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Original Paper

Adsorption of bovine serum albumin *in situ* using SPR on modified thiol-PEG-acetic acid surface

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ABSTRACT

Hydrophilic surfaces modified with polymer brushes have attracted significant attention due to their applications in biomedical fields, particularly for reducing non-specific protein adsorption. In the current study, different concentrations of thiol-poly(ethylene glycol)-acetic acid (thiol-PEG-acetic acid) were used to modify gold surfaces using surface plasmon resonance (SPR), aiming to reduce non-specific protein adsorption. Different grafting densities were obtained by using different concentrations of thiol-PEG-acetic acid, and the results showed that the highest concentration of thiol-PEG-acetic acid produced a high thiol-PEG-acetic acid grafting density. All modified surfaces were characterized using advanced analytical techniques, including X-ray photoelectron spectroscopy (XPS), SPR, and water contact angle measurements. Furthermore, all surfaces were tested in a bovine serum albumin (BSA) solution. The study found that a low grafting density of thiol-PEG-acetic acid resulted in high protein adsorption on the modified gold surface.

Keywords: *BSA adsorption, Gold surface modification, SPR, Thiol-PEG-Acetic acid*

1 INTRODUCTION

Surfaces that can reduce protein adsorption while also resisting bacterial adhesion have attracted great attention [1, 2]. Surface modification using polymers has attracted the attention of many researchers due to biomedical applications, including implants in the human body, tissue engineering, drug delivery systems, and biosensing [3, 4]. The hydrophilic behavior and neutral charge of polymers used for modification are key properties that control protein adsorption on modified surfaces [5]. Surfaces modified with PEG terminated with thiol groups show increased hydrophilicity because of the chemical composition of PEG molecules [6]. Therefore, the final PEG grafting density and the achieved brush thickness are the main factors controlling interactions at biointerfaces [7–9]. Over the last few decades, researchers have found that surfaces modified with PEG-brush polymers provide antifouling properties and reduce non-specific protein adsorption [10, 11].

To improve the biocompatibility of PEG-brush polymers, it is important to understand how protein adsorption occurs and how this complex process is influenced by the conformation of PEG polymer chains. The hypothesis is that optimal antifouling properties result from increased hydration of PEG with increasing graft density, reaching an optimal point [12–14]. PEG is non-toxic, biocompatible, and soluble in a wide range of solvents. In addition, PEG can be classified as a family of thermally separable polymers with high cloud-point values exceeding 100 °C. From a physicochemical perspective, this polymer is hydrophobic, and its side chains are amphiphilic. Changing the side-chain length alters hydrophilicity and, thus, the hydration state and

conformation, which can influence behavior at certain temperatures. This phenomenon laid the foundation for a series of comprehensive reviews that discuss how the molecular size of pOEGMA affects its thermoresponsive properties in solution and in biomaterials [15]. Recent research on protein adsorption onto PEG brush polymers with controlled graft density and molecular weight is in excellent agreement with theoretical predictions [16].

In this study, we systematically tested the adsorption of bovine serum albumin (BSA) onto thiol-PEG-acetic acid layers with different grafting densities and identified an optimal surface-modification strategy for thiol-PEG-acetic acid applications in the biomaterials field. This was achieved using a "grafting-to" approach, which alters the surface density of functional groups and can be used to adjust the surface density of PEG chains and to attach them to the substrate.

2 MATERIALS AND METHODS

SPR gold sensors with dimensions of 1 × 2 cm and a thickness of 50 nm were obtained from BioNavis Ltd. (Finland). Sigma-Aldrich (Australia) provided thiol-poly(ethylene glycol)-acetic acid (M_w 3000) and phosphate-buffered saline (PBS). Invitrogen (USA) supplied bovine serum albumin (BSA) (cat # 16000044).

2.1 Thiol-PEG-acetic acid layer *in situ* using SPR

The SPR gold sensor was ultrasonically cleaned with acetone, dried under a flow of N_2 gas, and then treated with UV/O_3 (Bio Force Nanoscience, Salt Lake City, UT) for about 20 min. Different concentrations of polymer brushes (0.1, 0.5, 1, and 2.0 mM) were prepared in PBS (pH 7.4), and the *in situ* reaction period was adjusted to 10 min for each concentration. Sensograms were measured using a gold sensor attached to a flow cell and washed with PBS as long as required until a stable baseline was reached. The flow rate was adjusted to 30 µL min^{-1}, and the thiol-PEG-acetic acid solution was allowed to form on the sensor surface within the flow cell for approximately 1 h [17]. All modified gold surfaces were characterized by XPS, SPR, as well as water contact angle measurements.

2.2 Protein adsorption

2.2.1 BSA adsorption to the thiol-PEG-acetic acid layers

The BSA solution was prepared at a concentration of 0.1% in PBS (pH 7.4). BSA adsorption on the modified surfaces was assessed using SPR by injecting the diluted mixture at a flow rate of 30 µL min^{-1} for 10 min, followed by rinsing with PBS for about 10 min. Protein adsorption was evaluated using the variation in total internal reflectance (ΔTIR) to calculate the adsorbed surface mass density using the de Freiter method (Equation 1), which depends on baseline differences before and after adsorption [18].

$$\Gamma_p = \frac{\Delta\theta \cdot k \cdot dp}{dn/dc} \quad (1)$$

For the SPR Navi 210 instrument, the k.dp values in water-based buffers with Au-based sensors are approximately: 670 nm = $1.0 * 10^{-7}$nm/degree. For proteins, with dn/dc = 0.182 cm^3/g, this means: 670 nm = $\Delta\theta$ * 550 ng/cm^2 [18].

2.2.2 Contact angle measurements

The water contact angle of the modified surface was measured by placing a static sessile drop of Milli-Q water (0.5 µL) on the surface using a contact angle goniometer (FTA1000 Instrument, USA). Three distinct spots on each surface were analyzed for each sample (n = 3).

2.3 Advanced surface characterization techniques

2.3.1 Surface plasmon resonance (SPR)

The MP-SPR Nav™ 210A VASA (BioNavis Ltd, Finland) device was utilized to study grafting of the PEG-thiol molecule onto a gold sensor. The instrument is equipped with a type (II) laser diode (600–1000 nm, tunable) and an optical range of 40–78° (internal reflection). All measurements were carried out at the same location using two wavelengths, 670 nm and 785 nm. This allowed determination of changes in the critical angles throughout the measurements, which were then used to estimate the thickness of the PEG-thiol layer. The overall thickness was determined by fitting a reflectance spectrum to a Fresnel model using the MP-SPR Nav™ Layer Solve™ software [19].

2.3.2 X-ray photoelectron spectroscopy (XPS)

XPS spectra were obtained using an AXIS Nova spectrometer (Kratos Ltd, Telford, UK) with a monochromatic aluminum source (Al $K\alpha$, 1486.6 eV) operated at 150 W. The pressure inside the analysis chamber throughout the measurements was approximately 10^{-1} mbar. Three different data points were evaluated for each sample surface. For each sample, an elliptical area of approximately 0.7 mm × 0.4 mm was analyzed. All elements were detected using survey spectra acquired at a pass energy of 160 eV. To obtain additional details such as

oxidation state, high-resolution spectra were collected for selected peaks at a pass energy of 20 eV. Data analysis was performed using CasaXPS version 2.3.15 along with the instrument sensitivity factors. High-resolution spectra were fitted using a Gaussian-Lorentzian function after linear background subtraction. Spectra were referenced by assigning the C–C/C–H bond energy to 285.0 eV [20].

2.3.3 Contact angle measurements

Substrate wettability was determined by measuring static sessile drops of Milli-Q water (0.5 µL) using the contact angle goniometer (FTA1000 Instrument, USA). A total of three different spots were analyzed for each sample (n = 3) [21].

3 RESULTS AND DISCUSSION

3.1 SPR analysis of thiol-PEG-acetic acid grafting *in situ*

To measure the binding effect of different concentrations of thiol-PEG-acetic acid on the gold surface and determine the surface coverage area, full-width scanning reflectance spectra were recorded by SPR in PBS. The variation in total internal reflectance (ΔTIR) before and after exposure of the Au slide to PEG thiol is shown in Figure 1 (baseline shown to indicate the actual value). The change in the angle of reflection measured by SPR confirms the binding of thiol-PEG-acetic acid to the gold surface [22].

Figures 2 and 3 indicate that the critical angle for thiol-PEG-acetate bonding at different concentrations changes with exposure duration. The Au sensor slide is immediately covered with thiol-PEG-acetic acid bonds, and the PEG layer forms rapidly within the first 2–5 min. The magnitude of the ΔTIR variation followed the sequence 2.0 > 1.0 > 0.5 > 0.1 mM. This trend is likely due to denser packing of PEG molecules on the gold surface at higher thiol-PEG-acetic acid concentrations.

3.2 Thiol-PEG-acetic acid thickness

The overall thickness was calculated using the refractive index of PEG-thiol (n = 1.45), and the protein coverage area was also calculated [12]; the results are presented in Table 1. The thickness of each PEG-modified surface increased with increasing concentration of the PEG-thiol solution.

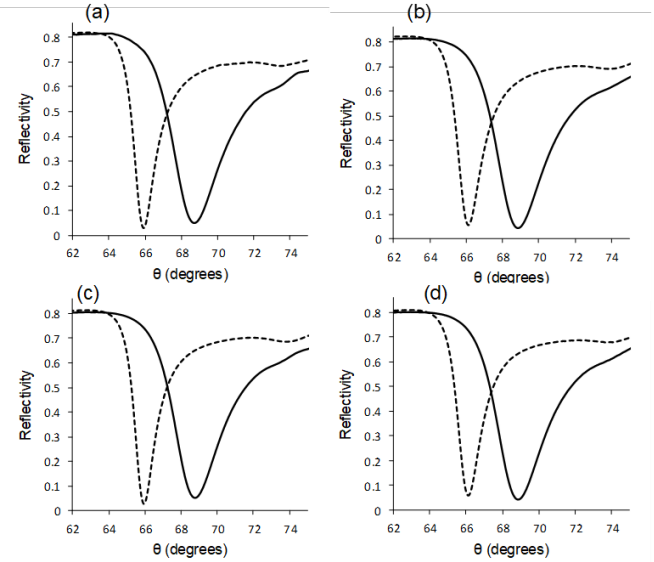

Fig. 1 Angular spectra for the Au controls both before and after adding the HS-PEG-COOH at different concentrations: (a) 0.1 mM, (b) 0.5 mM, (c) 1.0 mM, and (d) 2.0 mM at 20 °C

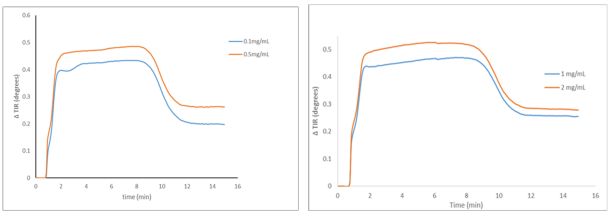

Fig. 2 The SPR sensograms were recorded for coating Au slides with PEG-SH-COOH at (a) 0.1, 0.5 mg/ml, (b) 1.0, 2.0 mg/ml at 20 ºC

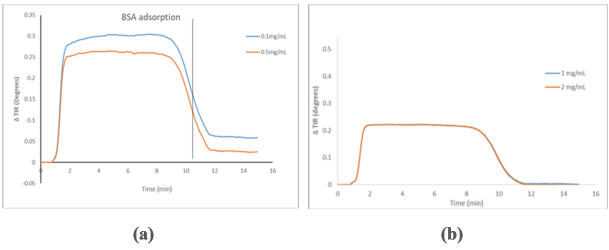

Fig. 3 The SPR sensograms recorded for adsorption of BSA on PEG-SH-COOH at (a) 0.1, 0.5 mg/ml and (b) 1.0, 2.0 mg/ml at 20 ºC

Table 1 The total thickness of thiol_PEG-acetic acid at different concentrations

C_{peg}	PEG thickness (nm)	Δ TIR (degrees)	BSA surface coverage (ng/cm^2)
0.1 mM	2.33	0.05844	32.1
0.5 mM	2.55	0.02505	13.8
1.0 mM	3.78	0.00008392	<0.55
2.0 mM	4.08	-0.003044	<0.55

3.3 XPS analysis

XPS analysis was performed for all thiol-PEG-acetic acid–treated surfaces. The presence of carbon and oxygen on the Au control indicates contamination from sample cleaning and transport to the XPS spectrometer. Although this contamination is unavoidable, its effect on the PEG-coated surfaces is minimal because the thiol-PEG-acetic acid layer was prepared on a freshly cleaned Au slide. As expected for PEG-layer grafting, increasing the concentration of thiol-PEG-acetic acid in solution led to overall increases in carbon and oxygen signals, accompanied by a decrease in the Au signal, consistent with an increase in overlayer thickness. All results are shown in Table 2.

Table 2 XPS elemental compositions were found upon PEG-thiol grafted surfaces under various conditions at 20 °C

Sample	%*Au**	%C*	%O*
Au (control)	58.5 ± 1.5	35.2 ± 1.1	6.2 ± 0.4
HS-PEG-COOH_0.1 mM	45.2 ± 0.8	37.3 ± 2.2	17.4 ± 1.4
HS-PEG-COOH_0.5 mM	43.10 ± 4.0	38.8 ± 3.0	17.7 ± 0.3
HS-PEG-COOH_1.0 mM	42.2 ± 1.9	40.2 ± 1.4	18.4 ± 0.5
HS-PEG-COOH_2.0 mM	38.03 ± 4.3	42.7 ± 5.4	18.7 ± 1.1

Collected from C 1s, O 1s and Au 2p spectra

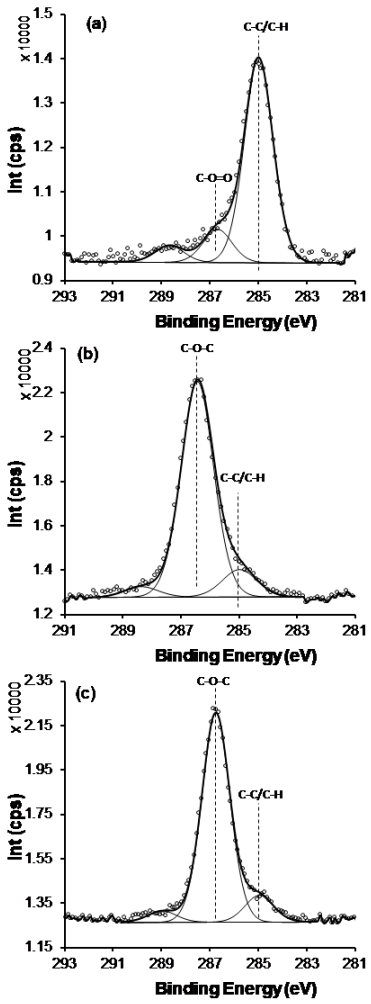

Fig. 4 High-resolution C 1s spectra for (a) Au control (b) HS-PEG-COOH in (0.1) mM and (c) HS-PEG-COOH in (2.0) mM

High-resolution XPS investigation of the C 1s region was performed for all thiol-PEG-acetic acid–modified surfaces, providing further evidence of successful grafting due to the presence of an abundant ether bond (C–O) at 286.5 eV (20). The presence of a C–C/C–H peak at 285.0 eV indicates unavoidable residual hydrocarbon contamination on these surfaces. However, consistent with the O 1s and Au 4f signals, the ether content increased with increasing thiol-PEG-acetic acid concentration in solution (Figure 4).

3.4 Water contact angle measurements

Contact angle measurements were performed to evaluate the wettability of the thiol-PEG-acetic acid–modified surfaces. The results are shown in Table 3. The surfaces became more hydrophilic after polymer grafting compared with the Au control surfaces [23]. Using the highest thiol-PEG-acetic acid concentration (2.0 mM) reduced the static sessile-drop contact angle of the Au control from 91.55° to 42.14°, 37.60°, 34.16°, and 26.91°, respectively (Figure 5), consistent with previous reports. The PEGylated layer is known to increase the hydrophilicity of the modified surface and to adhere firmly to the substrate. Therefore, covalent attachment is the preferred approach for achieving prolonged adhesion of PEG on modified surfaces [24].

Table 3 Comparison of Classification Results.

Sample	C_{pEG}	Water contact angle (θ)
Au (control)	-	91.55 ± 2.1
HS-PEG-COOH	0.1 mM	42.14 ± 1.1
	0.5 mM	37.60 ± 3.0
	1.0 mM	34.16 ± 2.4
	2.0 mM	26.91 ± 0.4

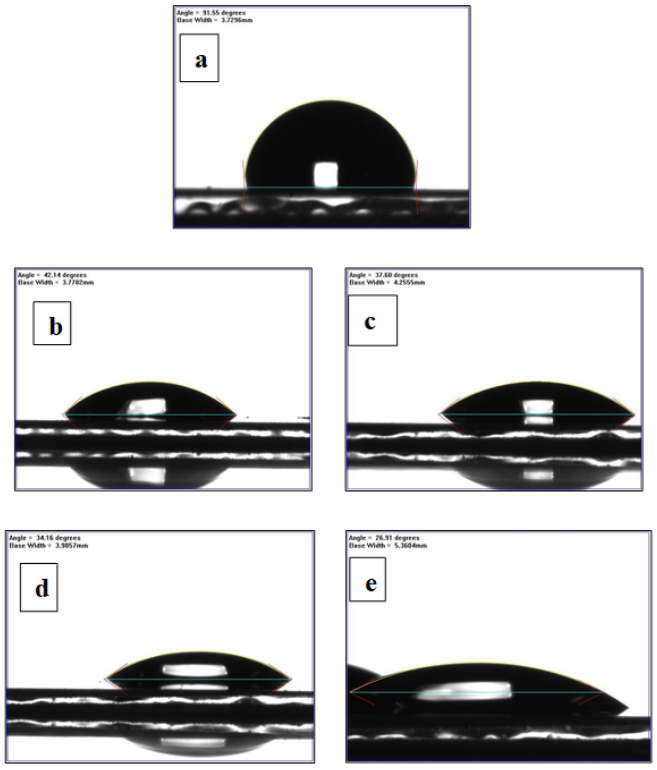

Fig. 5 Water contact angles of (a) Au controls, (b) HS-PEG-COOH, at (0.1) mM, (c) HS-PEG-COOH at (0.5)mM, (d) HS-PEG-COOH at (1.0) mM and (e) HS-PEG-COOH in (2.0) mM

4 CONCLUSION

To investigate the role of grafting density in protein adsorption, gold surfaces were modified in situ using SPR at varying thiol-PEG-acetic acid concentrations. The surfaces were also characterized using XPS and contact angle measurements to determine surface density under both wet and dry conditions. We found that the grafting density of the modified surface is a critical factor controlling protein adsorption. High protein adsorption was observed on surfaces prepared with the lowest thiol-PEG-acetic acid concentrations.

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Data availability

N/A

DECLARATIONS

Conflict of interest

The authors state that this study has no conflicts of interest.

Consent to publish

N/A

Ethical approval

N/A

REFERENCES

[1] Zhang D, Ren B, Zhang Y, Liu Y, Chen H, Xiao S, et al. Micro- and macroscopically structured zwitterionic polymers with ultralow fouling property. Journal of Colloid and Interface Science. 2020;578:242–253. 10.1016/j.jcis.2020.05.122

[2] Heggestad JT, Fontes CM, Joh DY, Hucknall AM, Chilkoti A. In Pursuit of Zero 2.0: Recent Developments in Nonfouling Polymer Brushes for Immunoassays. Advanced Materials. 2019;32(2). 10.1002/adma.201903285

[3] Guo S, Zhu X, Loh XJ. Controlling cell adhesion using layer-by-layer approaches for biomedical applications. Materials Science and Engineering: C. 2017;70:1163–1175. 10.1016/j.msec.2016.03.074

[4] Sánchez-Alvarado AB, Iturbe-Ek J, Mamidi N, Sustaita AO. Polymer Brush-Based Thin Films via Cu(0)-Mediated Surface-Initiated Atom Transfer Radical Polymerization for Sensing Applications. ACS Applied Polymer Materials. 2021;3(11):5339–5354. 10.1021/acsapm.1c01037

[5] Rahmati M, Mozafari M. Protein adsorption on polymers. Materials Today Communications. 2018;17:527–540. 10.1016/j.mtcomm.2018.10.024

[6] Jeon SI, Lee JH, Andrade JD, De Gennes PG. Protein—surface interactions in the presence of polyethylene oxide. Journal of Colloid and Interface Science. 1991;142(1):149–158. 10.1016/0021-9797(91)90043-8

[7] Rikiyama K, Maehara N, Abe H, Nishimura Y, Yukawa H, Kaminaga K, et al. Quantification of Poly(ethylene glycol) Crowding on Nanodiamonds toward Quantum Biosensor for Improved Prevention Effects on Protein Adsorption and Lung Accumulation. Langmuir. 2024;40(18):9471–9480. 10.1021/acs.langmuir.3c03988

[8] Pawar AS, Ghahremanzadeh A, Ghaffari Sharaf M, Unsworth LD. Effect of Uremic Toxins and Methoxy-PEO Chain Density on Plasma Protein Adsorption. ACS Biomaterials Science & Engineering. 2024;11(1):322-9

[9] Ortiz R, Olsen S, Thormann E. Salt-Induced Control of the Grafting Density in Poly(ethylene glycol) Brush Layers by a Grafting-to Approach. Langmuir. 2018;34(15):4455–4464. 10.1021/acs.langmuir.8b00030

[10] Yousif EA, Majeed AS, Salih NA. Synthesis, characterization and preliminaryin vitroantibacterial screening activity of metal complex derivatives of 2-[(5-styryl-[1,3,4]thiadiazol-2-ylimino)-methyl]-phenol. Journal of Taibah University for Science. 2014;8(1):26–30. 10.1016/j.jtusci.2013.09.003

[11] Wang F, Zhang H, Yu B, Wang S, Shen Y, Cong H. Review of the research on anti-protein fouling coatings materials. Progress in Organic Coatings. 2020;147:105860. 10.1016/j.porgcoat.2020.105860

[12] St Hill LR, Tran HV, Chinwangso P, Lee HJ, Marquez MD, Craft JW, et al. Antifouling Studies of Unsymmetrical Oligo(ethylene glycol) Spiroalkanedithiol Self-Assembled Monolayers. Micro. 2021;1(1):151–163. 10.3390/micro1010012

[13] Win Yf, Yousif E, Majeed A, Ha St. Synthesis, characterization and in vitro antimicrobial activity of Co (II), Cu (II), Zn (II), Cd (II) and Sn (II) ions with {[5-(4-bromophenyl)-1, 3, 4-oxadiazol-2-yl] thio} acetic acid. Asian Journal of Chemistry. 2011;23(11):5009

[14] Win YF, Yousif E, Ha ST, Majeed A. Synthesis, Characterization and Preliminary in vitro Antibacterial Screening Activity of Metal Complexes Derivatives of 2-[5-(4-Nitrophenyl)-1,3,4-thiadiazol-2- ylimino]methylphenol. Asian Journal of Chemistry. 2013;25(8):4203–4206. 10.14233/ajchem.2013.13901

[15] Akar I, Keogh R, Blackman LD, Foster JC, Mathers RT, O'Reilly RK. Grafting Density Governs the Thermoresponsive Behavior of P(OEGMA-co-RMA) Statistical Copolymers. ACS Macro Letters. 2020;9(8):1149–1154. 10.1021/acsmacrolett.0c00461

[16] Emilsson G, Schoch RL, Feuz L, Höök F, Lim RYH, Dahlin AB. Strongly Stretched Protein Resistant Poly(ethylene glycol) Brushes Prepared by Grafting-To. ACS Applied Materials & Interfaces. 2015;7(14):7505–7515. 10.1021/acsami.5b01590

[17] Albers WM, Vikholm-Lundin I. In: Surface Plasmon Resonance on Nanoscale Organic Films. Springer New York; 2010. p. 83–125. 10.1007/978-1-4419-6169-3_4

[18] Green RJ, Frazier RA, Shakesheff KM, Davies MC, Roberts CJ, Tendler SJB. Surface plasmon resonance analysis of dynamic biological interactions with biomaterials. Biomaterials. 2000;21(18):1823–1835. 10.1016/s0142-9612(00)00077-6

[19] Homola J. Surface Plasmon Resonance Sensors for Detection of Chemical and Biological Species. Chemical Reviews. 2008;108(2):462–493. 10.1021/cr068107d

[20] Greczynski G. Binding energy erencing in X-ray photoelectron spectroscopy: Expanded data set confirms that adventitious carbon aligns to the sample vacuum level. Applied Surface Science. 2024;670:160666. 10.1016/j.apsusc.2024.160666

[21] Kwok DY, Neumann AW. Contact angle measurement and contact angle interpretation. Advances in Colloid and Interface Science. 1999;81(3):167–249. 10.1016/s0001-8686(98)00087-6

[22] Lapitan LDS, Drozd M, Malinowska E, Pietrzak M. Implications of surface architecture towards the development of a rapid and label-free Glypican-3 immunosensor. Microchemical Journal. 2025;209:112665. 10.1016/j.microc.2025.112665

[23] Liu W, He S, Liu H, Shou Z, Huo K, Xiang H, et al. A green, versatile, and facile strategy for anti-biofouling surface with ultra-high graft density polyethylene glycol. Journal of Nanobiotechnology. 2024;22(1). 10.1186/s12951-024-03026-x

[24] Sheng K, Gao Y, Bao T, Wang S. Covalent coating strategy for enhancing the biocompatibility and hemocompatibility of blood-contacting medical materials. Pharmaceutical Science Advances. 2023;1(1):100001. 10.1016/j.pscia.2022.100001

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