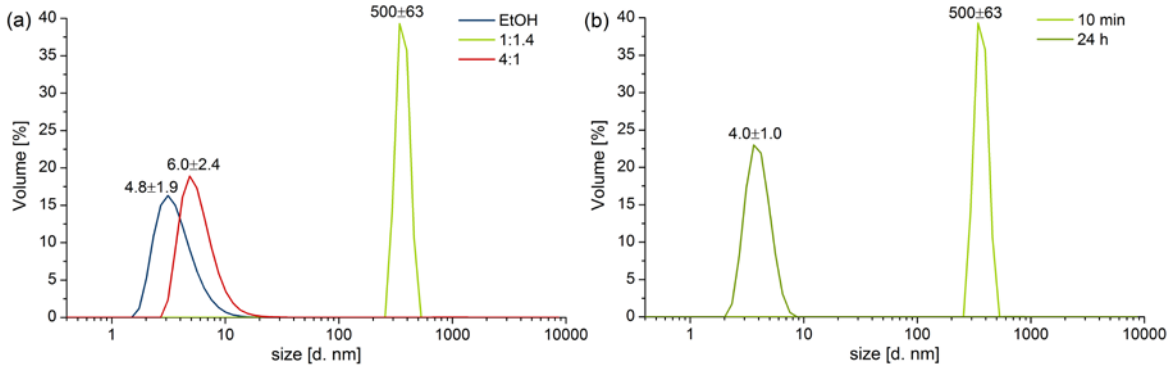

Figure S2. (a) Size distribution by volume of PGE-AA measured by DLS at a concentration of 10 mg mL^{-1} in ethanol and in mixtures of ethanol/MOPS buffer of 4:1 (FSG conditions) and 1:1.4 (CPG conditions). (b) Size distribution by volume of PGE-AA in ethanol/MOPS buffer 1:1.4 after 10 min and after 24 h. Numbers indicate the mean diameter size in nm together with their standard deviation from three replicated measurements.

In ethanol and the solvent mixture ethanol/MOPS buffer of 4:1, which resembles FSG conditions, single polymer coils of PGE-AA with a hydrodynamic diameter of around 5 nm were detected. In the solvent mixture ethanol/MOPS buffer 1:1.4, which resembles CPG conditions, aggregates of PGE-AA with a diameter of around 500 nm were formed within the first few minutes after mixing. After a few hours, however, the turbid solution turned clear again. Therefore, the hydrodynamic diameter was assessed again by DLS in the ethanol/MOPS mixture (1:1.4) after 24 h at room temperature. Figure S2(b) illustrates that the polymer size after 24 h under CPG conditions was similar to the diameter observed for single polymer coils in ethanol or under FSG conditions. For surface coatings under CPG conditions always freshly prepared polymer solutions were applied and the adjusted turbidity of the polymer solution was maintained throughout the whole grafting process.

Ellipsometry data of covalently attached PGE-AA coatings on PDA-precoated Si-wafers

Due to the high surface roughness of the PDA precoating it is difficult to determine the precise layer thicknesses of PGE-AA coatings with an additional PDA adhesion layer on glass or silicon wafers. When fitting the measured ellipsometry data no meaningful fits were obtained. The results applying a simple Cauchy layer representing PGE-AA with a fixed refractive index of n = 1.495, but neglecting the surface roughness of the underlying PDA layer also fitted by a Cauchy layer are summarized in Figure S3(a). In addition, results by applying the Lorentz oscillator as a dispersion model in order to fit the rough PDA layer as described by Pop-Georgievski et al.[6] are summarized in Figure S3(b).

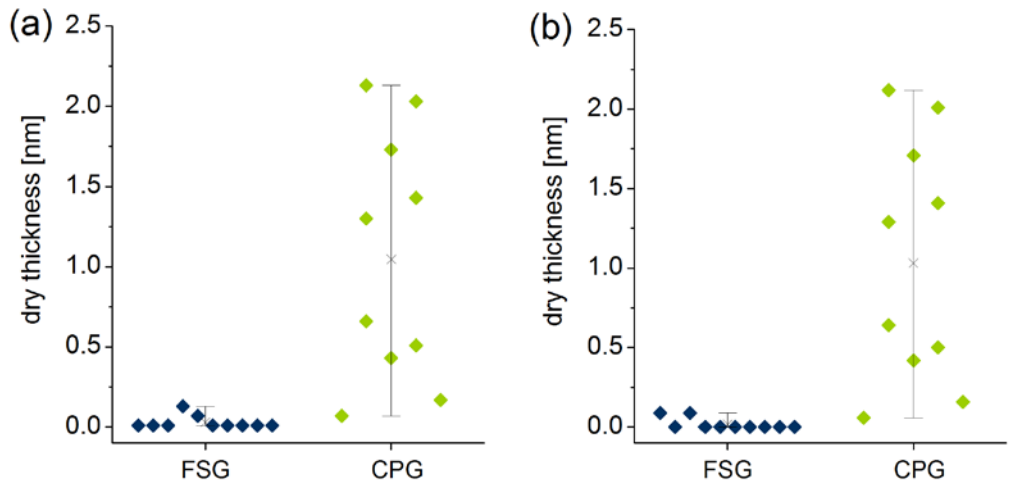

Figure S3. Compilation of the dry thickness of PGE-AA chains assembled on PDA-precoated silicon wafers as determined via ellipsometry. The PDA layer thickness was either determined by a Cauchy layer fit (a) or by using the Lorentz oscillator (b). Coatings were prepared either by grafting from solutions of ethanol/MOPS buffer of 4:1 (FSG) or 1:1.4 (CPG). Values are shown for each performed measurement (diamonds) together with their mean value (black cross) and their 90% CI (whiskers).

For surfaces coated under full solubility, thickness values at the lower limit of the fitting model (0.1 nm) where obtained while data from cloud point grafted surfaces resulted in a strong scattering of fitted values. AFM measurements revealed a high surface roughness of R_q = 5.8 nm for PDA-precoated substrates (Figure 3) with an average layer thickness of approximately 8 nm. At such a high roughness an average effective refractive index is needed which requires complex modeling and simulation of the rough surface in order to obtain correct layer thicknesses.[2-5]

XPS measurements on PGE-AA coated glass

In order to assess the stability of self-assembled and physically attached PGE-AA monolayers on bare glass, we measured XPS spectra of (i) bare glass, (ii) PGE-AA coated glass via FSG and (iii) PGE-AA coated glass after cell culture (Figure S4). The latter sample was prepared

for harvesting a cell sheet via a thermal trigger. The rational of the experiment was to reveal whether the PGE-AA coating remains on the glass surface after harvesting the cell sheet.

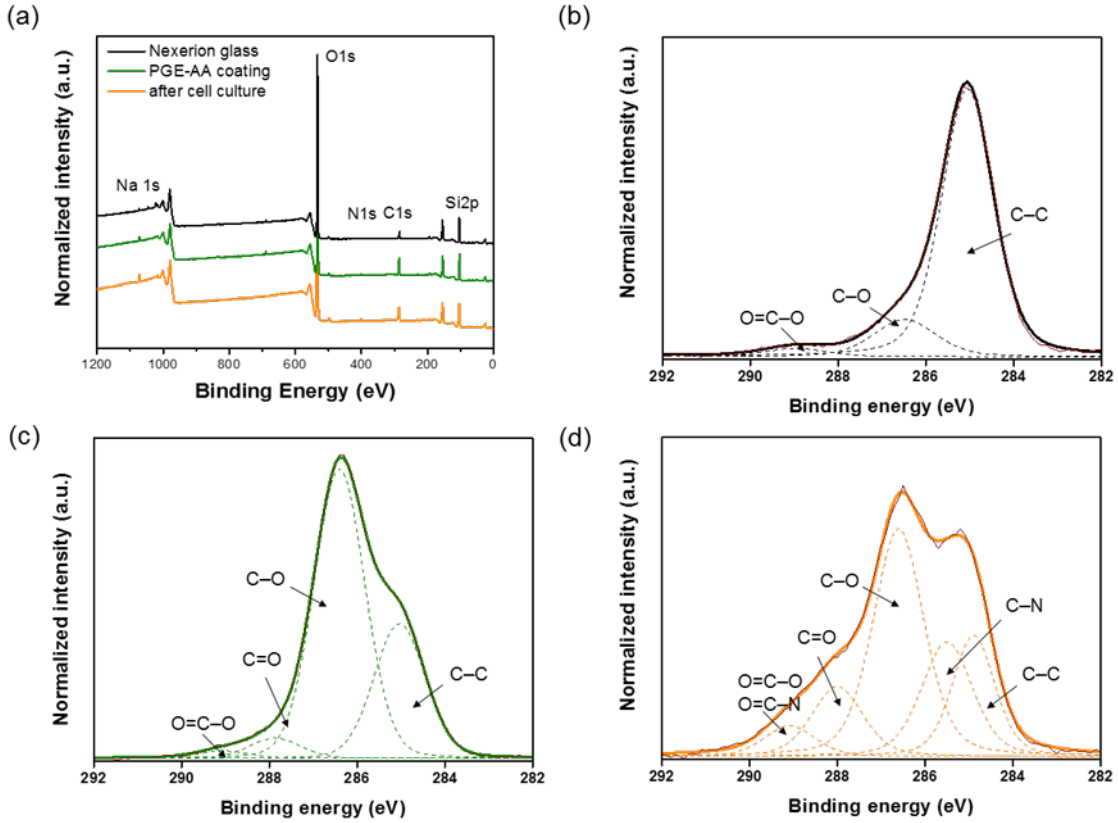

Figure S4. (a) Survey XPS spectra of the bare glass substrate (Nexterion; borosilicate), self-assembly of PGE-AA on glass via FSG, and self-assembly of PGE-AA on glass via FSG after thermally-triggered cell sheet detachment and additional trypsinization. (b)-(d) Highly resolved C1s spectra of bare glass, PGE-AA coating and PGE-AA coating after cell sheet detachment and trypsinization, respectively. For assignments see Supporting Information Tables S1b.

The observed relative chemical composition of the bare glass, the PGE-AA coated glass and the coated glass after cell culture, cell sheet detachment and trypsination in order to remove residual ECM proteins from the surface are summarized in Table S1a. Assignments of the fitted components in the highly resolved XPS C 1s spectra are summarized in Table S1b.

Table S1a. Relative chemical composition in atom%.

	glass	PGE-AA	trypsinized
C1s [%]	8.3	17.6	16.8
N1s [%]	0.4	0.8	1.5
Si2p [%]	25.8	21.0	22.7
O1s [%]	62.1	56.8	58.2

Table S1b. Assignments of the fitted components in the highly resolved XPS C 1s spectra.

Sample	Spectrum	Binding energy	L-G Mixing	FWHM	Interpretation	Relat. Area	Abs. Area [cps*eV]
Glass substrate	C1s	285.0	0.32	1.39	C–C sp^3	0.83	3988
		286.5	0.32	1.67	C–O	0.14	665
		289.0	0.32	1.67	O–C=O	0.03	149
PGE-AA	C1s	285.0	0.32	1.37	C–C sp^3	0.29	2467
		286.4	0.32	1.37	C–O	0.66	5541
		287.8	0.32	1.37	C=O	0.05	258
		289.0	0.32	1.37	O–C=O	0.02	119
Trypsinized PGE-AA	C1s	285.0	0.32	1.14	C–C sp^3	0.19	1164
		285.6	0.32	1.37	C–N	0.21	1319
		286.7	0.32	1.37	C–O	0.42	2627
		288.1	0.32	1.37	C=O	0.13	810
		289.2	0.32	1.37	N–C=O, O–C=O	0.05	356

The presence of the PGE-AA coating was clearly observed by the elemental composition of the analyzed surface when compared to that of the Nexterion glass. As expected, the carbon fraction increased from 8.3 at% to 17.6 at% and that one of N from 0.4 at% to 0.8 at% after coating. However, after cultivation of cells on PGE-AA surfaces with subsequent cell sheet detachment (and additional trysinization to remove the majority of residual proteins), the carbon fraction did not change significantly (from 17.6 at% to 16.8 at%) while that one of N increased from 0.8 at% to 1.5 at% indicating that the physically attached thermoresponsive coating remains stably on the surface.

The thickness of polymer layer was also estimated by running the SESSA 2.0 simulation software using experimental XPS data from survey spectra of the pristine glass and PGE-AA film (see Table S1a). SESSA covers a standard reference database that is delivered by the National Institute of Standards and Technology. This database contains all the data needed for quantitative simulations of XPS spectra.[7,8]

The amount of Si and C on the pristine surface of glass according to XPS survey spectra were 25.8 at% and 8.3 at%, respectively. Therefore, the atomic ratio of Si/C components was 3.1. According to that experimental ratio the thickness of carbon material (adsorbed hydrocarbons), that covers the surface of glass was calculated as the first step. Assuming the density of that material to be 1 g/cm^3, the thickness of the carbon material surface layer that corresponds to the experimental Si/C atomic ratio was 0.8 nm.[8]

Experimental XPS survey spectra of the PGE-AA film on glass showed that the amount of Si and C was 21.0 at% and 17.6 at%, respectively. From the overall stoichiometry of PGE-AA the Si/C atomic ratio was calculated to be 1.2. In the next step, SESSA was used to simulate spectra for a double layer PGE-AA on carbon material on top of the glass substrate. The polymer PGE-AA layer was modelled with defined numbers of repeating units (x (GME) = 62, y (EGE) = 187, z(AGE-Amin) = 11) and a polymer density of 1.2 g/cm^3. The best simulation result was reached assuming a mean thickness of the PGE-AA polymer top layer of 0.60 nm.[8] Thit result is in good agreement with the ones measured by ellipsometry on dried PGE-AA coatings at FSG condition (Figure 3).

ABBREVIATIONS

AFM, atom force microscopy; CPG, cloud point grafting; DLS, dynamic light scattering; DMEM, Dulbecco's modified Eagle medium; EGE, ethyl glycidyl ether; FSG, full solubility grafting; GME, methyl glycidyl ether; MOPS, 3-(*N*-morpholino)propanesulfonic acid; PDA, polydopamine; PGE-AA, poly(glycidyl ether) with an amine-containing anchored block; QCM-D, quartz crystal microbalance with dissipation; XPS, X-ray photoelectron spectroscopy.

REFERENCES

1. D. D. Stöbener, M. Uckert, J. L. Cuellar-Camacho, A. Hoppensack, M. Weinhart, *ACS Biomater. Sci. Eng.* 2017**, 3** (9), 2155-2165.
2. P. Petrik, L. P. Biró, M. Fried, T. Lohner, R. Berger, C. Schneider, J. Gyulai, H. Ryssel, *Thin Solid Films* 1998**, 315** (1), 186-191.
3. C. A. Fenstermaker, F. L. McCrackin, *Surf. Sci.* 1969**, 16**, 85-96.
4. S. Marsillac, M. N. Sestak, J. Li, R. W. Collins, Spectroscopic ellipsometry. In *Advanced Characterization Techniques for Thin Film Solar Cells*, Wiley-VCH Verlag GmbH & Co. KGaA: 2011, pp 125-149.
5. D. Lehmann, F. Seidel, D. R. Zahn, *SpringerPlus* 2014**, 3** (1), 82.
6. O. Pop-Georgievski, Š. Popelka, M. Houska, D. Chvostová, V. Proks, F. Rypáček, *Biomacromolecules,* 2011, **12**, 3232-3242.
7. W. Smekal, W. S. M. Werner, C. J. Powell, *Surf. Interface Anal.* 2005, **37**, 1059−1067.
8. W. S. M. Werner, W. Smekal, C. J. Powell, *NIST Database for the Simulation of Electron Spectra for Surface Analysis (SESSA)*, Version 2.0; U.S. Department of Commerce/NIST: Gaithersburg, Maryland, 2014. http://www.NIST.gov/srd/NIST100.cfm (accessed Jan 29, 2018).