Electronic Supplementary Material (ESI) for RSC Advances

Electronic Supplementary Information

On-chip detection of multiple serum antibodies against epitopes of celiac disease by an array of amorphous silicon sensors

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1. Equipment

The thickness of PHEMA layers at various functionalization steps was measured using atomic force microscopy (AFM). Samples were measured in air by employing a Dimension Icon atomic force microscope with a Nanoscope V Controller (Bruker AXS, Germany) in tapping mode. High resolution Rotated-Tapping Mode-etched silicon probes (RTESP) with 8 nm radius of curvature, resonant frequency around 300 kHz and a nominal spring constant of 50 N/m was employed for measurements in air. All the AFM images were analyzed with the free software Gwiddion, and the only correction was the background subtraction by a plane leveling, assuming the glass surface, which is uncovered by the polymer film and by photoresist, as reference value. The film thickness was calculated as the average of a set of 512 horizontally leveled sampling profiles. FTIR was performed using 410 Jasco spectrometer equipped with a conductive ceramic coil mounted in a water-cooled copper jacket source and a KBr beamsplitter, an optical path purged continuously with gaseous nitrogen and a TGS detector, with spectral resolution up to 1 cm. Spectra were recorded in transmission mode using a silicon oxide substrate as the blank for background subtraction.

Filed emission scanning electron microscopy (FESEM) images were recorded with an Auriga 405 system (Zeiss), using low operating voltage and current conditions, to eliminate charging effects and to avoid material damage.

2. AFM-imaging

AFM images of the polymer film on glass surfaces after functionalization with peptides, primary antibody and secondary antibody marked with HRP (Figure 1).

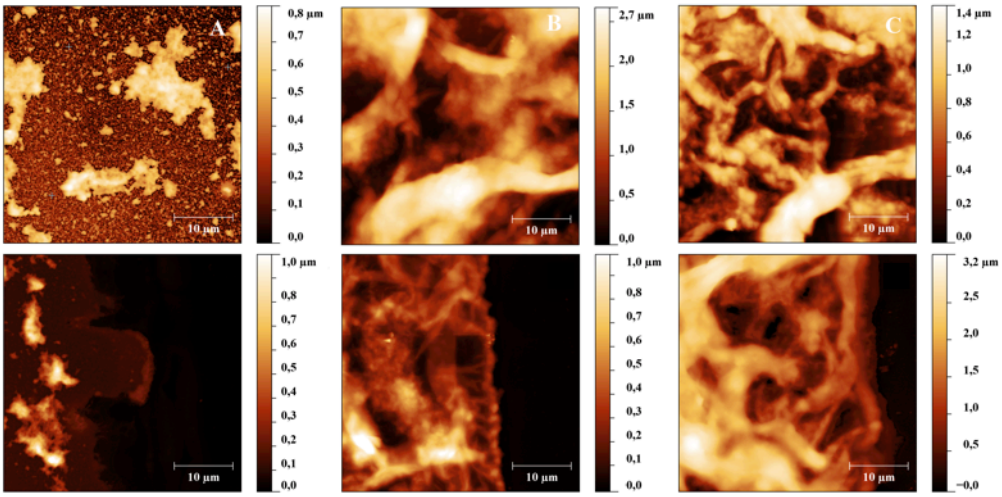

Figure 1: AFM images of PHEMA film after immobilization of A) peptides, B) primary antibody and C) secondary antibody marked with HRP.

Table 1: Thicknesses of the film at different steps of functionalization as measured by the AFM analysis.

Film	Thickness (nm)
PHEMA	187 ± 4
PHEMA+VEA	496 ± 9
PHEMA+VEA+anti-VEA	626 ± 11
PHEMA+VEA+anti-VEA+ Ig-HRP	646 ± 10

3. FESEM-imaging

FESEM-images of the polymer film on glass surfaces after functionalization with peptide, primary antibody and secondary antibody marked with HRP (Figure 2).

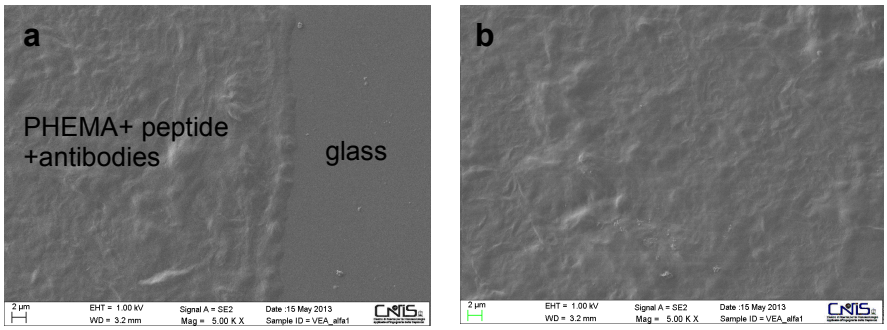

Figure 2: FESEM images of patterned PHEMA functionalized with peptide and antibodies on **a)** the edge, and **b)** the bulk part of the film (glass substrate).

4. FTIR-spectroscopy

Silicon oxide substrates for FTIR spectroscopy were functionalized using the same procedure described for glass slide, without performing the photolithographic step.

FTIR analysis was executed after each functionalization step (Figure 3,4). Spectra of the PHEMA brush film displays two mayor absorption bands at 1700 and 3500 cm^{-1} relative to the stretching of the carbonyl and hydroxyl (OH) groups, respectively (Figure 3a). Upon reaction with succinic anhydride (SA)**,** spectrum evidences the disappearing of the OH-related bands, confirming the formation of the carboxylic acid function along the brush structure (Figure 3b). Carboxylic groups react with n-hydroxysuccinimide (NHS) forming NHS-ester groups, which display two characteristic absorption peaks at 1710 and 1720 cm^{-1} (Figure 3c).[1]

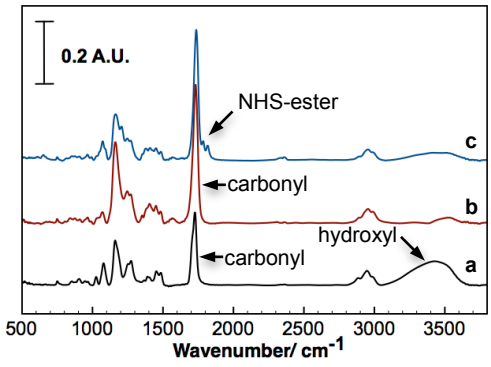

Figure 3: FTIR spectra of **a)** PHEMA, **b)** PHEMA after reaction with SA (PHEMA-SA), and **c)** PHEMA-SA after reaction with NHS (PHEMA-SA-NHS).

After reaction with the peptide, FTIR spectrum (Figure 4a) shows the formation of two peaks at 1600-1700 and 1550-1450 cm^{-1} that correspond to amide I (C=O stretching and NH-bending) and amide II (N-H bending) vibration respectively, confirming the immobilization of the peptide.[2] Beside amide bonds, the FTIR spectrum displays the presence of the absorption peaks relative to the NHS-ester unreacted groups. These NHS-ester groups may interfere with other species present in the serum, therefore they were blocked soaking the PHEMA functionalized substrates in a phosphate solution of ethanolamine (pH= 7.5, 25 mM) for 20 min. Analysis by FTIR of this layer (Figure 4b) shows the disappearance of the NHS-ester absorption peaks and the increase of signal intensity relatives to amide I and II.

As expected, spectra of PHEMA functionalized film (blocked with ethanolamine) after reaction with the primary antibody and Ig-HRP did not show the appearance of additional absorption bands, while displaying the signal relative to the absorption of amide I and II (Figure 4c,d), demonstrating the stability of the sandwich like structure.

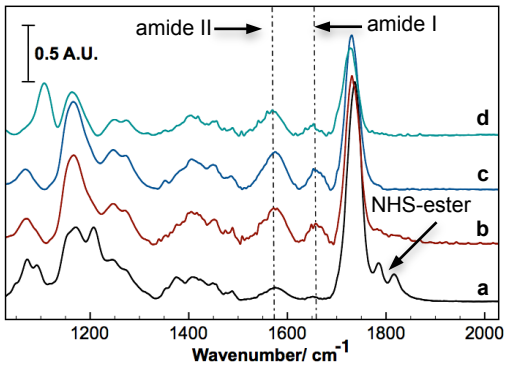

Figure 4: FTIR spectra of **a)** PHEMA-SA-NHS after reaction with the peptide (PHEMA-peptide), **b)** PHEMA-peptide after reaction with ethanolamine, **c)** PHEMA-peptide after reaction with ethanolamine and primary antibody, and **d)** after reaction with Ig-HRP.

(1) Costantini, F.; Tiggelaar, R. M.; Sennato, S.; Mura, F.; Schlautmann, S.; Bordi, F.; Gardeniers, H.; Manetti, C. *Analyst*, **2013**, 138, 5019-5024.
(2) Jung, C. *J. Mol. Recognit.* **2000**, *13*, 325.