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Delayed Sensor Activation Based on Transient Coatings: Biofouling Protection in Complex Biofluids

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Supporting Information

ABSTRACT: Transient polymeric coatings with a programmable transiency behavior are used for delayed exposure of fresh surfaces of multi-electrode sensor arrays at preselected times. Such delayed sensor actuation is shown to be extremely attractive for addressing severe biofouling characteristic of electrochemical biosensors in complex biofluids. Controlled coating dissolution and tunable sequential actuation of the individual sensing electrodes are achieved by tailoring the characteristics of the coating (density and thickness). The unique features offered by these delayed sensors allowed direct glucose monitoring in untreated blood and saliva samples over prolonged periods. This attractive delayed-sensor exposure concept, offering time-tunable sequential activation of multiple sensors with remarkable anti-biofouling properties, indicates considerable promise for operating sensors continuously in complex body fluids.

Electrochemical biosensors offer distinct advantages for on-body monitoring of biomarkers,^{1–4} in connection to implantable^{5–7} and wearable devices.^{8–10} However, despite tremendous progress, biofouling problems—associated with gradual passivation of the transducer surface due to accumulation of sample macromolecules—have hindered the continuous electrochemical monitoring in complex biofluids.^{11–15} As a result, direct electrochemical detection in biological media remains a major challenge.^{5,16,17} Efforts to minimize surface biofouling have focused primarily on the development of permselective protective coatings, such as Nafion,^{18,19} polyvinyl chloride (PVC),²⁰ or *o*-phenylenediamine (OPD),^{12,21} that allow transport of target analytes while excluding large fouling macromolecules. While these coatings have been shown useful to minimize electrode fouling, the sensor performance degrades gradually, and the device needs to be replaced periodically.^{5–7,19,22}

In this Communication we demonstrate an attractive approach for addressing the biofouling challenge of electrochemical biosensors. Rather than protecting the sensor during the operation in complex biofluids, the new approach relies on turning “ON” different sensors sequentially, at preselected times, and hence ensuring no biofouling even after prolonged contact with biological media (Figure 1, left side). Such operation is realized by using transient methacrylate-based coatings, with different dissolution times, that lead to delayed

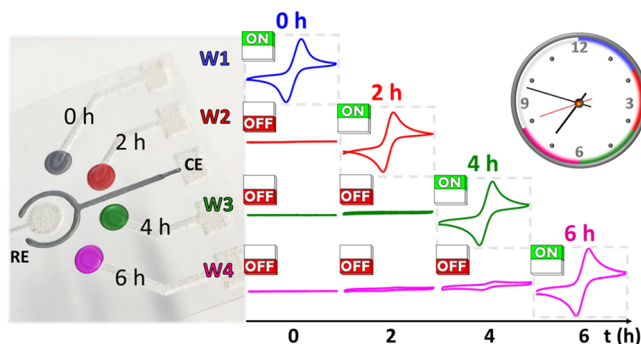


Figure 1. Controlled delayed sensor activation concept based on transient coatings that dissolve completely within a programmed time to expose fresh transducer surfaces. Left: Image showing the design of the four-electrode sensor array with delayed activation using dissolvable coatings of different thicknesses on the working electrodes W1–W4, with 0, 1, 2, and 3 layers of the 16% polymeric coating, respectively. Right: CVs illustrating the activation of the different electrodes at specific times. CV: 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ in 0.1 M PBS (pH 6.5), scan rate = 100 mV s^{-1} .

exposure of the transducer surface. However, unlike the common use of transient materials which fully disappear into the surrounding environment with non-traceable residues after completing their operation,^{23–26} the present approach relies on using transient coatings to expose a fresh transducer surface for starting the sensing operation in the surrounding biofluid sample. Specifically, among the wide variety of Eudragit (Evonik Industries) polymers with different pH response, the L100 polymer, which dissolves above pH 6.0,²⁷ was selected here for coating the multi-electrode sensor array (see Materials and Methods in Supporting Information). As illustrated from the cyclic voltammograms (CVs) of the $[\text{Fe}(\text{CN})_6]^{4-/3-}$ redox marker (Figure 1, right side), tuning the transient dissolution characteristics of the coating allows precise control of the sensor activation time following 2, 4, and 6 h exposure to the sample solution. Such sequential sensor activation holds considerable promise for continuous monitoring of important analytes in complex environments, not only in connection with electrochemical devices. Beyond sensing applications, these highly tunable multi-functional coatings—with remarkable anti-biofouling properties, pH-responsive behavior, and tailor-

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made dissolution—could benefit a wide range of biomedical devices (ranging from ingestible capsules to drug delivery vehicles) and a variety of industrial and environmental systems demanding clean surfaces and long-term stability.

Controlling the transiency behavior of the methacrylate-based protective coatings can be achieved by tailoring the factors that affect their dissolution time. Different experiments were performed to demonstrate the precise temporal control of the sensor activation through changes in the characteristics of the transient coating. Figure 2a,b displays CVs obtained using

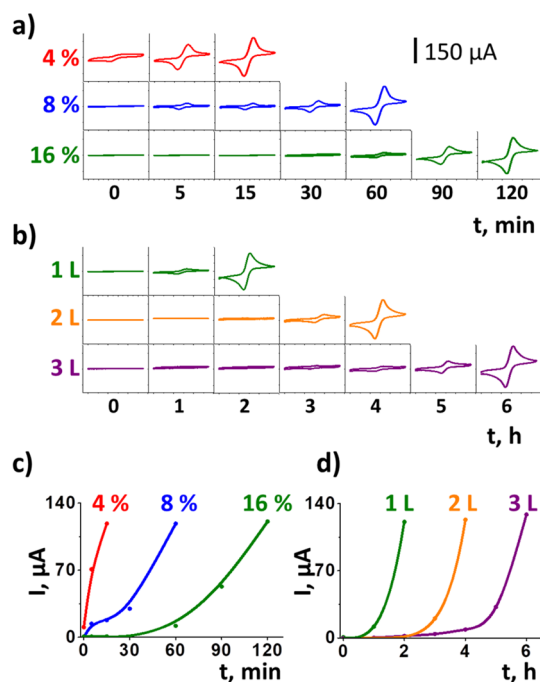


Figure 2. Control of the coating dissolution time and exposure of a fresh electrode surface. CVs (a,b) and oxidation peak currents (c,d) indicating the activation time of different electrodes coated with one layer of different coating densities (4, 8 and 16%) (a,c), and with different numbers of layers (1, 2, and 3 L) of a 16% coating (b,d). CV: 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ in 0.1 M PBS (pH 6.5), scan rate = 100 mV s^{-1} .

electrodes modified with coatings of different densities or thicknesses. The effect of the film density was examined using different working electrodes (WEs) coated with single layers containing different percentages of the methacrylate polymer in the precursor solution (4, 8, and 16%, Figure 2a,c). Figure 2a displays the CVs obtained at these coated WEs within a 120 min period. As expected, the sensor response was dependent on the coating density, enabling the sequential activation of the three electrodes, as indicated from the increased $[\text{Fe}(\text{CN})_6]^{4-/3-}$ redox peaks with time. Data comparing the corresponding oxidation peak currents with the coating dissolution time (Figure 2c) illustrate that the longest delayed sensor activation time (i.e., complete exposure of the electrode surface at 120 min) was achieved using the highly dense (16%) coating, compared to the shortest sensor activation within 15 min observed with the low-density (4%) film. Aiming at evaluating the extent of the sensor delay time, we examined the influence of the thickness of the 16% film in connection to different numbers of layers. Figure 2b,d displays the CVs and corresponding oxidation peaks currents obtained for three different WEs modified with 1, 2, and 3 layers of the 16%

coating. These results illustrate the possibility to delay the sensor activation up to 6 h by increasing the number of coating layers over the WE. As shown in Figure S1, the coating dissolution time depends linearly ($R^2 = 0.9984$) upon its thickness (expressed in Table S1 as mass loading (g/cm^2)). Overall, the results displayed in Figure 2 demonstrate the possibility of precisely controlling the sensor activation time by tuning the transient characteristics and timed disappearance of the protective coating.

Additional characterization of the coating process and dissolution time was performed by adding the fluorescent dye fluorescein (FITC) to the 16% coating solution (Figure S2). For this study, the four WEs were coated with different numbers of layers of the dye-labeled coating. After different incubation times in 0.1 M PBS (pH 6.5), the coated WEs were observed under a fluorescence microscope. Similar to the previous CV results, the fluorescence intensity decreased progressively with the incubation time until displaying a negligible signal, thus confirming the total dissolution of the coating and complete exposure of the electrode surface to the solution.

The reproducibility of the coating process was evaluated by comparing four different electrodes, modified identically with a single layer of the 16% coating. Figure S3 shows the CVs and oxidation peak currents obtained for the different electrodes at specific activation times. These results, along with the calculated RSD values ($<6.0\%$), indicate that the system offers good reproducibility toward a controlled delayed sensing applications.

To examine the potential application of the delayed sensors in biological fluids, the anti-biofouling protective properties of the transient coatings were investigated in relevant sample solutions (schematic illustration in Figure 3a). The responses of uncoated and coated electrodes (single 16% coating layer) were thus compared after incubation in different complex fluids. Figure 3b displays the CVs obtained for uncoated and coated electrodes exposed for 120 min to surfactant solutions containing 50 mg L^{-1} gelatin, 5000 mg L^{-1} Triton X-100, or undiluted human serum, respectively. It can be observed that after 120 min incubation in each medium (solid lines), the CV current signals of the uncoated electrodes decreased significantly compared to the initial value (measured at 0 min; dotted lines). In contrast, an increased response is observed at coated electrodes (red vs black CV plots). The relative (%) current signals (shown in Figure 3c), calculated every 30 min, also confirm that the coating protects the electrode surface from biofouling and ensures that 100% of the response is maintained after 2 h incubation in different complex fluids, compared to the 50% signal obtained at the uncoated electrodes. These results confirm further the important protective role of the transient coating layer after prolonged incubations in complex matrices.

After examining the anti-biofouling properties provided by the transient coating, we tested the protected sensors toward different biosensing applications. A preliminary application for prolonged glucose detection was carried out using glucose oxidase (GOx)-modified Prussian Blue (PB) electrodes (see Supporting Information) due to their high selectivity toward the hydrogen peroxide product of the enzymatic reaction (Figure S4a).²⁸ To perform this study, the chronoamperometric glucose signals were measured at four WEs (one uncoated and the other three modified with different numbers of 16% coating layers) at specific times (0, 2, 4, and 6 h)

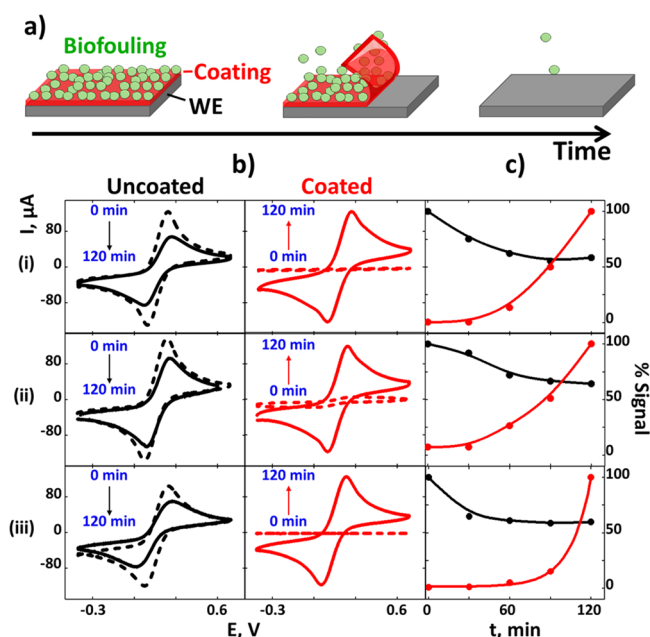


Figure 3. Anti-biofouling properties of the delayed sensor activation system. (a) Schematic illustration of the anti-biofouling protection of the sensor using the dissolvable coating. (b) CVs obtained for uncoated (black) and coated (red) electrodes at 0 and 120 min (dotted and solid lines, respectively). (c) Comparison of the relative (%) response in the presence of 50 mg L⁻¹ gelatin (i), 5000 mg L⁻¹ Triton X-100 (ii), and undiluted serum (iii) at different incubation times. Black and red arrows on the CV plots indicate the decrease or increase of the current intensity peaks, respectively. Coating: single 16% layer. CV: 5 mM [Fe(CN)₆]^{4-/3-} in 0.1 M PBS (pH 6.5); scan rate = 100 mV s⁻¹.

(Figure S4). As demonstrated by the current signals (Figure S4b) and the corresponding chronoamperograms (Figure S4a), there is a sequential activation of the electrodes (depending on the film thickness) within the 6 h incubation time in the glucose solution (pH 6.5). These results demonstrate the ability to delay the glucose detection to preselected times by tuning the transiency properties of the coatings. It is important to mention that although these experiments were performed using a fixed pH of 6.5, the dissolution rate of the L100 polymer coating increases with the pH of the solution (described in the specifications of the commercial polymers²⁹ and in the experimental data of Figure S5).

Finally, aiming to mimic a prolonged glucose sensing in an *in vivo* system, we carried out another experiment involving the monitoring of glucose in undiluted saliva and blood samples (Figure 4, panels a,c and b,d, respectively). In this study, the spiked glucose response was measured every 30 min at the uncoated and coated (single 16% coating) GOx-PB electrodes. The chronoamperograms (Figure 4a,b), and the corresponding relative changes of the glucose signal (Figure 4c,d) further confirm the anti-biofouling activity imparted by the dissolvable coating. These data illustrate that after 2 h of incubation in these biological media, the coated electrodes offered their maximum sensitivity, compared with 29.6% and 34.6% of the original sensitivity achieved at the uncoated electrodes. Hence, it is possible to detect important analytes (such as glucose) after prolonged exposure to complex undiluted biological samples using such transient coatings without compromising the sensor sensitivity.

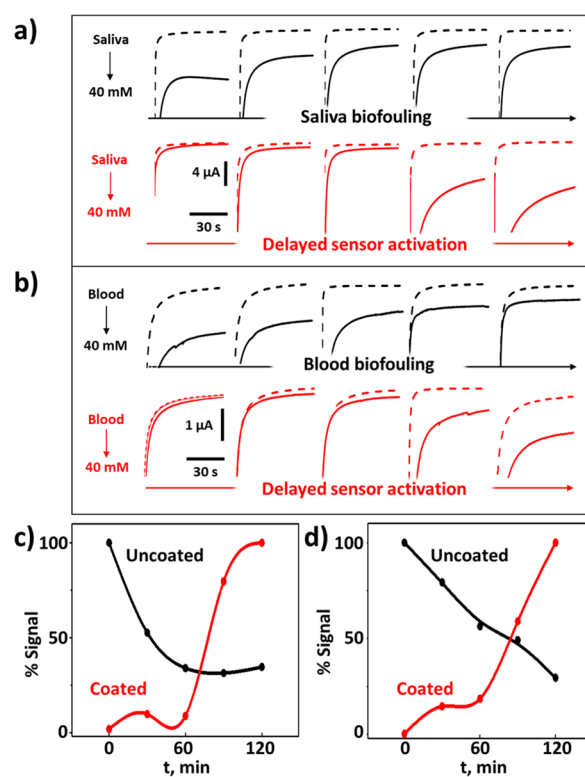


Figure 4. Biosensing-model application of the delayed sensor and anti-biofouling activity of dissolvable coating. Chronoamperograms obtained in undiluted saliva (a) or blood (b) before (dotted lines) and after (solid lines) spiking 40 mM glucose at 0, 30, 60, 90, and 120 min incubation times (from left to right, respectively). $E_{app} = -0.2$ V (vs Ag/AgCl), 60 s. Relative (%) response of the GOx-PB sensor in undiluted saliva (c) and blood (d), corresponding to the uncoated (black) or coated (single layer with 16%; red) GOx-PB electrodes at specific incubation times.

In summary, we presented the design and operation of a multi-electrochemical sensor array with controlled delayed sensing capability achieved by modifying the individual electrode surfaces with transient polymeric coatings. These delayed sensors are extremely attractive for addressing severe biofouling observed in complex biofluids. Protective coatings with different programmable transiency behaviors (provided by different densities or thicknesses) have thus been used to offer sequential activation, exposing fresh sensor surfaces at preselected times (up to 6 h), and impart remarkable anti-biofouling properties. Such operation and the excellent anti-biofouling characteristics have been illustrated for enzymatic monitoring glucose in blood and undiluted saliva samples over a 2 h period. Future efforts will be aimed at evaluating different coating materials (including other types of pH-responsive polymers), soluble under different conditions, that may extend the range of applications and the sensor delay time toward prolonged *in vivo* monitoring of important analytes in a variety of body fluids. Such new capabilities pave the way to the design of highly stable on-body multi-sensor systems with “built-in” programmed delayed response to determine important analytes in untreated complex fluids. The concept can be readily adapted to other electrochemical sensing platforms (e.g., sequential activation of different microneedles arrays) as well as for the protection of other sensing devices involving different transduction modes. This delayed actuation concept can benefit also a wide variety of biomedical devices, ranging

from implantable devices and ingestible capsules to microscale gastrointestinal delivery vehicles. The highly tunable and dual nature of these unique and robust coatings, merging pH-responsive temporal behavior and remarkable antifouling properties, could lead to multifunctional devices that address unmet challenges in many other fields.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/jacs.8b08894](https://doi.org/10.1021/jacs.8b08894).

Experimental details, Table S1, and Figures S1–S5 (PDF)

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Notes

The authors declare no competing financial interest.

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