Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-019-01979-9

RESEARCH PAPER

Maleic anhydride and acetylene plasma copolymer surfaces for SPR immunosensing

Ekaterina Makhneva[1,2] · Zdeněk Farka[3] · Matěj Pastucha[3,4] · Adam Obrusník[1,2] · Veronika Horáčková[3] · Petr Skládal[3,4] · Lenka Zajíčková[1,2]

Received: 30 March 2019 / Revised: 8 June 2019 / Accepted: 13 June 2019

Abstract

We report on the successful application of carboxyl-rich plasma polymerized (PP) films as a matrix layer for bioreceptor immobilization in surface plasmon resonance (SPR) immunosensing. Composition and chemical properties of the carboxyl-rich PP films deposited from a mixture of maleic anhydride and acetylene were investigated. Changes in the films stored in air, water, and buffer were studied and the involved chemical changes were described. Performance in SPR immunosensing was evaluated on interactions of human serum albumin (HSA) with a specific monoclonal antibody. The comparison with the mixed self-assembled monolayer of mercaptoundecanoic acid and mercaptohexanol (MUA/MCH) and one of the most widely used surfaces for SPR, the 2D and 3D carboxymethylated dextran (CMD), was presented to show the efficacy of plasma polymerized matrix layers for biosensing. The PP film-based SPR immunosensor provided a similar detection limit of HSA (100 ng/mL) as MUA/MCH- (100 ng/mL) and 3D CMD (50 ng/mL)-based sensors. However, the response levels were about twice higher in case of the PP film-based immunosensor than in case of MUA/MCH-based alternative. The PP film surfaces had similar binding capacity towards antibody as the 3D CMD layers. The response of PP film-based sensor towards HSA was comparable to 3D CMD-based sensor up to 2.5 μg/mL. For the higher concentrations (> 10 μg/mL), the response of PP film-based immunosensor was lower due to inaccessibility of active sites of the immobilized antibody inside the flat PP film surface. We have demonstrated that due to its high stability and cost-effective straightforward preparation, the carboxyl-rich PP films represent an efficient alternative to self-assembled monolayers (SAM) and dextran-based layers in label-free immunosensing.

Keywords Plasma polymerization · Carboxyl-rich films · Immobilization · Surface plasmon resonance biosensor · Label-free detection

Introduction

Biosensors are being extensively developed and applied for biomedical and environmental studies. Quartz crystal microbalance (QCM) [1] and surface plasmon resonance (SPR) [2] biosensors proved to be very promising tools for sensitive label-free determination of various analytes including proteins and bacteria [3]. Efficient immobilization of the biorecognition molecules onto the sensor surface is always required for the biosensor development. Coatings containing chemical groups capable of the formation of covalent linkages between biomolecules and the surface are of high interest for biological applications [4–6]. A sufficiently high surface concentration of functional groups as well as the layer stability under various pH is required.

Plasma polymerized (PP) films are extensively used in the field of biomedical applications. The amine-rich PP films are

Published in the topical collection *New Developments in Biosensors* with guest editors Francesco Baldini and Maria Minunni.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00216-019-01979-9) contains supplementary material, which is available to authorized users.

✉ Zdeněk Farka
farka@mail.muni.cz

[1] RG Plasma Technologies, CEITEC MU, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

[2] Department of Physical Electronics, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

[3] RG Nanobiotechnology, CEITEC MU, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

[4] Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

Published online: 27 June 2019

the most widely used and known to be convenient for immobilization of biomolecules and binding of cells [7–11]. There were plenty of reports describing the use of amine-rich PP films in biosensing, cell proliferation, and other biological applications [12–15]. Compared to the conventionally used layers, such as carboxymethylated dextran (CMD), the plasma polymerization can provide faster and cost-effective layer preparation. Furthermore, PP surfaces provide good adhesion to common substrates, which results in stable signal and good regeneration capabilities. Carboxyl-rich PP films are typically generated by plasma polymerization of various acrylates [16, 17]. Examples of using gas mixtures of CO_2 and ethylene were also presented [18, 19]. Another approach relied on the use of PP films deposited from maleic anhydride (MA), which provided highly reactive coatings, but the process required fine tuning, because the level of stability was initially not sufficient [20]. There were several impressive results for biological applications using the grafting of MA onto hydrocarbon surfaces [21, 22]; however, these PP layers were not yet employed as a matrix layer for biomolecule immobilization.

Because the carboxyl-based layers are otherwise the gold standard within matrices for label-free biosensing [23, 24], our work was dedicated to the development of effective carboxyl-rich PP films. The preliminary tests were carried out to show the potential of PP films in SPR immunosensing [25]. Two different types of carboxyl-rich PP films were prepared at different plasma conditions by polymerization from MA/C_2H_2/Ar gas mixture and from CO_2/C_2H_4/Ar gas mixture. Their binding capacity towards monoclonal anti-human serum albumin (HSA) antibody was evaluated and the potential to bind antigen was demonstrated by applying HSA solution (single concentration of 5 μg/mL). However, the CO_2/C_2H_4 PP film-based immunosensor provided lower stability and smaller binding capacity towards the antibody, which resulted in constant drift of the baseline and a low level of response towards HSA. On the other hand, the MA/C_2H_2 PP film was able to efficiently bind the antibody, provided a stable baseline during the measurements, and the response towards HSA was selective and much larger compared to the CO_2/C_2H_4 PP film-based immunosensor.

Based on the obtained results, we continued the studies of the MA/C_2H_2 PP film and its properties with the aim of developing a robust immunosensing layer. Here, we present a detailed study on the characterization of chemical properties of the MA/C_2H_2 PP film, its stability and use as a matrix suitable for bioreceptor immobilization in SPR immunosensing. Chemical composition of the obtained PP films was analyzed by Fourier transform infrared spectroscopy (FTIR) and the chemical changes were followed right after the deposition, after storage in air, water, and buffer. The changes of surface topography and stiffness were evaluated by atomic force microscopy (AFM). The performance of the developed PP film-based immunosensor was characterized and compared with sensors based on mixed self-assembled monolayer (SAM) of mercaptoundecanoic acid and mercaptohexanol (MUA/MCH) and on the widely used CMD.

Materials and methods

Chemicals and materials

Maleic anhydride (MA) (98%), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), bovine serum albumin (BSA), and human serum albumin (HSA) were purchased from Sigma-Aldrich (Germany). Sodium hydrogen phosphate, sodium dihydrogen phosphate, and sodium chloride for preparation of the phosphate-buffered saline (PBS; 50 mM phosphate, 150 mM NaCl, pH 7.4) were supplied from Penta (Czech Republic). Anti-HSA monoclonal antibody (clone AL-01, purified immunoglobulin G) was obtained from Exbio (Czech Republic). Argon (99.998%) was supplied by Messer (Czech Republic). Double-side polished single crystalline silicon (c-Si) wafers (⟨111⟩, N-type phosphorus doped, resistance 0.5 Ω cm) from ON-SEMI (USA) were cut into 10×15 mm^2 substrates. MP-SPR (multi-parametric surface plasmon resonance) Navi gold sensors, SPR102 Au were purchased from BioNavis (Finland). All substrates were cleaned by sonication in isopropanol (Penta, 99.8%) for 10 min. All buffers were filtered using vacuum filtration system Stericup with 0.22-μm polyethersulfone (PES) membrane (Merck Millipore, USA).

Plasma polymerization in atmospheric pressure dielectric barrier discharge

The mixture of MA/C_2H_2/Ar was plasma polymerized in an atmospheric pressure dielectric barrier discharge (AP-DBD) reactor. The reactor was powered by 4 kHz AC voltage with peak-to-peak voltage of 4 kV. The reactor geometry and a detailed study of the correlation of plasma parameters with the PP film properties was reported before [26]. The polymerization time was adjusted to obtain the PP films with a thickness of 20 nm (0.5 min) for the SPR tests and AFM measurements, and 120 nm (3 min) for the FTIR studies [25].

FTIR analysis

Vertex 80v spectrometer (Bruker Optik, Germany) was used to obtain the FTIR spectra of the PP films. The measurements were carried out in the transmission mode on the IR-transparent Si substrates using parallel beam transmittance accessory in the spectral range from 400 to 4500 cm^{-1}. The reference spectra were recorded from a bare double-side

polished Si wafer. Absorbance was calculated based on the measured transmittance and data were normalized by thickness. The FTIR spectra are plotted in the range of 1000–4000 cm^{-1}, and there were no significant absorption peaks of the PP films identified outside the given range [25]. The spectrum assignments were done according to [27].

Ellipsometric measurements

The Jobin Yvon UVISEL ellipsometer (Horiba, Japan) was used to determine the thickness of PP films. The data were collected in the spectral range from 0.6 to 6.5 eV at the incidence angle of 65°. The model of one homogeneous non-uniform film using a PJDOS dispersion model assuming a wedge-shaped non-uniformity was used to fit the optical data obtained for the as-deposited PP films and dried PP films after immersion in PBS [11]. In all cases, the thickness determination was possible with a sufficient level of precision.

Atomic force microscopy

The surface topography and stiffness were studied using AFM. Dimension FastScan Bio (Bruker, USA) with FastScan-A probe was used to scan the surface of bare gold SPR chips, chips with freshly deposited MA/C_2H_2 PP film and chips with PP film after overnight immersion in PBS. The data were evaluated in software Gwyddion (Czech Metrology Institute, Czech Republic).

SPR immunosensing

For the SPR studies, system MP-SPR Navi 210A (BioNavis, Finland) was used; the injection of samples was controlled using the SPR-Navi software. The sensor response was evaluated as the surface plasmon resonance angle determined in angular scan mode by a *centroid* fitting function. Prior to insertion into BioNavis system, the PP-modified SPR chips were washed using PBS, followed by washing with water and drying by nitrogen. First, the baseline signal was established with the degassed PBS as a running buffer with a flow rate of 20 μL/min. Afterwards, the freshly prepared mixture of EDC (200 mM) and NHS (50 mM) in water was injected for 7 min to activate the sensor surface. The anti-HSA antibody was diluted in acetate buffer (pH 4.5) to the concentration of 50 μg/mL and injected to the binding channel for 20 min using the flow rate of 10 μL/min. The reference channel was modified by a solution of BSA under the same experimental conditions using a parallel injection mode. The blocking of reactive surface was performed in both channels using 2 mg/mL solution of BSA in PBS (10 min, 10 μL/min). For the immunosensing experiments, the samples of HSA were injected for 10 min (20 μL/min) with another 10 min as a dissociation time [25].

The performance of PP film-based layer was compared with the surface based on SAM and with commercial CMD chips. For the SAM formation, the gold SPR chip was first washed in acetone (10 min) and isopropanol (10 min) under ultrasonication. The chip was then immersed overnight in a mixture of 3 mM 11-mercaptoundecanoic acid (MUA) and 7 mM 6-mercapto-1-hexanol (MCH). The commercial 2D (CMDP) and 3D (CMD50) CMD-based chips (XanTec bioanalytics, Germany) were used without prior pre-treatment. The conditions of activation using EDC/NHS, antibody binding and surface blocking remained the same as in case of the PP-based sensor.

The binding kinetics was fitted in software TraceDrawer (Ridgeview Instruments AB, Sweden). The one-to-one binding model was employed, and the k_a and k_d values were fitted globally from binding curves shown in graphs.

Results and discussion

Chemical composition and properties of MA/C_2H_2 plasma copolymers

The primary challenge for the development of effective biosensors using plasma polymerization is the creation of thin polymer layers stable in an aqueous environment, which would at the same time be suitable for the immobilization of biomolecules. In this paper, copolymerization of maleic anhydride with acetylene was employed to create stable carboxyl-rich PP films suitable for antibody immobilization. Acetylene is known as one of the most reactive hydrocarbons in terms of plasma polymerization [28]. The use of maleic anhydride and acetylene mixture allowed obtaining plasma copolymers consisting of anhydride groups incorporated along unsaturated hydrocarbon chains. Such copolymerization approach allowed obtaining coatings with desired properties.

To study the chemical changes in the MA/C_2H_2 PP films, FTIR analysis was employed (Fig. 1), which showed that anhydrides, α,β-unsaturated esters, and carboxylic acids were present in the as-deposited films. When the films were immersed in water or PBS right after the deposition, the stability was very poor and the films disappeared from the substrate due to the rapid hydrolysis of the anhydride groups. Due to this fact, the as-deposited films were stored in air and the slow hydrolysis of the anhydride groups was observed by an increase of carboxylic acid signals (at 1725 and 1690 cm^{-1}) and a decrease of anhydride group signals (at 1865 and 1780 cm^{-1}), which completely disappeared after approximately 5 days (120 h) of storage in air. No changes in FTIR spectra were observed during further storage in air. The FTIR spectra of MA/C_2H_2 PP films after various times of storage in air are shown in Fig. S1 in the Electronic Supplementary Material (ESM). After stabilization in air (196 h), the films were

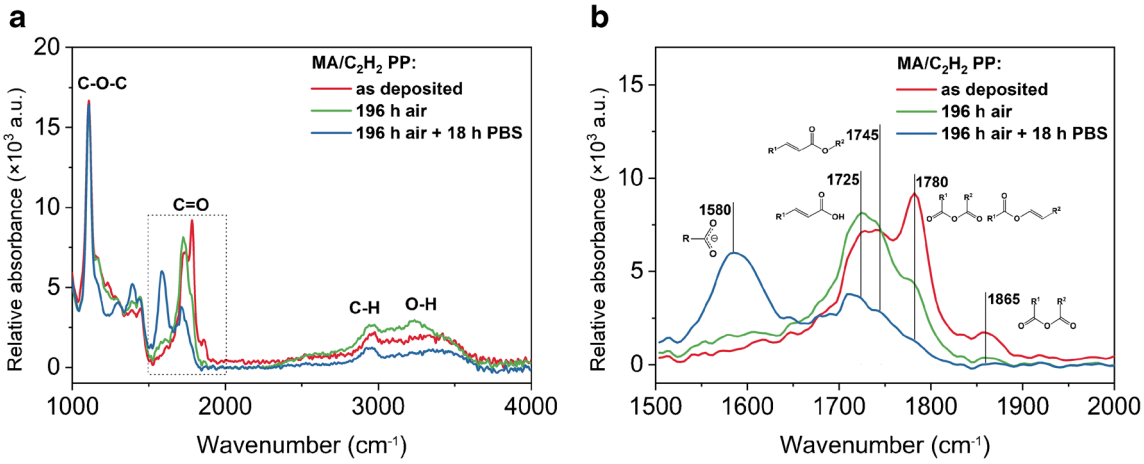

Fig. 1 FTIR spectra of MA/C_2H_2 PP films: **a** full range spectra, **b** double bonds region

immersed in PBS, and after 18 h of immersion, they exhibited approximately 28% thickness loss. During the immersion in PBS, the formation of carboxylate ions was observed, which was indicated by the appearance of a signal at 1580 cm^{-1}.

The 20-nm-thick (as determined by ellipsometry) MA/C_2H_2 PP layers deposited on gold SPR chips were studied using AFM (Fig. 2). Compared to the bare gold, surface roughness increased after the PP film deposition, followed by flattening after the overnight immersion in PBS. Particles observed at the surface of MA/C_2H_2 PP layers were formed due to the production of dust particles in the plasma gas phase that, after reaching a critical size, fell down and stuck to the surface. Acetylene-containing plasmas are well-known for their high production of dust particles and the formation of

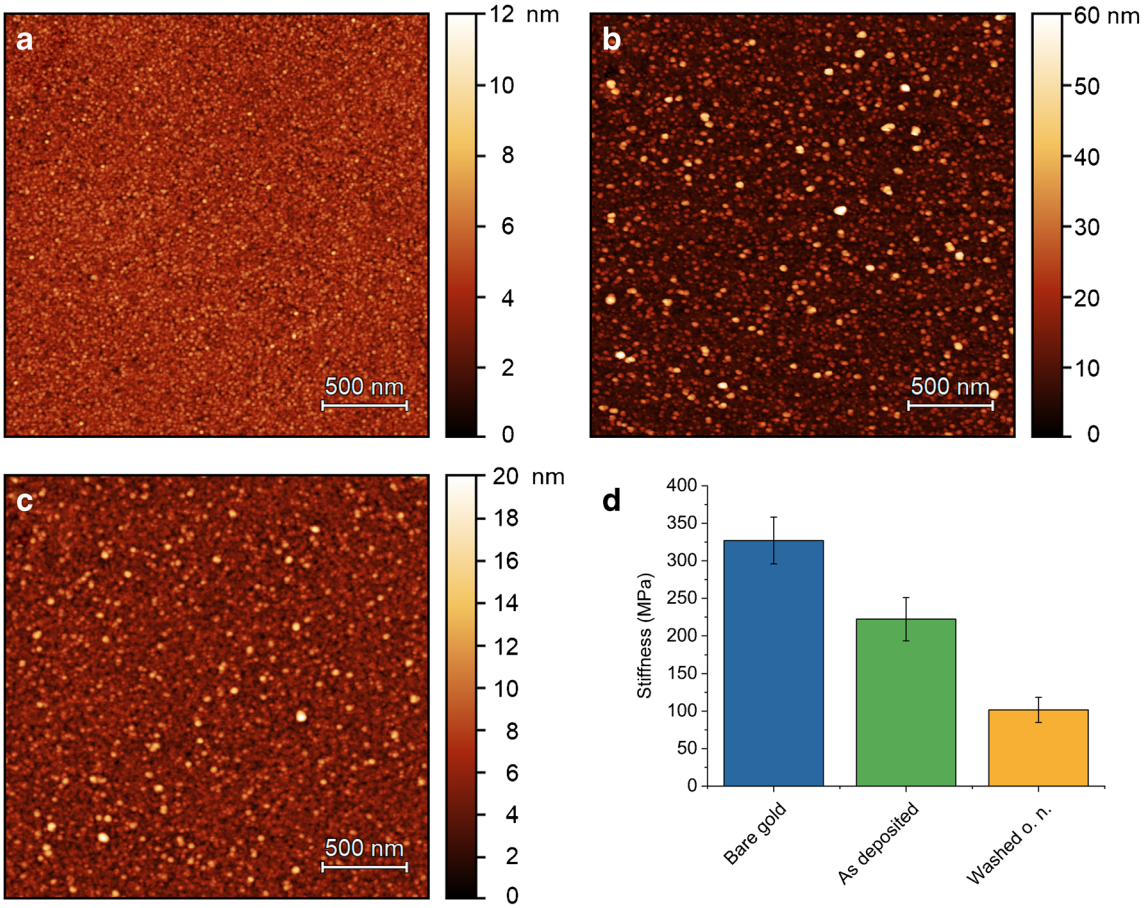

Fig. 2 AFM images of **a** bare gold surface of SPR chip, **b** as-deposited MA/C_2H_2 PP film, and **c** MA/C_2H_2 PP film after overnight immersion in PBS. **d** Surface stiffness evaluated using the AFM

rough films during MA/C_2H_2 plasma polymerization was studied in detail previously [29]. The PP surface flattening after overnight immersion in PBS was caused by the thickness losses of the PP layers, which resulted in partial removal of the loosely bound particles from the surface. After the PP film deposition, the stiffness of the surface decreased, which is connected with covering of the rather rigid gold surface with soft polymer layer. Further decrease of stiffness was observed after the overnight immersion in PBS, which indicates loosening of the PP film structure. Such change was generally beneficial as it provided higher flexibility during the interactions with soft biomolecules.

Performance of MA/C_2H_2 PP films in SPR immunosensing

Performance of the PP layers in SPR immunosensing was evaluated by probing the interactions of immobilized AL-01 antibody and HSA antigen as a model pair. It is known that this pair provides a reliable immunocomplex formation under different conditions and assay formats [30, 31]. SPR chips with the deposited 20-nm-thick MA/C_2H_2 PP films were stabilized in air and by overnight immersion in PBS and inserted into the system. Before antibody immobilization, the PBS was flown to establish a stable baseline signal. After this step, the sensor surface was activated by the reaction with EDC/NHS, which transformed the carboxyl groups into esters reactive towards amino groups of the antibody (Fig. 3).

After the antibody immobilization (Fig. 4a), reactions with HSA solutions of different concentrations were carried out (Fig. 4b). After the individual HSA injections, 10 mM HCl solution was applied for 2 min to regenerate the chip surface. The immunocomplex was dissociated during the regeneration, the antigen molecules were washed away revealing the active sites of antibodies, and the sensor surface was fully active again. Regeneration allowed to carry out multiple measurements with a single SPR chip, the sensorgram of sensor surface regeneration is shown in Fig. S2 in the ESM.

Limit of detection (LOD; evaluated as $S/N > 3$) of 100 ng/mL of HSA was achieved with the MA/C_2H_2 PP film-based chips, which is comparable to the conventionally used SAM-based sensors [32, 33]. For HSA concentrations above 10 μg/mL, saturation of the sensor surface was observed. The raw signals from the measuring and reference channels are shown in Fig. S3 A-B in the ESM. The negligible signal changes in the reference channel confirm the high efficiency of the used blocking conditions and the low level of non-specific interactions. Overall, the developed sensor provided excellent level of stability and selective response towards analyte.

To critically evaluate the suitability of the developed sensor for immunosensing, a comparison with the standard MUA/MCH self-assembled monolayer (Fig. 4c) and with the most widely used CMD-based sensors (Fig. 4d) was carried out using exactly the same procedure for the sensor preparation and the same antibody-antigen pair. The raw signals from the measuring and reference channel are shown in Fig. S3 C-F in the ESM. The signals obtained during the antibody immobilization are summarized in Table 1.

The MUA/MCH mixed self-assembled monolayer was formed based on carboxyl bearing mercaptoundecanoic acid (MUA) and inert mercaptohexanol (MCH). Compared to the MA/C_2H_2 PP film, the MUA/MCH-based sensor provided lower capacity towards antibody binding, which resulted in subsequent lower capacity towards binding of HSA antigen (lower signals and working range limited to 5 μg/mL). Compared to PP film- and CMD-based sensor surface

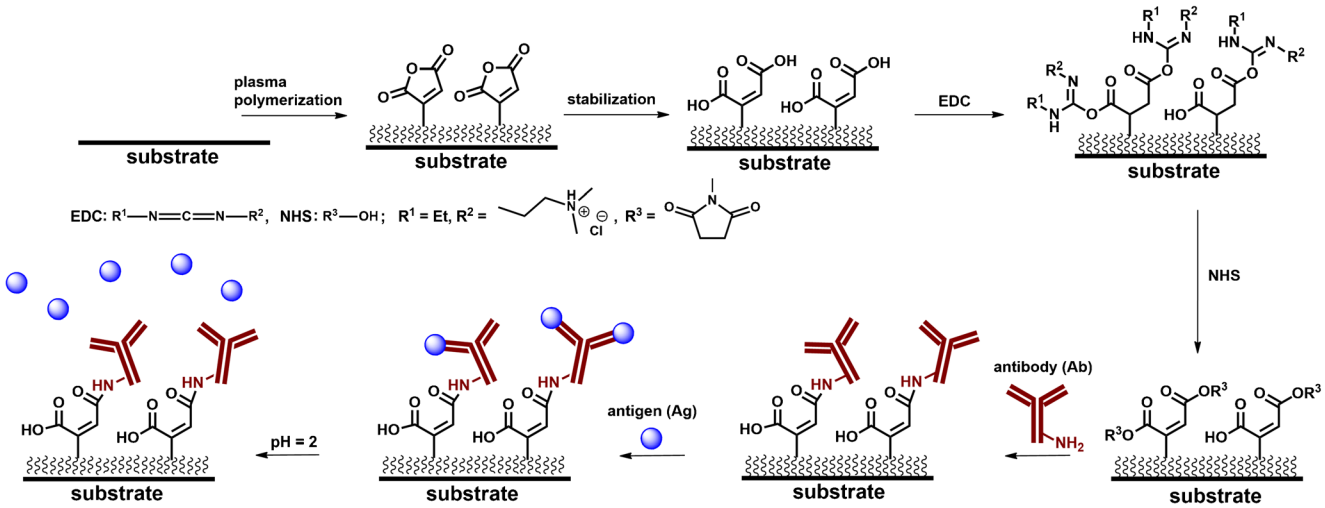

Fig. 3 Individual steps of the immunosensor surface preparation and measurement. Anhydride groups deposited during the plasma polymerization of MA were transformed to carboxyl groups during the stabilization in air. The carboxyl groups were then activated by the EDC/NHS, which allowed to covalently bind the amino groups of the antibody. After the capture of the antigen, the immunocomplex can be regenerated by the introduction of acidic pH, which dissociates the antigen and allows further experiments

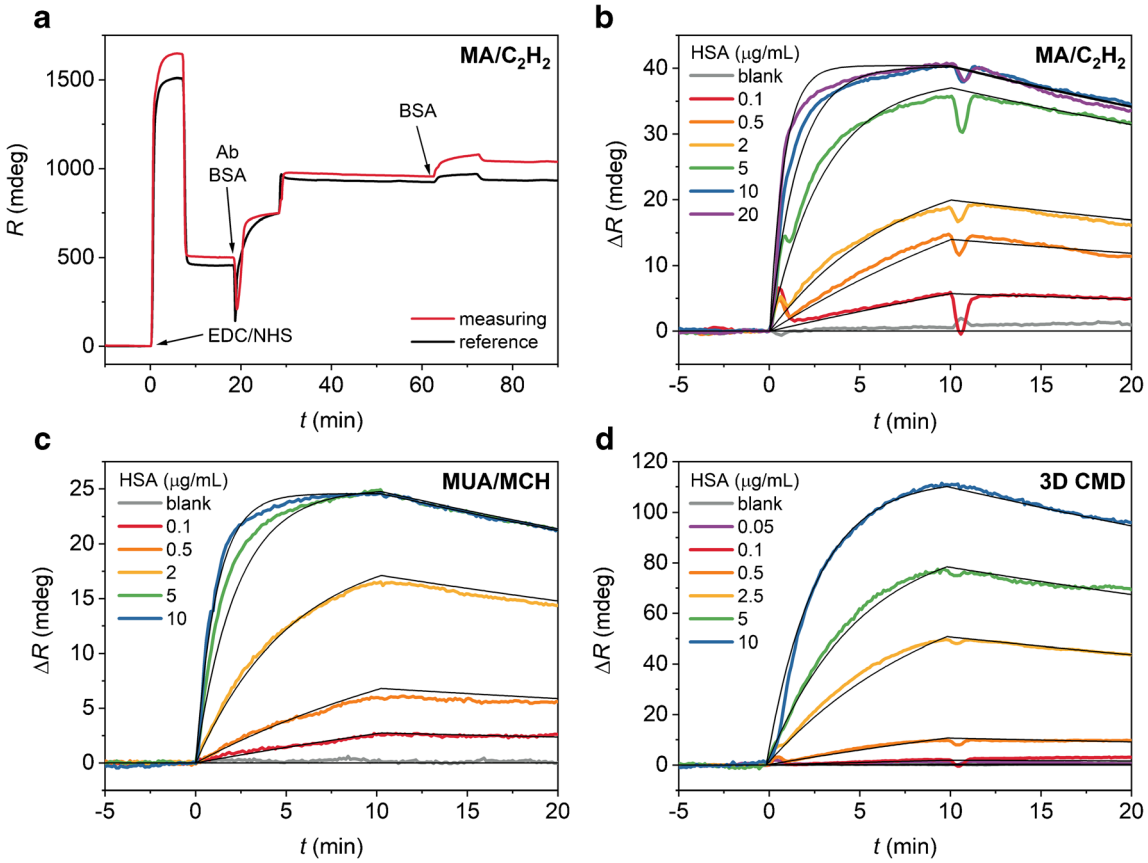

Fig. 4 **a** SPR sensorgram of the antibody immobilization on MA/C_2H_2 PP film surface. SPR binding curves of HSA on antibody-modified gold SPR chip based on **b** MA/C_2H_2 PP film, **c** MUA/MCH, and **d** 3D CMD. Differential signals (ΔR; calculated as the difference between channel with immobilized antibody and reference) are shown. The black lines correspond to the fitted kinetics model

matrices, the highest amount of BSA was adsorbed during the blocking step to the binding channel of the MUA/MCH sensor, suggesting there was still space for further protein immobilization. Different pH values of the immobilization solution were tested to enhance the electrostatic interaction and the efficiency of the antibody immobilization; however, no significant changes in immobilization were observed. To optimize the sensor surface blocking, the approaches based on binding of BSA (2 mg/mL) and ethanolamine (1 M, pH 8.5) were compared (Fig. S4 in the ESM). Both methods provided low level of non-specific protein adsorption, blocking by BSA was used for further experiments due the possibility to be performed at neutral pH.

In case of the CMD, the comparison with 2D CMD layer was done first (since the MA/C_2H_2 PP films are also 2D surfaces), followed by the same tests with the 50-nm-thick 3D CMD layer. PP films and CMD layers performed differently during the sensor preparation procedure. PP films exhibited a much larger response to the reaction with EDC/NHS mixture compared to the CMD layers, whereas the response during the

Table 1 Relative SPR responses (mdeg) after individual steps of antibody immobilization procedure

Type of matrix layer (channel)	Activation by EDC/NHS	Immobilization of antibody (binding channel) and BSA (reference)	Blocking by BSA	Binding of HSA (0.5 μg/mL)
MA/C_2H_2 (binding)	501	455	83	13
MA/C_2H_2 (reference)	456	460	11	−1
MUA/MCH (binding)	139	158	162	5
MUA/MCH (reference)	117	252	23	0
2D CMD (binding)	27	30	9	2
2D CMD (reference)	21	260	2	1
3D CMD (binding)	17	405	2	9
3D CMD (reference)	17	690	−2	0

antibody immobilization was comparable for the PP films and 3D CMD. The worst performance during the antibody immobilization was observed with the 2D CMD layer; the very low signal change suggests that only very limited number of antibody molecules was immobilized. Due to this fact, no response was observed during further tests with HSA solutions for the 2D CMD-based sensor and no sensorgram was obtained.

After the successful antibody immobilization onto the 3D CMD layer, tests with HSA solutions were carried out (Fig. 4d). The 3D CMD-based sensor provided LOD of 50 ng/mL, which is similar to MA/C_2H_2 PP film-based sensor. The level of response to relatively small concentrations (up to 0.5 or 2.5 μg/mL of HSA) was also comparable. However, the response to the concentration of 10 μg/mL of HSA was about twice lower in case of the MA/C_2H_2 PP film-based sensor. Interesting to note that the amount of immobilized antibody on the surfaces of MA/C_2H_2 PP film-based sensor and 3D CMD-based sensor was almost the same, which means that about a half of antibody molecules immobilized on the MA/C_2H_2 PP film-based sensor were not binding the analyte. This could be due to the 2D structure of the MA/C_2H_2 PP film with relatively large—but not selective—binding capacity. A sufficient amount of antibody molecules was capable of binding to the MA/C_2H_2 PP film surface, but the active sites of the antibody were not accessible for the analyte. The 3D CMD layers have "tree-like" structured brushes, where many of antibody molecules can be effectively immobilized—active sites of antibody molecules are more accessible for the antigen [34]. The comparison of the measured signals for all tested sensor types is shown in Fig. 5. The kinetic parameters of the interaction between AL-01 antibody and HSA antigen for the different layers are summarized in Table 2. The MUA/MCH-based chip provided the fastest association reaction, probably due to the perfectly plain surface and no re-binding. Also the MA/C_2H_2 PP film-based immobilization resulted in quite fast association, providing significantly higher k_a value compared to the CMD surface. The evaluated K_D values did not significantly differ among the layers, confirming the potential of the MA/C_2H_2 PP film-based surface for kinetic studies.

Table 2 Comparison of the evaluated kinetic parameters for the interaction between anti-HSA antibody AL-01 and HSA antigen on different surfaces

Type of matrix layer	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)
MA/C_2H_2	7.0×10^4	3.0×10^{-4}	4.3×10^{-9}
MUA/MCH	9.5×10^4	2.5×10^{-4}	2.6×10^{-9}
3D CMD	3.9×10^4	2.5×10^{-4}	6.3×10^{-9}

Conclusions

Plasma copolymerization of MA and acetylene in DBD reactor is an efficient method for the preparation of carboxyl-containing films that are proposed as an alternative to widely used SAMs and CMD layers on the gold surfaces of SPR sensors. The obtained MA/C_2H_2 PP films were highly reactive surfaces; their chemical properties in air and aqueous environment were studied by FTIR. After stabilization in air (5 days of storage), approximately 30% thickness loss was observed during the immersion in PBS due to the partial hydrolysis of the MA/C_2H_2 polymer film. Nevertheless, a stable signal was obtained during the subsequent SPR immunosensing and a selective response towards analyte was recorded.

The comparison of the performance of MA/C_2H_2 PP films, MUA/MCH, 2D and 3D CMD layers in SPR immunosensing of human serum albumin was carried out. The MA/C_2H_2 PP films showed enhanced performance compared to MUA/

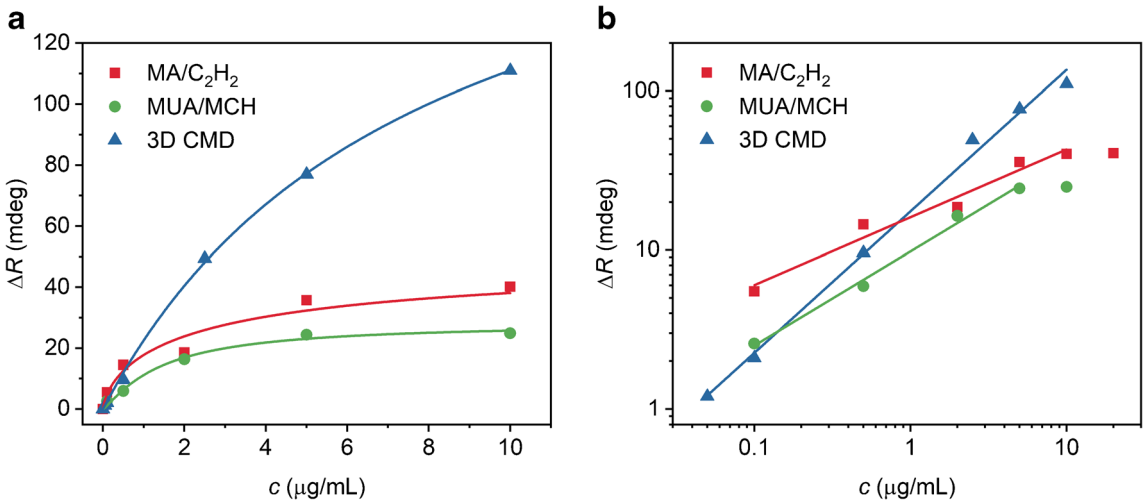

Fig. 5 Comparison of the obtained signals for HSA detection using surfaces based on MA/C_2H_2 PP film, MUA/MCH, and 3D CMD. **a** Linear scale fitted by the Langmuir model and **b** double-logarithmic plot fitted by line (the points in saturation were not included in the fit)

MCH and 2D CMD layers. The LOD achieved on the MA/C_2H_2 PP-based sensor (100 ng/mL) was similar to the MUA/MCH- and 3D CMD-based sensors. The level of response of the MA/C_2H_2 PP film-based sensors was about twice larger than in the case of MUA/MCH-based sensors. However, the level of response of the MA/C_2H_2 PP film-based sensor was lower compared to the 3D CMD-based sensor, especially in case of high antigen concentrations. This is due to the random distribution of functional groups in PP film, smaller surface area compared to the 3D CMD surface, and random orientation of the antibody molecules immobilized via amino groups. The achieved results demonstrate that plasma polymerization is a one-step eco-friendly procedure (not requiring the use of aggressive chemicals) for creation of highly reactive surfaces and prove a great potential of carboxyl-containing PP films as a matrix for immobilization of bioreagents in the field of biosensing.

Funding information This research has been financially supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601). CIISB research infrastructure project LM2015043, funded by Ministry of Education, Youth and Sports of the Czech Republic, is gratefully acknowledged for financial support of the measurements at CF Nanobiotechnology.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Skládal P. Piezoelectric biosensors. Trends Anal Chem. 2016;79:127–33.
2. Caucheteur C, Guo T, Albert J. Review of plasmonic fiber optic biochemical sensors: improving the limit of detection. Anal Bioanal Chem. 2015;407(14):3883–97.
3. Farka Z, Jurík T, Kovář D, Trnková L, Skládal P. Nanoparticle-based immunochemical biosensors and assays: recent advances and challenges. Chem Rev. 2017;117(15):9973–10042.
4. Wadu-Mesthrige K, Amro NA, Liu GY. Immobilization of proteins on self-assembled monolayers. Scanning. 2000;22(6):380–8.
5. Howell S, Kenmore M, Kirkland M, Badley RA. High-density immobilization of an antibody fragment to a carboxymethylated dextran-linked biosensor surface. J Mol Recognit. 1998;11(1–6):200–3.
6. Jadhav SA. Self-assembled monolayers (SAMs) of carboxylic acids: an overview. Cent Eur J Chem. 2011;9(3):369–78.
7. Jung D, Yeo S, Kim J, Kim B, Jin B, Ryu DY. Formation of amine groups by plasma enhanced chemical vapor deposition and its application to DNA array technology. Surf Coat Technol. 2006;200(9):2886–91.
8. Crespin M, Moreau N, Masereel B, Feron O, Gallez B, Vander Borght T, et al. Surface properties and cell adhesion onto allylamine-plasma and amine-plasma coated glass coverslips. J Mater Sci Mater Med. 2011;22(3):671–82.
9. Chen Q, Forch R, Knoll W. Characterization of pulsed plasma polymerization allylamine as an adhesion layer for DNA adsorption/hybridization. Chem Mater. 2004;16(4):614–20.
10. Wu ZY, Yan YH, Shen GL, Yu RQ. A novel approach of antibody immobilization based on n-butyl amine plasma-polymerized films for immunosensors. Anal Chim Acta. 2000;412(1–2):29–35.
11. Makhneva E, Farka Z, Skládal P, Zajíčková L. Cyclopropylamine plasma polymer surfaces for label-free SPR and QCM immunosensing of *Salmonella*. Sensors Actuators B Chem. 2018;276:447–55.
12. Yang ZL, Wang J, Luo RF, Maitz MF, Jing FJ, Sun H, et al. The covalent immobilization of heparin to pulsed-plasma polymeric allylamine films on 316L stainless steel and the resulting effects on hemocompatibility. Biomaterials. 2010;31(8):2072–83.
13. Hu WJ, Xie FY, Chen Q, Weng J. Amine-containing film deposited in pulsed dielectric barrier discharge at a high pressure and its cell adsorption behaviours. Chin Phys B. 2009;18(3):1276–82.
14. Ren TB, Weigel T, Groth T, Lendlein A. Microwave plasma surface modification of silicone elastomer with allylamine for improvement of biocompatibility. J Biomed Mater Res A. 2008;86A(1):209–19.
15. Makhneva E, Manakhov A, Skládal P, Zajíčková L. Development of effective QCM biosensors by cyclopropylamine plasma polymerization and antibody immobilization using cross-linking reactions. Surf Coat Technol. 2016;290:116–23.
16. Batan A, Nisol B, Kakaroglou A, De Graeve I, Van Assche G, Van Mele B, et al. The impact of double bonds in the APPECVD of acrylate-like precursors. Plasma Process Polym. 2013;10(10):857–63.
17. Nisol B, Watson S, Lerouge S, Wertheimer MR. Energetics of reactions in a dielectric barrier discharge with argon carrier gas: III esters. Plasma Process Polym. 2016;13(9):900–7.
18. Korner E, Fortunato G, Hegemann D. Influence of RF plasma reactor setup on carboxylated hydrocarbon coatings. Plasma Process Polym. 2009;6(2):119–25.
19. Rupper P, Vandenbossche M, Bernard L, Hegemann D, Heuberger M. Composition and stability of plasma polymer films exhibiting vertical chemical gradients. Langmuir. 2017;33(9):2340–52.
20. Mishra G, McArthur SL. Plasma polymerization of maleic anhydride: just what are the right deposition conditions? Langmuir. 2010;26(12):9645–58.
21. Chu LQ, Zhang Q, Forch R. Surface plasmon-based techniques for the analysis of plasma deposited functional films and surfaces. Plasma Process Polym. 2015;12(9):941–52.
22. Pearson HA, Urban MW. Simple click reactions on polymer surfaces leading to antimicrobial behavior. J Mater Chem B. 2014;2(15):2084–7.
23. Rich RL, Myszka DG. Advances in surface plasmon resonance biosensor analysis. Curr Opin Biotechnol. 2000;11(1):54–61.
24. Johnsson B, Lofas S, Lindquist G. Immobilization of proteins to a carboxymethyldextran-modified gold surface for biospecific interaction analysis in surface-plasmon resonance sensors. Anal Biochem. 1991;198(2):268–77.
25. Makhneva E, Obrusník A, Farka Z, Skládal P, Vandenbossche M, Hegemann D, et al. Carboxyl-rich plasma polymer surfaces in surface plasmon resonance immunosensing. Jpn J Appl Phys. 2018;57(1):5.
26. Obrusník A, Jelínek P, Zajíčková L. Modelling of the gas flow and plasma co-polymerization of two monomers in an atmospheric-pressure dielectric barrier discharge. Surf Coat Technol. 2017;314:139–47.
27. Coates J. Interpretation of infrared spectra, a practical approach. Encyclopedia of analytical chemistry. 2006.
28. Nisol B, Watson S, Lerouge S, Wertheimer MR. Energetics of reactions in a dielectric barrier discharge with argon carrier gas: V hydrocarbons. Plasma Process Polym. 2017;14(8):9.

29. Zajíčková L, Jelínek P, Obrusník A, Vodák J, Nečas D. Plasma-enhanced CVD of functional coatings in Ar/maleic anhydride/C_2H_2 homogeneous dielectric barrier discharges at atmospheric pressure. Plasma Phys Control Fusion. 2017;59(3):13.
30. Navrátilová I, Skládal P, Viklický V. Development of piezoelectric immunosensors for measurement of albuminuria. Talanta. 2001;55(4):831–9.
31. Farka Z, Čunderlová V, Horáčková V, Pastucha M, Mikušová Z, Hlaváček A, et al. Prussian blue nanoparticles as a catalytic label in a sandwich nanozyme-linked immunosorbent assay. Anal Chem. 2018;90(3):2348–54.
32. Farré M, Kantiani L, Barceló D. Microfluidic devices: biosensors. Chemical analysis of food: techniques and applications; 2012. p. 177–217.
33. Farka Z, Kovář D, Přibyl J, Skládal P. Piezoelectric and surface plasmon resonance biosensors for *Bacillus atrophaeus* spores. Int J Electrochem Sci. 2013;8(1):100–12.
34. Lofas S, Johnsson B. A novel hydrogel matrix on gold surfaces in surface-plasmon resonance sensors for fast and efficient covalent immobilization of ligands. J Chem Soc Chem Commun. 1990;(21):1526–8.