pH-responsive SERS substrates based on AgNP-polyMETAC composites on patterned self-assembled monolayers

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Abstract
Patterned silver nanoparticle (NP)-poly[2-(methacyloyloxy)ethyl] trimethyl ammonium chloride (AgNP-polyMETAC) composites were prepared by electrochemical lithography, surface-initiated atom-transfer radical polymerization (SI-ATRP) and NP growth inside the polymer brushes. For this purpose, polymer brushes of poly[2-(methacyloyloxy)ethyl] trimethyl ammonium chloride (polyMETAC) were utilized as strong electrolyte brush system. These were introduced in form of patterned polymer brushes to create pH-responsive surface enhanced Raman scattering SERS substrates. It is well-known that the charges of strong polyelectrolyte chains are usually insensitive to pH changes, hence, rarely strong polyelectrolyte brushes have been utilized so far to study pH-responsive properties of such films. Here pH-insensitive polyMETAC brushes exhibit pH-sensitive properties and can be used as pH-responsive surfaces for SERS applications due to the embedding of AgNPs into the polymer brushes. When increasing the pH, the assembly of the AgNPs transfers from quasi two-dimensional (2D) aggregates, attaching mainly to the polymer surface, into a three-dimensional (3D) assembly, where the particles are penetrating into the brushes. These changes result in significant alterations of the SERS efficiency of the polymer brush composite. At pH 5, the enhancement of the Raman scattering approaches its maximum. The fabricated SERS substrates show a high sensitivity as well as good experimental reliability at different pH values. Moreover, electrochemical lithography was utilized to fabricate patterned SERS substrate, which allows an easy combination of multiple other functionalities in hierarchical structuring steps. In addition, the microstructure is in our studies beneficial because of a simplified and reliable characterization of the polymer brushes at defined sample areas. The introduction of the microstructured brush system is regarded moreover attractive for the development of high-throughput platforms for rapid, automated screening and analysis applications.
1. Introduction

Surface enhanced Raman scattering (SERS) has attracted tremendous interest due to its manifold applications in chemical, material, and life science applications [1–3]. SERS represents a promising technique which enhances Raman scattering of adsorbed molecules on SERS-active surfaces. After the first observation of SERS for pyridine prepared on roughened Ag electrodes [4], a large number of experimental and theoretical investigations have been conducted to develop more sensitive and reproducible SERS substrates. Next to roughened metal surfaces [4] and plasmonic nanostructures [5], metal nanoparticles (NPs) suspensions [6] as well as novel materials [7], like graphene and MoS$_2$, have been tested intensively as suitable SERS-active substrates. The aim of all of these developments is to establish a reliable, reproducible and cost-effective method to obtain high performance SERS platforms which ensure reproducibility of the SERS enhancement as well as stability of the substrates towards external environmental influence factors.

In particular plasmonic NPs suspensions have been widely studied for obtaining SERS substrates due to their high sensitivity, simple fabrication and low costs. Gold, silver, and copper are materials which are frequently utilized for the fabrication of SERS-active substrates. Because of the particularly strong localized surface plasmon resonance of Ag NPs, silver became the most popular material being utilized for SERS applications [8]. Nevertheless, there are important drawbacks of these metal NPs as they, e.g. frequently tend to agglomerate into larger clusters and are prone to precipitation. To circumvent these problems, strategy is to stabilize and to immobilize the NPs in matrices or at surfaces [9]. In the last decades, a wide range of studies have been performed to successfully immobilize metal NPs suspensions on solid substrates. These include the utilization of self-assembled monolayers (SAMs) [10, 11], the introduction of layer-by-layer coated multilayer substrates [12, 13], the in-situ nucleation and growth of NPs on surfaces [14–16], or block copolymer micelle nanolithography [17] among others. Beside these methods, attaching metal NPs onto polymer brushes has gained attention as a promising alternative method for the stabilization of NPs on various substrates [18, 19]. One of the most important strength of polymer brushes is that they can possibly offer a stimulus-responsive platform, which can be sensitive to changes of the external environment, such as, temperature and pH changes or different solvent polarities, etc., if suitable polymer systems are chosen. Due to conformational changes in the polymer brush chains, these polymer brushes can be used as templates for preparing plasmonic sensing devices [20]. Literature examples demonstrated that polymer brushes can be utilized as a substrate for the fabrication, fixation, and stabilization of metal NPs. For example, Ye et al [21] reported a method of an electrostatically driven self-assembly to deposit gold NPs on polystyrene (PS) with controllable gap size between adjacent NPs, which is controlled by adjusting the charge of the PS films. Three-dimensional (3D) Ag nanostructures were in-situ fabricated on poly(acrylic acid) polymer brushes grafted onto graphene oxide. The morphology of Ag nanostructures could simply be controlled by using different Ag precursor ions and reducing agents, which resulted in the formation of 3D, hierarchically organized flowerlike nanostructures. They proved to be efficient SERS substrates showing enhancement factors (EF) of $\sim 10^7$ [22]. Thermo-sensitive poly(N-isopropylacrylamide) (PNIPAM) brushes were integrated onto a substrate to adsorb gold NPs, leading to temperature-sensitive nanosensors by utilizing the phase transition of the polymer, which is triggered by changes in the temperature, and immobilization of AuNPs with tunable optical properties [23]. Significant efforts have been devoted to the fabrication of pH-sensitive devices based on pH-responsive polymer brushes. It emerges from these studies that preferably weak polyelectrolyte brushes can be used to establish this pH-responsive properties. Stamm et al [20] reported a novel nanocomposite fabricated by adsorbing Au and core–shell Au@Ag nanorods onto pH-responsive poly(2-vinyl pyridine) (P2VP) polymer brushes, which were utilized as pH-sensitive nanosensors. The authors exploited both the swelling and shrinking properties of the polymer brushes induced by changes of the pH value, as well as the tunable optical properties of the AuNPs. Poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) brushes with incorporated AgNPs were prepared by an approach utilizing photopolymerization and AgNp growth. The controllable thickness of the polymer film and the high density of AgNPs provided a large area substrate for SERS analysis. These PDMAEMA-Ag assemblies have been demonstrated to represent a well-suited SERS substrate, which also shows potential applications for pH-responsive devices based on the properties of the PDMAEMA [24]. However, recently also strong polyelectrolyte brushes were observed to act as suitable pH-responsive templates. Von Klitzing et al investigated the uptake of pH-dependent, negatively charged AuNPs within strong poly[2-(methacryloyloxy)ethyl trimethyl ammonium chloride (polyMETAC) polyelectrolyte brushes at different pH values [23]. The distribution of the Au NPs could be tuned and a transition from a two-dimensional (2D) to 3D aggregation was triggered by adjusting the pH value. The
prepared nanocomposites were identified as suitable pH-sensitive substrates [25–27].

Patterned surfaces have gained great attention in both fundamental investigation and industrial applications providing micro/nanostructures with spatially periodical or aperiodic morphology showing distinctive mechanical, photonic, electric, and biological properties [28]. Dynamic, patterned surfaces with controllable topographies represent surfaces with the possibility of serving as smart surfaces for enhanced and tunable optical devices [29, 30]. Besides, structured surfaces can also be combined with stimuli-responsive properties, such as towards the pH, which provide potential applications in smart nanocontainers and nanoreactors [31], medicine and biosensor systems [32]. Therefore, it is important to investigate patterned pH-responsive SERS substrates. Electrochemical lithography has been widely applied in the fabrication of micro/nanostructures due to its advantages of high accuracy, low cost and easy fabrication conditions [33]. Using this fabrication approach to pattern the surface allows easy combination of multiple other functionalities in hierarchical structuring steps. Besides, electrochemical lithography is a reliable method that can be employed in high-throughput experimental setups.

Following this strategy we developed an easy and versatile approach to fabricate patterned AgNP-polyMETAC composites by combining electrochemical lithography, surface-initiated atom-transfer radical polymerization (SI-ATRP) and AgNP nucleation and growth. This study aims on the development of a microstructured SERS active platform, which should provide reliable SERS enhancement, easy handling as well as a good reproducibility of its SERS performance. Therefore, both the electrostatic particle-particle interaction and the particle assembly formation within the polymer matrix under different pH conditions have to be considered.

These investigations have been performed on microstructured substrates which allowed the fabrication of micropatterned brush systems. While the microstructure is in our studies beneficial because of a simplified and reliable characterization of the polymer brushes at defined sample areas, the introduction of the microstructured brush system is regarded moreover attractive for the development of high-throughput platforms for rapid, automated screening and analysis applications [34]. In this contribution, the introduction of the microstructured polymer brushed provides mainly a good possibility to easily compare the influence of different pH value conditions on the SERS enhancement, independent of the brush morphology or the local filling factor of the Ag NPs.

2. Experimental details

2.1. Chemicals

Bicyclohexane (BCH), toluene, n-octadecyldichlorosilane (OTS), (3-aminopropyl)-trimethoxysilane (APTMS), tri-ethylamine, 2-bromo-2-methyl-N-[3-(trimethoxysilyl)propyl] propanamide (BTBAm) was prepared as initiator layer. The initiator precursors were synthesized according to a literature procedure [38]. These molecules were immobilized on the patterned areas of the OTS monolayer obtained by electrochemical oxidation lithography. These provide a pattern presenting carboxyl groups in the electrochemically oxidized areas. Self-assembly of the initiator molecules takes place exclusively in these areas of the surface pattern. Subsequently, the structured polyMETAC-brush [39] was prepared. The synthesis proceeded as follows: 4.63 g METAC were dissolved in 4.6 ml methanol at room temperature. The solution was stirred while being degassed for 20 min by passing a continuous stream of dry Ar through the solution. 2,2′-Dipyridyl (0.241 g), Cu(I)Cl (0.061 g), Cu(II)Cl₂ (0.0083 g) were added to the METAC solution. The mixture was stirred and degassed with a stream of dry Ar for another 20 min. The initiator-modified substrates were subsequently immersed in the polymerization solution and were continuously degassed by a stream of Ar. After 5 h reaction time, the samples were taken out, washed with water and methanol, and were finally dried with a stream of N₂.

2.2. Electrochemical lithography

Silicon wafers were pre-cleaned by sonication in a sequence of solutions of toluene, acetone, ethanol and deionized water, for 15 min each. Then the substrates were additionally cleaned in a plasma cleaner (Diener Electronics, N₂ influx) for 10 min directly before use. By immersing the cleaned silicon substrates in a solution of OTS (10 µl) in dry BCH (2 ml) for 1 min, subsequent sonication in toluene, and blow drying in N₂, OTS monolayers were prepared. A TEM grid (Plano) was used as a conductive stamp to perform electrochemical oxidation lithography. Chemically active surface patterns were obtained by exposing the OTS coated Si substrate to water vapor. Then the TEM grid was pressed onto the OTS surface. At the same time a bias voltage of −25 to −30 V was applied to the TEM grid for 10 s. After that, the stamp was removed [35–37].

2.3. PolyMETAC-brush fabrication

First, a SAM of 2-bromo-2-methyl-N-[3-(trimethoxysilyl)propyl] propanamide (BTBAm) was prepared as initiator layer. The initiator precursors were synthesized according to a literature procedure [38]. These molecules were immobilized on the patterned areas of the OTS monolayer obtained by electrochemical oxidation lithography. These provide a pattern presenting carboxyl groups in the electrochemically oxidized areas. Self-assembly of the initiator molecules takes place exclusively in these areas of the surface pattern. Subsequently, the structured polyMETAC-brush [39] was prepared. The synthesis proceeded as follows: 4.63 g METAC were dissolved in 4.6 ml methanol at room temperature. The solution was stirred while being degassed for 20 min by passing a continuous stream of dry Ar through the solution. 2,2′-Dipyridyl (0.241 g), Cu(I)Cl (0.061 g), Cu(II)Cl₂ (0.0083 g) were added to the METAC solution. The mixture was stirred and degassed with a stream of dry Ar for another 20 min. The initiator-modified substrates were subsequently immersed in the polymerization solution and were continuously degassed by a stream of Ar. After 5 h reaction time, the samples were taken out, washed with water and methanol, and were finally dried with a stream of N₂.

2.4. Silver nanoparticles loaded polyMETAC-brushes

The fabricated polyMETAC-brush modified substrate was immersed in a 10 mM aqueous solution of hydrogen tetrachloroaurate(III) trihydrate (HAuCl₄ • 3 H₂O) for 1 h. After incubation, the sample was removed, rinsed with water, and dried with a stream of N₂. Subsequently, the [AuCl₄]⁻ loaded polyMETAC-brush was incubated in freshly prepared 100 mM Cu(I)Cl, Cu(II)Cl₂, hydrogen tetrachloroaurate(III) trihydrate (HAuCl₄ • 3 H₂O), sodium borohydride (NaBH₄), hydrogen peroxide (H₂O₂), and a silver enhancer kit (solution A and silver enhancer solution B) were obtained from Sigma Aldrich. 4-Aminobenzenethiol (4-ATP) was purchased from Alfa Aesar. Double side polished p-type silicon wafers (100) were obtained from Siegert Wafer (resistivity: 10–20 Ohm•cm).
aqueous sodium borohydride solution for 5 min. After removal from the aqueous sodium borohydride (NaBH₄) solution, the sample was washed with water and dried with a stream of N₂. The silver NP loaded polyMETAC-brush was obtained by immersing the polyMETAC sample (with small Au seeds) in a 1:1 mixture of silver enhancer solution A and silver enhancer solution B (Silver Enhancer Kit, Sigma Aldrich) for 30 min. The whole sample surface was covered with a layer of AgNPs with high density. Unspecifically precipitated silver NPs were finally removed from the sample by water rinsing and Scotch tape cleaning. Atomic force microscopy (AFM, NT-MDT, NTegra Aura) was performed using commercial AFM tips (μ-Mash NCS35/AIBS) in tapping mode. Scanning electron microscopy imaging was conducted with a Zeiss Sigma VP utilizing the InLens and SE detector.

2.5. SERS measurements

The prepared samples were immersed in different concentrations of 4-ATP ethanol solution for 24 h prior to Raman measurements. SERS measurements were conducted at a laser excitation wavelength of 532 nm, and all experiments were carried out with a laser power of 5 mW and an integration time of 10 s (Bruker Senterra). Buffer solutions with pH values ranging from 2 to 10 were used to regulate the pH when Raman spectra were acquired under different pH value conditions to determine the SERS activity. Various solutions with different pH values were dropped on the sample surface. After each measurement the substrate was washed with a large amount of water and then dried by N₂ flow. The same position on the polymer brush was chosen for the Raman measurements under different pH value conditions, which can be easily identified due to the unique morphology of each microline of the AgNP-polyMETAC-brush composites by the optical detection system of the AFM and Raman instruments. The concentration of all applied buffer solutions was 0.1 M.

3. Results and discussion

Patterned AgNP-polyMETAC composites were prepared based on electrochemical oxidation lithography [35–37], SI-ATRP and AgNP nucleation and growth on the polymer brushes. Figures 1(A)–(F) illustrate the fabrication process of AgNP loaded polyMETAC-brushes. First, electrochemical oxidation lithography was applied for preparing microstructured, chemically active surface patterns.

In brief, electrochemical oxidation lithography (figure 1(B)) [35–37] is a patterning technique based on a tip or conductive stamp which generates chemically active, functional groups on OTS monolayers. This process represents an established method where a water meniscus is formed between the OTS coated substrate and a conductive stamp or an AFM tip, respectively. This formed liquid reservoir formed under ambient conditions resembles an electrochemical cell, which allows to perform an electrochemical oxidation reaction of the top functional groups of the OTS monolayer. The patterns fabricated here were generated by utilizing a TEM copper grid with a diameter of 3.05 mm consisting of parallel line features serving as a conductive stamp. Upon application of a negative bias voltage to the conductive stamp an electrochemical oxidation of the top functional groups of the OTS monolayer into carboxy functionalities takes place and forms a chemically active surface area, which can be used for further modification. The structural parameters of the generated patterns are tunable by controlling the dimension of the stamp and the patterning conditions, such as, the applied voltage, the pulse duration, and the relative humidity, which are all related to the size of the formed water meniscus. The applied voltage between the TEM grid and the modified silicon substrate was in the range of −25 to −30 V and the oxidation time was set to ∼10 s. By repeated oxidation and functionalization of different areas of the substrate, combinations of different materials can be created.

Afterward, polyMETAC brushes were grown on the patterned OTS monolayer substrate by utilizing SI-ATRP in aqueous solutions of [2-(methacyrloyloxy)ethyl] trimethyl ammonium chloride (METAC) (figures 1(C) and (D)). Details about the growth of the polymer-brushes can be found in literature [31]. The thickness of the polymer films is controllable by adjusting the polymerization time.
Figures 2(A) and (B) depict scanning electron microscopy (SEM) images of the patterned polyMETAC-brushes. The dark area in figure 2(A) represents areas of the unmodified OTS monolayer while the bright features represent the polyMETAC brush. Each line has its own unique morphology of the lines’ perimeters (seen in figure 2(A)), which results from the electrochemical lithography process. The peculiarities of each microline could be used in our investigations to easily determine the positions used for the subsequent characterization and SERS enhancement studies. The inset in figure 2(B) depicts a 3D AFM image representation, which displays the polyMETAC-brushes with a rather smooth surface with an RMS roughness of 4.9 nm as measured on a 10 × 10 µm² area on the brush. The AFM image acquired on the edge of the polymer brush, where it faces the OTS monolayer (figure 2(C)), enables to determine the height of the polymer brush which is in the range of 10–15 nm (figure 2(D)) albeit the peak-to-peak fluctuations appear to be large indicating the collapse of the brush structure when measured in the dry state. To further confirm that the polyMETAC-brushes were successfully grafted from the surface FT-IR investigations were performed. The obtained spectrum can be found in figure S1 (stacks.iop.org/NANO/31/465604/mmedia). A broad band around 3400 cm⁻¹ appeared in the spectra originating from the O–H absorption in the phenolic and aliphatic compounds, and the strong band at 2900 cm⁻¹ can be attributed to the C–H stretching vibration of the CH₂ groups. The bands at 1715 cm⁻¹, 1435 cm⁻¹, and 905 cm⁻¹ were assigned to the C=O stretching, C−N bending vibration, and the methyl groups of the quaternary ammonium in polyMETAC-brushes, respectively [40].

Finally, AgNPs were grown within the polyMETAC-brushes (schematic outline of the fabrication process is shown in figure 1(E)). Ag nanoparticles were prepared by first immersing the polyMETAC-brushes in an aqueous solution of HAuCl₄, which was used to form small Au seeds. The strong affinity of the quaternary ammonium groups for highly polarizable and weakly hydrated anions lead to the incorporation of the [AuCl₄]⁻ ions into the brushes. After absorption of the precursor gold salts into the polyMETAC-brushes, the substrates were reduced by aqueous NaBH₄ solution, which represents a well-known approach of reducing [AuCl₄]⁻ to form colloidal gold. Subsequently, silver enhancer solution was used to further develop the AuNPs by the electroless metal deposition of silver, which results in the further growth of the particles. The diameter of the AgNPs is controllable by adjusting the reaction time; as the reaction time increases, the AgNPs become larger.

In this work, the substrate was treated with the silver enhancer solution for 15 min. Figures 3(A) and (B) show the SEM images of patterned AgNP-polyMETAC composites. It is observed in figure 3(A) that the unpatterned areas remain free of any polymer or AgNPs. The thickness of the polyMETAC-brushes after growing the AgNPs largely increased to approximately 360 nm (figure 3(D), places where the height analysis has been performed are indicated in figure 3(C)). The surface structure of the AgNP-polyMETAC composites appears also to be rougher compared to the polyMETAC-brushes. The RMS roughness on a 10 × 10 µm² area of the brush increases to 35.7 nm. Energy-dispersive x-ray spectroscopy (EDX) was performed on the polyMETAC-brushes loaded with AgNPs in order
to determine the presence of silver within the brush layer after reduction (figure S2). Based on the EDX spectra (figure S2(B)), it can be concluded that the polymer-AgNP composite was composed of 61% silver, 17% carbon, 3% oxygen and 1% chlorine. The abundant presence of silicon originates from the silicon wafer used as substrate. Elemental EDX mapping of C, O and Ag reveals that the Ag is exclusively present in the patterned brush area whereas the OTS remains free of silver (figure S2(C)). Oxygen and carbon (figures S2(D) and (E)) are present in the brush as well as in the self-assembled OTS layer and are therefore found in all places on the surface.

3.1. pH effect on SERS of patterned AgNP-polyMETAC composites

After successful incorporation of silver NPs into the polyMETAC brushes, their response to solutions with different pH values was investigated. In general, the pH value represents an important factor in biomedical and chemical systems [41]. Hence, pH-responsive polymer materials have gained significant attention and, in particular changes induced by the pH value in polymeric brush systems are of particular interest. As such, the fabricated AgNP-polyMETAC composites could find potential use in, e.g. SERS sensor applications. Therefore, the pH value effect on the SERS activity of AgNP-polyMETAC composites was investigated here. Figure 4(A) depicts the Raman spectra obtained on the patterned AgNP-polyMETAC composites at different pH conditions ranging from pH 2 to pH 10 for the detection of $10^{-6}$ M 4-ATP, which was used as a test analyte. The peaks at 1076 and 1578 cm$^{-1}$ in the SERS spectra can be assigned to the benzene rings of the 4-ATP and originate from the benzene ring stretching vibration and the C−C stretching vibration, which are characteristic fingerprint peaks for 4-ATP. The intensity of the fingerprint Raman scattering peaks (figure 4(B)) increases with increasing pH value up to a pH of 5 and decreased again when the pH value exceeded this number (down to an intensity value of 50% of the maximum peak height that was observed at pH 5).

On the first glance, the pH-responsive property of the polyMETAC polymer brush is to some extend surprising. In general it is acknowledged that the strong polyelectrolyte brushes are pH-insensitive polymers [42]. However, recent studies [25–27, 43] have demonstrated that many properties of strong polyelectrolyte brushes are influenced and are governed by the pH value of their environment. E.g. the reorganization of the interchain hydrogen bonds resulting from the adsorption OH$^-$ or H$_2$O$^+$ to the polyelectrolyte brushes results to some extend in the pH-responsive properties of the polyMETAC-brushes. As a consequence, the loaded plasmmonic NPs redistribute in the strong polyelectrolyte brushes if the pH value of the environment changes. Thus, the polyMETAC/plasmmonic NP composites become pH-sensitive substrates in a reversible manner, which is shown in figure S3 [25]. The characterization of the spatial distribution of AgNPs in polyMETAC polymer brush is still a challenging task [18], therefore an explanation is mainly based on previous work [25]. Firstly, Raman spectra of pure polyMETAC brush were measured in various pH solutions (figure S4), demonstrating that the polymer brushes itself was insensitive to pH changes. Hence, different pH solutions are mainly influencing the spatial distribution of AgNPs in the polymer, which has already been proven by previous work [25] and Raman spectroscopy. In addition, Raman activities are directly related to the gap size of NPs when other parameters are set the same. So we suppose that at low pH values, the NPs tend to aggregate because of the lack of electrostatic stabilization and the aggregates form a 2D assembly attaching to the brush surface (figure 4(D)). With increasing pH value, the system is electrostatically stabilized and the plasmmonic NPs can penetrate the brush and assemble in three dimensions inside the brushes (figure 4(D)). The electrostatic attraction between the brush and the NPs plays an important role in the attachment of the plasmonic particles in both cases, which also leads to different gap sizes formed between adjacent NPs. In our work, the silver NPs distribute in a 2D assembly at pH 5 and the gap size approaches a minimum value under these conditions (figure 4(D)). It is in this context interesting to observe that the differences in the signal intensities at pH values <5 are less strong (signal intensities are only ~25% percent lower than at the maximum of pH 5) (figure 4(B)), indicating that the NPs are presumably still mainly attached to the surface of the polymer brush, albeit with a less pronounced order and, as a consequence, presumably larger distances between the particles. However, under these conditions the particle distances and hence the NP gaps are only moderately changed. A stronger dilution of the NPs is observed only when the NPs can penetrate into the brush and distribute in the 3D volume. In this case changes in the interparticle distances are expected which result in a stronger decrease of the Raman enhancement. At this stage we can only speculate about the reasons for the observed increase of the Raman intensities at a pH of 10. We assume that the capacity to distribute NPs inside the brush is saturated at this stage and a more dense arrangement, with consequently smaller particle distances, is found inside the brush which again would result in an increase of the observed
Raman intensities. These investigations have been performed at different pH values on the same area of the SERS substrate which could be identified by the characteristic shape of the patterns. This provides a good comparability for the SERS results since effects of inhomogeneities in the particle density or changes in the brush morphologies across the pattern can be minimized. The same trend of Raman activity was also found on other prepared SERS substrates as a result of increasing pH value (figure S5), which demonstrates that the fabrication of pH-responsive SERS surfaces is reproducible.

The patterned AgNP-polyMETAC composites not only act as a pH-sensitive SERS substrate but also show good reproducibility. Figure 4(C) presents a statistical analysis of mean, standard (SD) and relative standard deviation (RSD) of the obtained Raman intensities of the fingerprint peaks at 1076 and 1578 cm⁻¹ at different pH conditions, ranging from pH 2 to 10. More than 15 random points were measured for each pH value. The RSD for the Raman peak at 1076 cm⁻¹ ranges from 11.2% to 19.2% while at 1578 cm⁻¹ the RSD values differ from 8.0% to 17.6%. The statistical analysis demonstrates that the AgNP-polyMETAC composite substrate is stable as a SERS platform (wet state) and provides reproducibility at different pH values. Furthermore, the dry film of the composites was also statistically analyzed by mean, SD and RSD values, which are shown in figure S6.

For the purpose of investigating the limit of detection of the patterned AgNP-polyMETAC composites, 4-ATP molecules in a concentration range of 10⁻⁸–10⁻⁸ M were measured at pH 5. As shown in figure 5, the SERS intensity of the fingerprint peaks at 1076 and 1578 cm⁻¹ decreased as the concentration decreased and the fingerprint peaks were observed even at a concentration as low as 10⁻⁹ M in the Raman spectra. At a concentration of 10⁻¹⁰ M no Raman signals could be detected anymore. In summary, these investigations clearly demonstrate that this polymer-AgNP composite system can be used as an extremely sensitive pH-responsive SERS substrate.

4-ATP solutions could be measured down to a concentration of 1 × 10⁻⁸ M.

4. Conclusion

A promising composite substrate consisting of polyMETAC-brushes and AgNPs was successfully fabricated by electrochemical oxidation lithography. SI-ATRP and AgNP growth facilitated the fabrication of patterns with AgNP loaded polyMETAC brushes. The involved preparation processes are versatile and are of low cost. Due to the presence of the AgNPs, the pH-insensitive strong polyelectrolyte brushes (polyMETAC-brushes) start to exhibit pH-sensitive properties and can be used as pH-responsive SERS substrates. This is rationalized by assuming that upon an increase of the pH value the assembly of AgNPs transfers from a 2D aggregation state attached to the brush surface into a 3D assembly, which penetrates into the polymer brush. Moreover, the gap size between adjacent NPs reaches a minimum value when the pH value is 5, leading to the strongest SERS activity under these conditions. The composites could detect 4-ATP as analyte down to a concentration of 10⁻⁸ M at this pH value. Besides being used as a pH-responsive SERS substrate, the patterned AgNP-polyMETAC composites exhibit a good reproducibility as well, which renders this composite material suitable for potential applications in medicine, biology, chemistry and other fields. However, there are still improvements possible to further enhance the SERS performance of the patterned AgNP-polyMETAC composites in the future. This includes, e.g. controlling the size and distribution of the AgNPs among the polyMETAC-brushes. Moreover, the micropatterning, used in the present work mainly to enable the characterization of the polymerbrush system at a distinct area, is seen as a promising strategy to implement also platforms for automated parallel analysis and detection experiments by means of SERS investigations.

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