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Tuning of Surface Charge of Functionalized Poly(Carboxybetaine) Brushes Can Significantly Improve Label-Free Biosensing in Complex Media

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Poly(carboxybetaine) brushes are excellent antifouling platforms allowing facile functionalization with biorecognition elements via carbodiimide/N-hydroxysuccinimide (EDC/NHS) chemistry. However, residual active NHS esters and the loss of zwitterionic balance after the conjugation may impair initially excellent antifouling properties. This problem has so far been addressed either by using spontaneous hydrolysis or deactivation of residual NHS esters by the reaction with a small amino compound bearing hydroxyl or carboxyl groups. In contrast to this approach, and instead of using a single deactivator, here the use of tailored mixtures of deactivating agents containing carboxyl groups and sulfo or sulfate groups with permanent negative charge that allow to tune surface charge balance is investigated. The approach is applied to poly(carboxybetaine acrylamide) (pCBAA) and copolymer of carboxybetaine methacrylamide with N-(2-hydroxypropyl) methacrylamide [p(CBMAA-co-HPMAA)]. The fouling from undiluted blood plasma or crude minced meat is suppressed by one order of magnitude compared to commonly used deactivation protocols, while the label-free surface plasmon resonance detection of *E.coli* O157:H7 in crude minced meat shows the limit of detection improved by two orders of magnitude (3.2×10^3 CFU mL^{-1} in a direct detection assay format). The reported approach may be applied to other zwitterionic platforms which suffer from impaired antifouling properties after the platform modifications.

1. Introduction

Advances in the fields of bioanalysis, biomedicine or food safety call for efficient functional biointerface platforms that are resistant to nonspecific adsorption (fouling) from complex real-world media.[1–8] Besides the resistance to fouling, the platform should be easily functionalized, i.e., conjugated with molecules having specific biological activity, usually a high affinity for specific targets.[9–16] Applications that require such functionalized antifouling platforms span from rapid detection of chemical and biological species, coatings of nanoparticles used in drug delivery, membranes for separation and cleaning technologies, to scaffolds for tissue engineering.

Poly(carboxybetaine) (pCB) brushes are outstanding antifouling platforms allowing facile functionalization with biorecognition elements (BREs) via EDC/NHS (carbodiimide/N-hydroxysuccinimide) chemistry.[17–19] Their extraordinary properties stem from their high hydrophilicity and overall electroneutrality, which makes them resistant to hydrophobic as well as electrostatic adsorption from contacted media. However, their net electric charge is pH dependent. The positive charge of quaternary ammonium group is permanent but pKa of pCB carboxyl group is somewhere between 2 and 4.[17,20,21] The measurements of zeta potential of the surface bound poly(carboxybetaine methacrylamide) (pCBMAA),[22] indicated that its isoelectric point (pI) is around 8.5 and thus it is positively charged at lower pH values. The net positive charge of functionalized pCB brushes is further enhanced due to the consumption of betaine carboxyl groups following BRE conjugation reaction (**Figure 1**). It should be taken into consideration that conjugated BRE may also induce charge shifts and thus an effective optimization of the platform surface charge balance is of importance.[16]

The overall reaction scheme for the BRE conjugation via EDC/NHS is shown in Figure 1. The carboxyl group of pCB is converted to active NHS ester which readily reacts with amino group of BRE to create stable amide bond. However, not all NHS esters buried inside pCB brushes are able to react with bulky BREs that cannot penetrate below the surface, but can react nonspecifically with other smaller amino compounds present in complex biological media. Therefore, all residual NHS esters must be eliminated (deactivated) as otherwise they could

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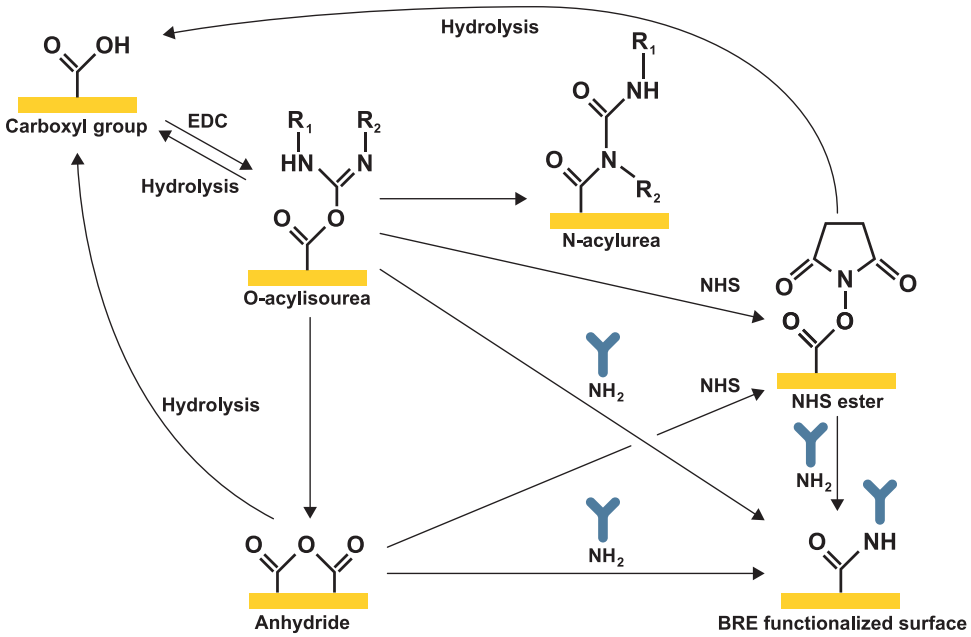

Figure 1. The general course of BRE conjugation to carboxy-functional platform via EDC/NHS coupling.

significantly impair initially excellent antifouling properties of pCBs and lead to false results if the platform is used for analytical purposes.[16]

It has been supposed that unreacted NHS esters (and *O*-acylisourea) rapidly hydrolyze in slightly alkaline solutions and all carboxyl groups can be fully recovered.[23,24] This was evidenced even if NHS esters were confined on top of a planar platform such as carboxyl terminated oligo(ethylene glycol) self-assembled monolayers.[19] However, a significant decrease in the rate of active ester hydrolysis at surfaces caused by in-plane confinement effects was reported.[25]

This problem has so far been addressed by deactivating residual NHS esters by reaction with a small amino compound (deactivator) bearing carboxyl group. (In this paper the terms "deactivation" and "deactivator" are further used to denote the elimination of active NHS esters and not the deactivation of BRE.) In our previous paper,[19] we discussed the effect of deactivation of NHS esters in various pCB platforms with glycine and 2-(2-aminoethoxy)acetic acid (AEAA). However, such approach does not fully compensate for the loss of negative charge resulting from the consumption of betaine carboxyl groups by the conjugation reaction. We demonstrated the residual net positive charge persisting deactivation with these agents by monitoring the interaction with polyanion dextran sulfate.

In contrast to previous approaches using a single deactivator with carboxyl group, in the present paper we investigated the use of tailored mixtures combining multiple deactivators bearing carboxyl groups and deactivators carrying sulfate or sulfo groups. While the ionization of carboxyl groups, and so the charge, is dependent on the pH of microenvironment, sulfate and sulfo groups are almost completely ionized over a wide pH range. It is supposed that the addition of a small amount of deactivator with permanent negative charge can help to better tune and optimize the surface charge balance.

The reported deactivation approach was applied to poly(carboxybetaine acrylamide) (pCBAA), and random copolymer of carboxybetaine methacrylamide with N-(2-hydroxypropyl) methacrylamide [p(CBMAA-*co*-HPMAA)] that have been shown previously to provide a great combination of antifouling and functionalizable capabilities.[19,26] The tested deactivators included AEAA and glycine as reference,[19,27] and the mixtures containing 2-aminoethyl hydrogen sulfate (D1) or aminomethanesulfonic acid (D2) with glycine. The ratios of glycine and D1 or D2 were varied to determine the optimal composition for a given application.

The surface plasmon resonance (SPR) was employed to assess the impact of the deactivation procedures on the resistance to fouling from undiluted human blood plasma, commonly accepted as a standard for resistance comparisons,[1] and from crude minced meat that is relevant for the detection of pathogens in food.[28] The optimized deactivation procedures of pCBAA functionalized with antibody were utilized in the label-free SPR detection of *Escherichia coli* O157:H7 (*E.coli*) in the crude minced meat samples.

2. Results and Discussion

The influence of investigated deactivation procedures on fouling is demonstrated on two pCB brushes: pCBAA and p(CBMAA-*co*-HPMAA). The choice of the two polymers is based on previous investigations which have shown that while pCBAA is slightly better resistant to fouling than pCBMAA, it cannot be randomly copolymerized with HPMAA by surface-initiated atom transfer radical polymerization (SI-ATRP).[19,26] We have suggested and demonstrated that p(CBMAA-*co*-HPMAA) exhibits an excellent resistance to fouling which is due to the combination of the two resistant monomer units of which only one undergoes functionalization reaction. Changing the ratio of the two

Table 1. The abbreviations used for the components of deactivation agents and their mixtures.

Deactivation solution	Abbreviation
1 M 2-(2-aminoethoxy)acetic acid	AEAA; A
1 M glycine	G
0.8 M 2-aminoethyl hydrogen sulfate	D1
0.8 M aminomethanesulfonic acid	D2
960 mM G + 32 mM D1	96G:4D1
900 mM G + 80 mM D1	90G:10D1
500 mM G + 400 mM D1	50G:50D1
960 mM G + 32 mM D2	96G:4D2
900 mM G + 80 mM D2	90G:10D2
500 mM G + 400 mM D2	50G:50D2

units makes it possible to control the number of functionalizable groups in the polymer brush and thus to optimize the extent of chemical modification to a minimum level indispensable for binding a required amount of BRE. The ratio of CBMMA to HPMAA 15/85 mol% was found to be advantageous for certain biosensing applications because of the optimum balance of the fouling resistance and functionalization capacity.[19,26]

In SPR experiments, the pCB-brushes were functionalized with anti-*E.coli* O157:H7 antibody. Its surface mass density on the pCBAA brush (350 ± 50 ng cm^{-2}) was higher than on the p(CBMAA-*co*-HPMAA) copolymer brush (81 ± 35 ng cm^{-2}) due to the higher amount of carboxyl groups available for the immobilization on the pCBAA brush.

After the anti-*E.coli* antibody immobilization, the brushes were incubated with deactivation solutions of glycine, or AEAA, or mixtures of glycine with D1 or D2. To find an optimum composition, we tested various mixtures overviewed in **Table 1**. The reaction scheme is illustrated in **Figure 2**.

2.1. Fourier Transform Infrared Reflection-Absorption Spectroscopy (FT-IRRAS) Spectra

Basic features of FT-IRRAS spectra of pCB brushes have already been discussed in our previous paper[19] and therefore herein we focused mainly on new aspects. The spectra are presented in Supporting Information.

The spectra of pCBAA, pCBMAA and p(CBMAA-co-HPMAA) show a weak but discernible absorption band at about 1730 cm^{-1} indicating the presence of some protonated carboxyl groups (Figure S2, Supporting Information) This is likely due to charge interactions between the chains of closely packed brushes,[17] so that some carboxyl groups may not find a quaternary ammonium counterpart and remain in a protonated form. In our experience, this may vary depending on polymerization conditions. The band does not disappear after re-swelling during repeated hydration and drying, which suggests that the arrangement of pCB chains in the brush has been fixed during polymerization and the subsequent drying/hydration does not lead to significant rearrangement (spectra not shown). The EDC/NHS activation, i.e., the consumption of carboxyl groups, is most notably reflected in the decrease in intensity of the bands of ionized carboxyl groups at 1608 cm^{-1} and 1366 cm^{-1}. The kinetics of activation assessed from the decrease of intensity of the band 1608 cm^{-1} is illustrated for pCBAA in **Figure 3**. For example, it indicates that under the given conditions ≈27% of pCBAA carboxyl groups should be activated after 30 min. The dependence for pCBMAA is nearly the same. However, in our experience the course of activation may greatly depend on the thickness of pCB brush and the quality of EDC and NHS used. It is to be noted that it is desirable to achieve only such degree of activation that is optimal for binding the required amount of BRE and not the maximum activation because superfluous not deactivated residual NHS esters could increase fouling.

The reactions of activated carboxybetaines with D1 and D2 can be well monitored thanks to the absorption bands of sulfate groups at 1253 cm^{-1}, 1229 cm^{-1} and 1010 cm^{-1} and the bands of sulfo groups at 1203 cm^{-1} and 1028 cm^{-1}. The spectra of pCBAA activated with EDC/NHS and deactivated by the reaction with D1 or D2 are shown in Figures S3 and S4, Supporting Information, respectively. A striking feature is the additional decrease in the intensity of the ionized carboxyl groups (1608 cm^{-1}, 1366 cm^{-1}) suggesting that once D1 or D2 is attached, sulfate or sulfo groups can displace certain fraction of betaine carboxyl groups from their interaction with quaternary ammonium cation. The same finding applies to pCBMAA and its copolymer p(CBMAA-co-HPMAA) (spectra not shown).

In the spectra of pCBs brushes deactivated with glycine or AEAA or with mixtures of glycine and D1 or D2 the attachment of glycine or AEAA cannot be well monitored, because their

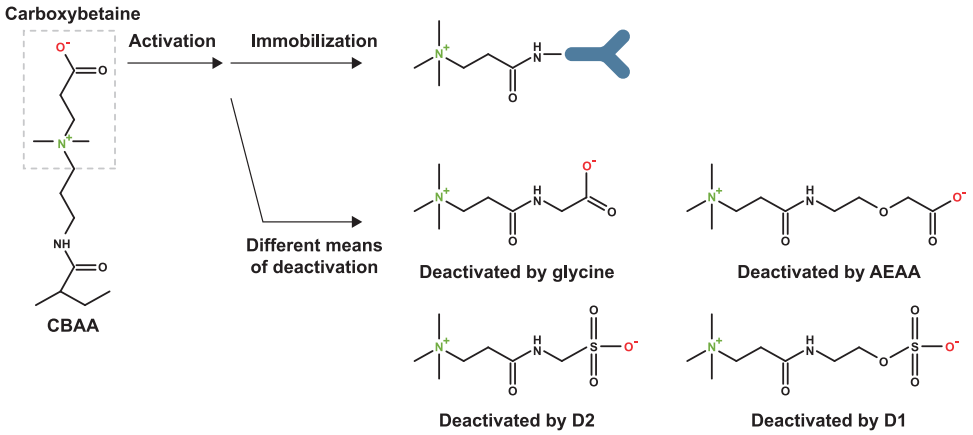

Figure 2. The reaction scheme of pCB deactivation with glycine, AEAA, D1 and D2.

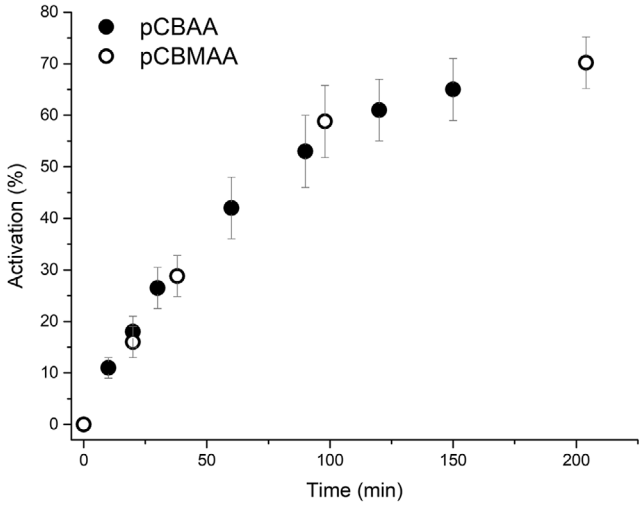

Figure 3. The time dependence of the degree of pCBAA (circle) and pCBMAA (cross) activation by EDC/NHS assessed from the decrease in intensity of the band 1608 cm^{-1}. (The band intensity of unmodified brushes was taken as the point of zero activation).

groups, i.e., carboxyl and amide groups, are already present in large excess in the initial structure of pCBs. However, the attachment of D1 or D2 was evidenced as described above.

2.2. Effect of Deactivation Agents on Fouling Resistance of pCBAA and p(CBMAA-co-HPMAA) Brushes

The fouling from model complex samples of bodily fluids (pooled undiluted human blood plasma) and food derived matrices (minced meat sample) was evaluated from SPR sensorgrams (**Figure 4**) as the difference between SPR signal baseline before and after 10 min of the sample injection over the chip surface. The contact time of 10 min was chosen as it is within the range of commonly used values for fouling assessment and comparison.

A summary of the fouling from blood plasma and minced meat on pCBAA and from blood plasma on p(CBMAA-*co*-HPMAA) is shown in **Figure 5**.

Both pCBs functionalized with anti-*E.coli* antibody were either hydrolyzed in borate buffer (pH 8.0, 30 min) or reacted with one of the agents marked on the x axis. Besides glycine and AEAA, the mixtures with varying ratios of glycine to D1 or D2 were tested. Figure 5 shows apparent trends of the fouling dependence on the treatment: hydrolysis is the least effective and significantly outperformed by individual glycine and AEAA. The using of both mixtures of glycine with D1 or D2 further suppressed the fouling more efficiently than the above treatments with distinctive minima of the fouling at the low content of D1 (96G:4D1) or D2 (90G:10D2) while higher contents of D1 or D2 resulted in deterioration of antifouling properties again. This is clearly seen in the upper part of Figure 5 showing the fouling on pCBAA. The trends did not indicate a principal difference between D1 and D2 mixed agents. The fouling on p(CBMAA-*co*-HPMAA) after hydrolysis and treatment with glycine and AEAA was significantly lower than on pCBAA and thus the effect of the treatment with the mixed agents was less distinctive. Such behavior is due to the fact that the prevailing component HPMAA (85 mol%) does not undergo EDC/NHS activation. The copolymer contains much lower amount of carboxyl groups available for

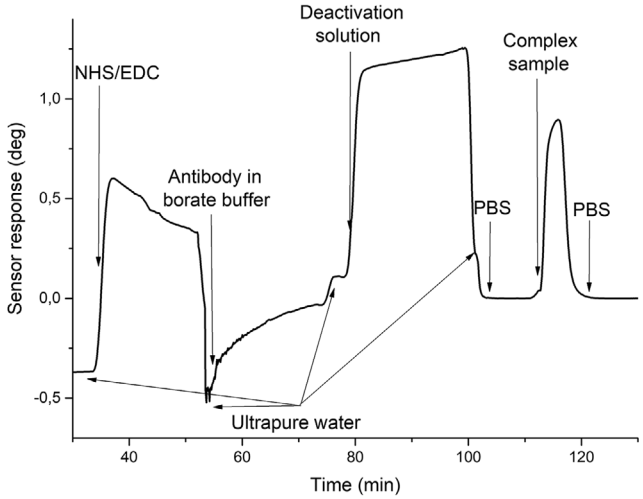

Figure 4. Typical SPR sensorgram of pCBAA functionalized with anti-E. coli antibody, after deactivation (here with 96G:4D)1 and exposed to complex sample (here undiluted blood plasma).

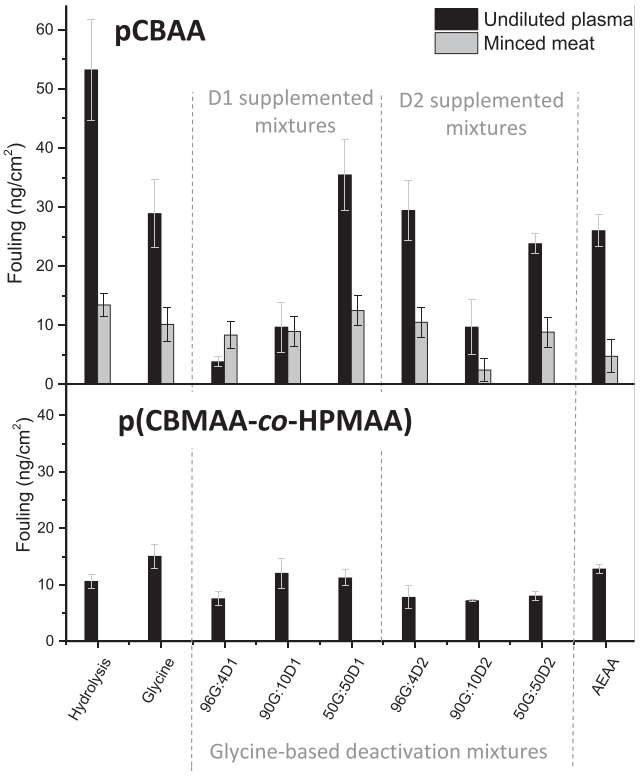

Figure 5. SPR measurements of fouling from undiluted blood plasma and minced meat on anti-*E.coli* antibody functionalized pCBAA and from undiluted blood plasma on anti-*E.coli* antibody functionalized p(CBMAA-*co*-HPMAA) deactivated by hydrolysis or using different deactivation agents.

functionalization than pCBAA (it binds 4.3 times less antibody) and so an apparent deterioration/improvement of the fouling due to the functionalization and the following treatment is less apparent. Figure 5 further shows comparisons of the fouling on pCBAA from blood plasma and the minced meat sample. It documents differences between the two types of media with the generally lower fouling from meat but the response to the post functionalization treatment exhibits similar trend. The fouling on pCBAA from blood plasma was suppressed from 29 ng cm^{-2} for glycine to 3.8 ng cm^{-2} for 96G:4D1 and the fouling from meat was suppressed from 9.7 ng cm^{-2} to 2.4 ng cm^{-2} for 90G:10D2. These results confirm the effectivity of the application of deactivating agents with added permanent negative charge.

It needs to be strongly emphasized that for the assessment of fouling we have not used any additional washing step using a higher ionic strength solution. Such procedure is commonly used to release deposits kept by electrostatic interactions. However, this procedure, if used for biosensing applications, may also release analytes and thus lead to false results. This must be taken into account when assessing the fouling and comparing our data with some data published elsewhere.

2.3. Enhancing Biorecognition Capability of Optimally Deactivated pCBAA Brush

The potential of the selected deactivation protocols in affinity biosensor applications was further investigated using the functionalized and deactivated pCBAA brushes for the label-free SPR detection of *E.coli* O157:H7 in minced meat samples (we remind here that the term "deactivation" as used in this paper refers solely to the converting of residual active NHS esters and never to the deactivation of BRE.) This foodborne bacterial pathogen and the medium were chosen as a model system due to their importance for food safety applications and the chance to compare the data with the results published earlier.[26,28] The pCBAA brush was functionalized by covalent attachment of anti-*E.coli* O157:H7 antibody with the surface mass density of 350 ± 50 ng cm^{-2} and subsequently deactivated by one of the three deactivation agents described above: 96G:4D1, 90G:10D2 and AEAA. The deactivation with glycine and by hydrolysis was used as the reference to already published results and standard procedures.[19,27]

Figure 6 shows calibration curves for the label-free *E.coli* detection using the above deactivation procedures. **Table 2** summarizes fouling from negative control (blank meat sample with no added *E.coli*) and the limits of detection (LODs) determined according to standard "3σ" approaches (see also Supporting information).[29,30]

Table 2 shows similar trends in LOD of the detection assay and the fouling from the negative control. The LOD and fouling from the negative control using glycine, 96G:4D1, or hydrolysis deactivations were comparable, LOD ≈ 10^5 CFU mL^{-1} and the fouling level 9–13 ng cm^{-2}) (Table 2). The optimal deactivation method (90G:10D2) improved LOD up to two orders of magnitude (LOD ≈10^3 CFU mL^{-1}) by reducing the fouling 5 to 6 times. The detection assays differed only in the method of deactivation used, resulting in the different fouling resistance of the detection platform.

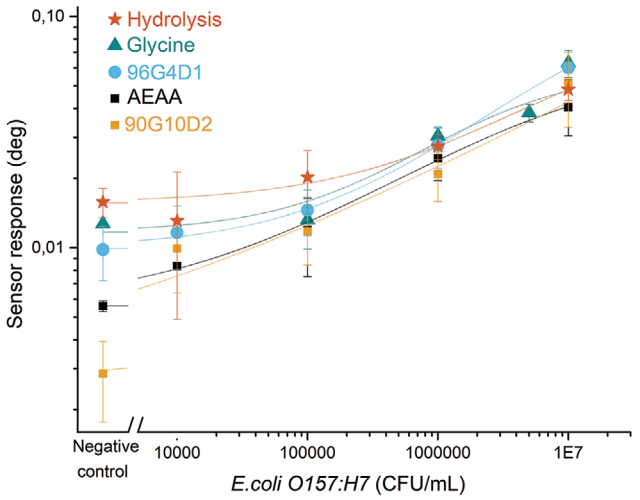

Figure 6. Calibration curves of the primary detection of *E.coli* O157:H7 in minced meat using functionalized pCBAA brush deactivated by different deactivation agents. Both axes are shown in logarithmic scale.

These results further highlight the impact of the procedures used for deactivation of residual NHS esters in functionalized pCB platforms. Even though the fouling from the complex samples was reasonably low for most of the deactivation methods tested, further reduction in the fouling proved to be effective in significantly improving LOD.

Typically, detectable concentrations and LODs for *E.coli* O157:H7 detection achieved by label-free direct optical or non-optical biosensors are in the order of 10^2 to 10^5 CFU mL^{-1} in buffer solutions.[31–39] As shown in Table 2, functionalized pCBAA brush optimally deactivated achieves comparable LOD values in a complex crude food sample, without any additional sample preconditioning or extra after-detection washing steps, in the reasonably short time (≈20 min).

3. Conclusions

A novel efficient approach is reported for recovering antifouling properties of zwitterionic pCB brushes after their functionalization with biorecognition elements via EDC/NHS chemistry. The deactivation of residual active esters by reaction with tailored composition of mixed multiple agents bearing amino groups with carboxyl and sulfate or sulfo groups carrying permanent negative charge makes it possible to tune the charge

Table 2. LODs for the label-free detection of *E.coli* O157:H7 in the minced meat sample, estimated for different deactivation agents.

Deactivation	Fouling from negative control [ng cm^{-2}]	LOD [CFU mL^{-1}]
Hydrolysis	13.4 ± 1.9	3.7×10^5
Glycine	10.8 ± 2.5	3.7×10^5
96G:4D1	9.0 ± 2.6	3.4×10^5
AEAA	4.8 ± 2.8	2.9×10^4
90G:10D2	2.4 ± 0.9	3.2×10^3

layout of the resulting biointerface. This functionality is of utmost importance for suppressing the fouling from complex biological media and thus it significantly advances the performance of label-free biosensing in such media.

4. Experimental Section

Reagents: All aqueous solutions were prepared using ultrapure water (18.0 MΩ cm, Milli-Q system, Merck). Stock chemicals for the preparation of phosphate-buffered saline (PBS, 0.01 M phosphate, 0.138 M sodium chloride, 0.0027 M potassium chloride, pH 7.4 at 25 °C), borate buffer (10 mM sodium borate, pH 8.0 at 25 °C), glycine, 2-aminoethyl hydrogen sulfate, aminomethanesulfonic acid were from Sigma-Aldrich, Czech Republic. 2-(2-aminoethoxy)acetic acid (AEAA) was from VWR International, Czech Republic. Carboxybetaine acrylamide, carboxybetaine methacrylamide, and N-(2-hydroxypropyl) methacrylamide monomers were from Specific Polymers, France. 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane (Me4Cyclam, 98%), CuCl (≥99.995%), $CuCl_2$ (99.999%), methanol (≥99.9%) and ethanol (99,9%) were from Sigma-Aldrich. Tetrahydrofuran (THF, ≥99.9%) was from Penta, Czech Republic. N-hydroxysuccinimide (NHS) and N-ethyl-N'-(3-diethylaminopropyl)carbodiimide (EDC) were from AP Czech, Czech Republic. Anti-*E.coli* O157:H7 antibody (anti-*E.coli*) and heat killed *E.coli* were from KPL Inc., USA. The procedure for the preparation of the minced meat sample (pH 6.8) is described in the Supporting Information. Human blood plasma (pooled, mixed gender, pH 7.9) in sodium citrate was purchased from VWR International, Czech Republic.

Preparation of Polymer Brushes on SPR Chips: pCBAA (wet thicknesses 100 ± 15 nm), pCBMAA (wet thicknesses 70 ± 15 nm) and p(CBMAA-*co*-HPMAA) (wet thicknesses 80 ± 20 nm) polymer brushes on SPR chips (BK7 glass coated with 5 nm titanium and 50 nm gold layers) were prepared by SI-ATRP according to the procedure described elsewhere.[40] The random copolymer p(CBMAA-*co*-HPMAA) contained 15 mol% CBMAA and 85 mol% HPMAA. The successful polymerization was checked by FT-IRRAS and spectroscopic ellipsometry. A detailed procedure is described in Supporting Information.

Characterisation of Polymer Brushes by Spectroscopic Ellipsometry: The wet thickness of pCB brushes was characterised using spectroscopic ellipsometry measurements in water environment. A spectroscopic ellipsometer VASE, J.A.Woollam, Lincoln, USA was employed in the spectral range of 3.4 – 1.5 eV with step of 0.008 eV and a 70° incidence angle. Data were taken using a custom made cell[41] and analyzed using WVASE32 software. Single oscillator model (Lorentz oscillator) was employed for data analysis.

Preparation of Deactivation Solutions: AEAA was dissolved in water (1 M, pH set to 7), aliquoted, and stored at −20 °C till used. The solution of glycine (1 M, pH set to 7) was prepared fresh every week and stored at 6 °C till used. 2-aminoethyl hydrogen sulfate (D1) and aminomethanesulfonic acid (D2) were dissolved in water (both 0.8 M, pH set to 8,5) and stored at 6 °C till used. The following Table 1 shows composition of the deactivation solutions and the abbreviations used for their names.

Preparation of Minced Meat Sample: A beef hamburger-meat was delivered by a local fast-food restaurant. A slice was homogenized with water (1:9) for 2 min using a Masticator (IUL Instruments, BioTech, Czech Republic) following a previously published procedure.[42] The homogenized sample was centrifuged for 2 min at 1200 rpm. The supernatant above the sediment was collected and frozen until used.

Activation/Deactivation Procedure: The activation of pCBs with EDC/NHS and deactivation of NHS esters with various deactivation agents was performed in the same way as described in Surface plasmon resonance measurements.

FT-IRRAS: FT-IRRAS spectra of pCB brushes on SPR chips were measured using a Thermo Scientific Nicolet iS50 FTIR Spectrometer equipped with a Thermo Scientific Smart SAGA Accessory. The spectra were collected with 200 scans at 4 cm^{-1} resolution and 80° reflection angle. The samples for the measurement of infrared spectra were prepared in the same way as for SPR.

SPR Measurements: SPR experiments were performed using the angular multiparametric SPR with a 4-channel microfluidic system (BioNavis Ltd, Finland) and the LED source emitting at 670 nm. The SPR sensor response change measured in angular units was converted into the surface mass density using the relation of 0.001° corresponding to 0.85 ng cm^{-2}.

SPR experiments to investigate the fouling resistance of functionalized pCB brushes i) and to establish biorecognition calibration curves ii) were performed as follows: SPR chips were washed with ultrapure water, dried with a stream of air, and mounted into the SPR system. After a few minutes of washing the surface with water to establish a baseline in the SPR response, the aqueous solution of 0.5 M EDC and 0.1 M NHS was injected for 30 min (12 µL min^{-1}), followed by injections of water for 2 min, 50 µg mL^{-1} of anti-*E.coli* antibody in borate buffer (10 mM borate buffer, pH 8.0, 15 µL min^{-1}) for 20 min, and water for 2 minutes to reach baseline again. The residual NHS esters were deactivated by reaction with deactivation solution flowed at a flow rate of 12 µL min^{-1} over the surface for 30 min. Subsequently, ultrapure water (2 min, 30 µL min^{-1}) and PBS (pH 7.4, 10 min, 30 µL min^{-1}) were flowed over the sensor surface to establish a baseline in the SPR signal. Deactivation by hydrolysis was performed in the same way by contacting with borate buffer (10 mM, pH 8) without any deactivator.

Then, in i) experiments, the undiluted human blood plasma (annealed to 50 °C and cooled down to room temperature, pH 7.9) or the minced meat sample (pH 6.9) were injected for 10 min (30 µL min^{-1}). The fouling was evaluated from the difference between the PBS baselines established 10 min after the sample injection and before the sample injection.

In ii), the minced meat sample was spiked with heat-killed *E.coli* (10^4 – 10^7 CFU mL^{-1}) and flowed over the functionalized and deactivated surface (10 min, 30 µL min^{-1}). The analyte signal was evaluated in the same way as the fouling.

Statistical Analysis: The SPR data analysis was performed using software OriginPro 2016. All the data presented in this study were assessed as a mean ± standard deviation of at least 3 independent measurements (typically 5). The LOD values were determined according to standard "3σ" approach, in which σ is a standard deviation of the negative control (complex sample with no analyte of interest) measured on different chips. The data of the calibration curve were fitted by the logistic fit.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

antifouling zwitterionic polymer brushes, biosensors, EDC/NHS chemistry, N-hydroxysuccinimide (NHS) active ester deactivation, poly(carboxybetaine) functionalization

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