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In-line sensing of sodium ascorbate using a poly(ferrocenylsilane)-coated microfluidic device

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Abstract

Poly(ferrocenylsilane) (PFS) is a redox-active polymer that can be utilized in a variety of applications, such as sensing, nanoparticle foundries or dual-responsive hydrogels, due to the fact that it can be partially or completely oxidized, reversibly, by (electro)chemical means. In this research, PFS is tethered to a cysteamine-modified gold-coated microchannel electrode via amine alkylation, following a recent published procedure for conventional macro-sized electrodes. The PFS-coated microfluidic device is successfully employed as an electrochemical sensor for sodium l-ascorbate, or vitamin C, with a detection limit of 0.5 mM in aqueous solution. The fabrication of the device, that comprises gold-coated electrodes of micrometers in width, is discussed, as well as the static and in-line detection of sodium l-ascorbate. The analyte concentration is sensed through an electrocatalytic process in both static and in-line cases, by cyclic voltammetry and amperometry, respectively. The findings presented in this work open novel opportunities for local surface modification of chip-integrated gold electrodes by redox-responsive polymers used, e.g., as electrochemical sensors.

1. Introduction

By integrating smart, stimulus-responsive polymers as functional elements, the performance capabilities of controlled fluid delivery and sensing in microfluidic devices can be greatly improved. Stimulus-responsive polymers [1] are frequently used in microfluidics, both in poly(dimethylsiloxane) [2] and in glass devices [3], as sensors [4] and actuators [5]. These polymers are often triggered by external stimuli, e.g., variations in temperature [6], solvent composition [7], pH [8] or illumination with light [9]. Of a different class are electroactive polymers as they enable device miniaturization and precise local addressability. Electroactive polymers [10] have also been used in various microfluidic devices, for example, in electrically driven hydrogel actuators [11], in applying electro-responsive polymers for channel-width modulation [12] or as diaphragm actuator [13]. Of particular interest are redox-responsive polymers such as poly(ferrocenylsilane) (PFS) which offer fast, reversible switching and local addressability in combination with nanofabricated electrodes [14].

PFSs comprise a unique class of redox-active materials composed of alternating ferrocene and alkylsilane units in the main chain. They combine a high density of redox centers with excellent processability and reversible redox characteristics [15], in particular, it is of interest that the ferrocene moieties in the main chain render these polymers electroactive [16].

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to the substituent on the silane moiety in the main chain, PFS can be modified to have various properties such as water-solubility [17]. Water-soluble PFSs are used as polyelectrolyte coatings [18], or utilized as a redox-active gel for synthesis of silver [19] or palladium nanoparticles [20]. Actuators could be devised from PFS gels cross-linked poly(N-isopropylacrylamide), which reversibly collapse and expand upon isothermal oxidation [19].

Controlled in-chip electrode modification can be a challenge because of various limitations, such as minute volumes and channel confinement, leading to low diffusion and local accessibility of reagents. Mild procedures are required to prevent deterioration of the electrodes and channel walls. PFS can be grafted to surfaces by using sulfur-terminated PFS as self-assembled monolayers [21], by electrografting of PFS to gold [22] or using a cysteamine monolayer for the covalent attachment of PFS chains to an electrode surface, employing amine alkylation reactions [23], encompassing a procedure which has been established recently in our group. Via this procedure, robust, dense, redox-active organometallic PFS films can be formed on gold substrates. These PFS polymer films can be employed as an electrochemical sensor, exhibiting high sensitivity, stability, and reproducibility [22–24].

In this paper, we present a microfluidic device with gold-coated microchannels as electrodes, to which the redox-responsive PFS was covalently tethered. To test the sensing capabilities of the microfluidic device, vitamin C, in sodium -ascorbate form, was chosen as an analyte. Vitamin C is found in fruits and vegetables and is water-soluble. As nature’s widely available antioxidant, it has been studied intensively [25]. Therefore, ascorbic acid is an excellent test case for sensing applications [26], also where polymer-based sensors are concerned [27]. Detection of ascorbic acid is mainly done in electrochemical systems [28], with detection limits up to 23 nM [29]. However, these systems are mostly on macro-sized electrodes [30], while miniaturization, for example for lab on a chip applications, requires micro or nano-sized detectors, where detection limits are not even approaching the current macro-sized values [31], hence new types of detection layers should be investigated. In this work, we fabricated a microfluidic device with integrated gold channel electrodes. Subsequently, PFS, with an iodopropyl sidechain, was utilized for inner-surface modification via alkylation of cysteamine-modified channel-electrodes. Finally, the use of suchlike modified microfluidic devices for electrochemical sensing was elaborated by the electrocatalytic determination of sodium ascorbate.

2. Device fabrication

2.1. Gold-coated microchannels

The fabrication and characterization of the glass microfluidic device with integrated gold channel electrodes is explained in more detail in this section. Glass wafers were cleaned with fuming nitric acid for 10 min and coated with a Cr/Au (10 nm/100 nm) etch mask using an evaporator. After spin-coating with photoresist, illumination with UV light for 8 s through a lithography mask and development in tetramethylammonium hydroxide solution for 60 s, the wafers were etched using a 25% HF solution for 20 min yielding 27 µm deep features. Afterwards, the resist was removed with acetone and the etch mask was removed with a gold etchant and a chromium etchant. Afterwards, the wafers were thoroughly rinsed with water and dried by spin-drying. The glass wafers, with integrated microchannels, were cleaned again with fuming nitric acid for 10 min and spin-coated with a new layer of photoresist. Subsequent to illumination for 30 s through a second mask, and development, the wafers were immersed in buffered hydrofluoric acid solution (BHF) for 6 min to etch a 110 nm deep trench to embed the electrodes. 100 nm gold electrodes were evaporated on the photoresist. A 10 nm Cr layer was used as an adhesive layer for the glass. After a lift-off procedure in acetone using an ultrasonic bath, the wafers were cleaned using nitric acid for 10 min and Piranha solution (1:3 H₂O₂:H₂SO₄, 1 min) and subsequently bonded thermally (550 °C, 1 h) to a cover glass wafer, on which access holes had been fabricated using a powderblast technique. The whole process is schematically shown in Fig. 1.

Fig. 2 shows a scanning electron microscopy (SEM) image of the gold-coated channel, as well as an atomic force microscopy (AFM) image of the electrode. Fig. 2a displays an overview image of the section of interest of the device, and Fig. 2b displays a close-up of the gold-covered channel. It can be seen that the electrode is successfully evaporated on top of the...
microfluidic channels, as is also shown by the AFM image in Fig. 2c. The section plot, below the AFM image, corresponds to the blue line in the AFM image, and it can be seen that a 140 nm deep trench is etched by the BHF, therefore the electrode of height 110 nm is embedded into the glass, ensuring proper bonding with the top glass wafer in the fabrication step that followed. Prior to use with the polymer functionalization, the device performance was tested by analysis of potassium hexacyanoferrate(II), as shown in Fig. S1. From this can be concluded that the device worked as electrochemical sensor, by employing the gold-coated microchannels as working electrode.

2.2. Surface attachment of poly(ferrocenylsilane)

PFS, comprising iodopropyl and methyl sidegroups (PFS-I), was grafted to the cysteamine-modified gold surface of the microfluidic device, via amine alkylation. The grafting procedure is depicted in Scheme 1. The gold surface is first cleaned by oxygen plasma for 2 h (1) and then covered with a monolayer of cysteamine (2) flowing a 10 mM solution (ethanol) overnight at 30 µm h⁻¹. Afterwards, the PFS-I (5 mg mL⁻¹ in tetrahydrofuran) is drop-casted and soaked into the channel by capillary forces, and, after drying with nitrogen flow, heated overnight at 50 °C under vacuum (3). The channels are subsequently rinsed thoroughly with tetrahydrofuran, a good solvent for PFS-I, to remove any physisorbed polymer. A more in-depth study of this process can be found in Ref.[23] for flat substrates and Ref.[32] for microchannels. To confirm the attachment of the PFS-I, cyclic voltammetry experiments where performed. Fig. 3 shows two voltammograms, one of a non-coated device (red line) and one of a PFS-I-coated microfluidic device (blue line). The characteristic double wave of PFS can clearly be observed, proving that PFS is tethered to the working electrode. The double peak in the voltammogram is related to stepwise oxidation of the first (statistically every second) ferrocene in the main chain, followed by filling in the remaining sites at higher oxidation potentials [14a]. The faradaic current of the PFS layer exceeds the capacitive current of the device. To test the kinetics of the PFS layer, the scan rate was also varied, the results are shown in Fig. S2. Square-root dependence of the peak height on the scan rate is observed, hence can be concluded that the electrochemical response of the device is diffusion controlled. In contrast, on macro-sized electrodes, a linear relation was found in previous work from our group [22,23], which are characteristic for surface-confined layers [33]. However, the ability of the electrolyte to diffuse into the polymer layer has a strong effect on the kinetic behavior of the film [34]. Therefore, we attribute the diffusion control observed in our case to the confinement of the electrolyte due to the small dimensions compared to flat substrate/macro sized electrode studies. Adding to this effect is the large geometrical separation between the working electrode and counter and reference electrodes, namely around 18 mm, due to the design choices of the device.
3. Vitamin C sensing

3.1. Static sensing

The performance of the microfluidic device as electrochemical sensor was studied by electrochemical measurements in various concentrations of sodium ascorbate. To confirm the electrocatalytic behavior of the polymer layer, in stationary solution, cyclic voltammograms of the PFS-I-coated microfluidic device in the presence of sodium ascorbate were performed, as shown in Fig. 4. The concentration of sodium ascorbate used was varied from 0.5 to 2 mM. A clear increase in peak current, when adding sodium ascorbate, can be observed, proving that the microfluidic device can detect the presence of sodium ascorbate. The electrocatalytic reaction proceeds as follows [35]. After oxidation of the ferrocene moiety via the electrode potential, the ascorbate reduces ferric iron to ferrous iron, oxidizing into dehydroascorbic acid, allowing the ferrocene to once more oxidize to ferrocenium, resulting in an increase in current depending on the concentration of the ascorbate. However, due to the nature of the reaction, the amount of available ascorbate diminishes, resulting in lower current in each voltammetry cycle. To counteract this depletion a constant influx of ascorbate is required, leading to a steady state in current response. Hence, the electrochemical technique was changed from cyclic voltammetry to amperometry and a constant ascorbate inflow was realized.

3.2. In-line vitamin C sensing

Fig. 5 shows the amperometric response of the PFS-modified microfluidic device, under application of various concentrations of sodium ascorbate over an order of magnitude from 0.5 mM to 5 mM, at +0.70 V vs. Ag/AgCl. The different amperometric responses are shifted in time to overlap, because, due to the microfluidic setup, the exact time of arrival of the sodium ascorbate cannot be controlled. However, the electrocatalytic effect of the PFS is clear. The PFS-coated microchannel was used as working electrode, Ag/AgCl was used as reference electrode, platinum wire as counter electrode and no flow was applied (quiet electrolyte).

Fig. 4. Cyclic voltammograms (Current I vs. potential E) of a PFS-I-coated microchannel, with increasing concentration of sodium ascorbate (0, 0.5, 1 and 2 mM, inner to outer lines). The electrocatalytic effect of the PFS is clear. The PFS-coated microchannel was used as working electrode, Ag/AgCl was used as reference electrode, platinum wire as counter electrode and no flow was applied (quiet electrolyte).
ascorbate at the PFS-covered microelectrode is not known beforehand. Furthermore, the contribution of the PFS layer itself is filtered from the signal. (See Fig. S5 for the non-shifted curves). From Fig. 5 several conclusions can be drawn. Firstly, from the shape of the curve it can be concluded that a second order diffusion process takes place, as the sodium ascorbate has to diffuse from the bulk fluid to the wall of the channel, since, due to the no-slip boundary condition, there is no flow at the wall. To reach a plateau value approximately 100 s are required. Secondly, the increase in plateau height increases linearly with concentration (inset in Fig. 5) as can be expected from the hydrodynamic electrodes in the microfluidic flow cell. The observed amperometric currents (i_{max}) are governed by a convective transport (flux J) of the electroactive species or, as shown here, the electrocatalyzed sodium ascorbate [33,36]. Because flow rate, cell dimensions and diffusion coefficient are constant, the concentration change of the analyte reflects in a corresponding linear dependency to the detected current [36].

The limit of detection is calculated to be 12.7 μM, with a signal-to-noise ratio of 3. This value compares with the limit of detection found in literature on vitamin C detection in microfluidic devices: 5 μM in Ref. [37]; 5 μM in Ref. [38], 5 μM in Ref. [39] and 20 μM in Ref. [31]. To verify that the current response of the PFS is not influenced by the flow rate, cyclic voltammograms at various flow rates were performed, as well as amperometric sensing experiments, shown in Figs. S3 and S4, respectively. From these it can be concluded that the device is not influenced by the flow rate, apart from the time required to reach the plateau value, within the range of flow rates studied. To further improve the sensitivity of the device, the availability of in-channel platinum counter and Ag/AgCl reference electrodes will be explored, to further miniaturize this microfluidic device [40]. Furthermore, thickness control of the PFS in-chip layer will be investigated to improve the electron-transfer kinetics of the PFS layer. Moreover, other types of PFS will be investigated as active layers, such as a water soluble PFS [17,22].

4. Conclusion

PFS was successfully tethered to cysteamine-modified gold-coated microchannel electrodes. The microfluidic device was utilized to sense sodium ascorbate, with a detection limit of 0.5 mM concentration. This indicates that PFS is a strong candidate as active layer for electrochemical sensors in microfluidic devices. In future work, by controlling the layer thickness and device dimensions, we anticipate higher sensitivity of the device.

5. Experimental section

5.1. Reagents and solvents

MEMPax Glass wafers (100 mm diameter, 0.5 mm thickness) were purchased from Schott. From Arch Chemicals Inc., Olin OIR 907-17 was used as photosist, tetramethylammonium hydroxide solution as developer. 25% HF solution was made by diluting 50% HF solution purchased from BASF. Chromium etchant was purchased from Technic. BHF (1:7 HF:NH₄F) was purchased from Honeywell. Nitric acid was purchased from KMG Chemicals. Acetone, sulfuric acid and hydrogen peroxide were purchased from BASF. Potassium iodide, iodine, potassium ferrocyanide, cysteamine, sodium perchlorate and sodium ascorbate were purchased from Sigma-Aldrich. Tetrahydrofuran was purchased from Biosolve. Ethanol was purchased from Merck. All chemicals were analytical grade and used as received, except where noted otherwise. The synthesis of PFS-I is described elsewhere [18b,41].
5.2. Device fabrication and characterization

Chromium and gold were evaporated at 0.05 nm s⁻¹ and 0.2 nm s⁻¹, respectively, using a Balzers BAK 600. UV exposure was performed with an EVC 620 at 12 mW cm⁻². SEM experiments were performed using a JEOL JSM-6330F SEM. AFM experiments were done with a Bruker Dimension 3100 equipped with a hybrid scanner and a NanoScope VIa controller, using ‘tapping mode’ and a silicon nitride tip.

5.3. Electrochemical experiments

Cyclic voltammetry and amperometry were performed using a CH instruments 852D potentiostat. “In-chip” measurements were done as follows. The chip was first filled with the electrolyte solution (0.1 M NaClO₄ in H₂O) using a syringe pump, this is the electrolyte used in all experiments. A platinum wire and a Ag/AgCl electrode (BASi) were gently immersed into the now-formed droplets at the two outlets and used as counter and reference electrode, respectively. Finally, using a needle, the gold-coated channel was contacted via microfabricated contact pads and used as working electrode. The whole setup was placed on a stabilizing table. A photograph of the microfluidic device in the set-up can be found in Fig. S6.

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Appendix A. Supplementary material

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References


