

Thick-Film Carbon Electrode Deposited onto a **Biodegradable Fibroin Substrate for Biosensing Applications**

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This study addresses a proof-of-concept experiment with a biocompatible screenprinted carbon electrode deposited onto a biocompatible and biodegradable substrate, which is made of fibroin, a protein derived from silk of the Bombyx mori silkworm. To demonstrate the sensor performance, the carbon electrode is functionalized as a glucose biosensor with the enzyme glucose oxidase and encapsulated with a silicone rubber to ensure biocompatibility of the contact wires. The carbon electrode is fabricated by means of thick-film technology including a curing step to solidify the carbon paste. The influence of the curing temperature and curing time on the electrode morphology is analyzed via scanning electron microscopy. The electrochemical characterization of the glucose biosensor is performed by amperometric/voltammetric measurements of different glucose concentrations in phosphate buffer. Herein, systematic studies at applied potentials from 500 to 1200 mV to the carbon working electrode (vs the Ag/AgCl reference electrode) allow to determine the optimal working potential. Additionally, the influence of the curing parameters on the glucose sensitivity is examined over a time period of up to 361 days. The sensor shows a negligible cross-sensitivity toward ascorbic acid, noradrenaline, and adrenaline. The developed biocompatible biosensor is highly promising for future in vivo and epidermal applications.

biomedical applications.^[1,2] When applying such electronics onto human skin to detect. e.g., different ions, glucose, or lactate in sweat, the usage of skin-friendly and noninflammatory materials is a prerequisite.^[3-5] For this purpose, several synthetic materials such as polyethyleneglycol (PEG), polylactic acid (PLA), poly(vinyl alcohol) (PVA), poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), or polyhydroxvalkanoates (PHA) have been examined to build biocompatible sensor devices.[6-10] Due to their intrinsic biocompatibility, scientists have always sought inspiration by nature to use natural materials such as alginate, chitosan, collagen, or hyaluronic acid.^[11-14] While some of these materials are in daily use, the existing material combinations can still lead to foreign body responses, like inflammation, fibrous encapsulation, or biofouling, which also affect the overall biosensor performance.^[15] Therefore, researchers exploited silk as a material to build "green electronics" with the advantage of biocompatibility and biode-

1. Introduction

Within the last years, much effort has been put into the development of biocompatible and biodegradable electronic devices for

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silk worm Bombyx mori, this organic material benefits of superior mechanical properties such as a high tensile strength and large breaking strain or flexibility.^[17] Silk consists of two proteins,

gradability under physiological conditions.^[16] Obtained from the

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namely, fibroin and sericin. The fibroin protein can form various morphologies, like sponges, gels, powder, scaffolds, membranes, or fibers. $^{[18]}$

Several examples of fibroin-based biocompatible electronics have been published over the last years such as a thin-film transistor, a field-effect sensor, a thermally triggered drug delivery device, a resistive switching device, and a flexible pressure sensor.^[19-23] In addition, fibroin has been applied for the modification of a glassy-carbon electrode (GCE) to detect bisphenol A or DNA.^[24,25] Furthermore, the adjustable water solubility and biodegradability of fibroin was used to transfer biocompatible electronics onto several surfaces, like feline brain, tooth enamel, and different kinds of food.^[26-28] Our group has recently published a flexible, biocompatible amperometric biosensor chip for the detection of glucose in, e.g., Ringer's solution.^[29] This biosensor chip was fabricated by combining thick- and thin-film technologies and was able to detect glucose with good sensor characteristics. Furthermore, the produced fibroin membrane together with the deposited platinum thin-film electrodes did not induce a cytotoxic reaction and demonstrated a complete degradation by a protease from Streptomyces griseus, Type XIV, within 10 days. However, the fabrication of the Pt working electrode requires clean-room environment for physical vapor deposition (PVD). In addition, the life time of these sensors is limited, which could be explained by the detachment of the platinum thin-film electrode from the fibroin substrate. One possible explanation is given by the high-vacuum conditions during the PVD process $(<10^{-5} \text{ mbar})$, which stresses the water-containing membrane morphology.

To circumvent these drawbacks, the present work is focusing on the development of a biosensor consisting of a substrate made of fibroin in combination with a biocompatible carbon-based working electrode fabricated via thick-film echnology. To ensure biocompatibility, the whole sensor setup (including the conducting tracks) was encapsulated with a biocompatible silicone rubber (EcoFlex). In comparison to our previous work, thick-film technology allows a cost-effective production of the electrodes without the needs of a clean-room environment. In addition, thick-film carbon electrodes also possess a higher mechanical robustness compared to thin-film electrodes. To examine the morphology of the fabricated thick-film biosensor, scanning electron microscopy (SEM) was performed. The sensor was functionalized as a glucose biosensor by immobilizing the enzyme glucose oxidase on top of the thick-film carbon electrode. The biosensor was electrochemically characterized by amperometric/voltammetric measurements in phosphate-buffered solution (PBS). One important part of this work was to study the influence of the curing temperature (while drying the carbon paste) on the silk-fibroin properties, such as the sensor's flexibility: glucose concentrations were measured at different applied potentials to the working electrode (500-1200 mV), using sensors with carbon electrodes cured at different curing profiles (50 °C/24 h, 80 °C/24 h, and 160 °C/1 h). Furthermore, the long-term stability of the biosensor was studied as well as the cross-sensitivity toward ascorbic acid, adrenaline, and noradrenalin.

2. Experimental Section

2.1. Materials

Glycerol, ethanol, glucose monohydrate, and glucose oxidase (GOx) from Aspergillus niger (E.C. 1.4.4.), bovine serum albumin (BSA), disodium hydrogen phosphate dihydrate, and sodium dihydrogen phosphate monohydrate were purchased from Sigma-Aldrich (St. Louis, USA). Adrenaline solution (1 mg mL^{-1}) was bought from Infectopharm (Heppenheim, Germany), noradrenaline (250 mg mL^{-1}) was purchased from Carinopharm (Eime, Germany), and ascorbic acid was ordered from Merck (Darmstadt, Germany). Ecoflex 00-30 silicone rubber was obtained from KauPo Plankenhorn e.K. (Spaichingen, Germany). Directly before use, the two components of EcoFlex 00-30 silicone rubber were mixed in a 1:1 ratio and degassed in vacuum until all bubbles disappeared. Glutaraldehyde (25%) was purchased from Acros Organics (Geel, Belgium). PureSilk fibroin solution was provided by Fibrothelium GmbH (Aachen, Germany) and stored in the freezer at -20 °C. The conductive carbon paste 126-03(SP)A-B-0100 was purchased from Dico Electronic GmbH (Schwabach, Germany). Directly before use, the two components of the carbon paste were mixed in a ratio of 1:1. The silver conducting ink (article number: 530 042) was bought from Ferro GmbH (Frankfurt am Main, Germany).

2.2. Preparation of the Fibroin Membrane

For the fibroin substrate, 2 vol.-eq. of an 8 wt% solution of PureSilk was mixed with 1 vol.-eq. of 10 vol% ethanol and 1 vol.-eq. of 3 vol% glycerol.^[29] The final mixture (10 mL) was casted onto a Teflon surface with a diameter of 9 cm and dried under cleanroom conditions at 21.0 °C for 2 days. The dried membrane was carefully detached afterward from the Teflon surface using a scalpel. Further details about the fibroin membrane preparation can be found elsewhere.^[29]

2.3. Fabrication of the Biocompatible Biosensor via Thick-Film Technology

The carbon electrodes were screen-printed onto the fibroin membrane by means of a manual Sp002 Essemtec (Aesch/LU, Switzerland) screen-printing device, and dried in a preheated muffle oven with different curing profiles. The screen-printed carbon electrodes were either dried at 50 °C for 24 h, at 80 °C for 24 h, or at 160 °C for 1 h. The final carbon-working electrode is 19 mm in length with a 7 mm in diameter sensing area (see Figure 1a,c).

Even after the curing process, the carbon-based electrode was still flexible, which is demonstrated in Figure 1d, where the electrode was bended by 90°. The final carbon electrodes are separated, glued onto a printed circuit board (PCB) with the biocompatible EcoFlex 00-30 silicone rubber, and connected to the PCB trace via silver conductive ink and an aluminum foil. For the modification of the carbon-based electrode, the GOx enzyme membrane consisting of a 1–2–2 (enzyme–BSA–glutaraldehyde/glycerol) mixture with GOx (5 U μ L⁻¹)







and BSA (10 vol%), both solved in a PBS and glutaraldehyde/ glycerol solution (2 vol%), was freshly prepared and drop-casted onto the carbon electrode. The glutaraldehyde/glycerol mixture consisting of glutaraldehyde (8 vol%) and glycerol (10 vol%) is solved in deionized water.^[30] After drying at room temperature overnight, the sensor was encapsulated by EcoFlex 00-30 silicone rubber such that only the enzyme membrane was left open. Figure 1a shows a microscopic image of the encapsulated biosensor chip with the immobilized enzyme membrane (top view), and Figure1b presents a schematic cross section of the biosensor with the different layers.

Fibroin

Carbon

2.4. Measurement Procedure

The electrochemical experiments were carried out in a threeelectrode arrangement, containing the functionalized biocompatible thick-film carbon electrode as working electrode, a platinum wire as counter electrode (MaTeck, Jülich, Germany), and a conventional Ag/AgCl reference electrode (Deutsche METROHM GmbH & Co. KG, Filderstadt, Germany). The three electrodes were connected to a potentiostat (PalmSens, Palm Instruments BV, GA Houten, The Netherlands). For all electrochemical measurements, the software tool PSTrace was used. All measurements were performed in a stirred PBS solution (pH 7.4, corresponding to the pH value in human blood) at 21.0 °C. Prior to amperometric measurements, cyclic voltammetric (CV) measurements in PBS were carried out applying potentials from -1.2to 1.2 V with a scan rate of 100 mV s⁻¹, to determine the plateau region of the applied sensor. With each differently cured carbon electrode (50 °C/24 h, 80 °C/24 h, or 160 °C/1 h), a CV diagram was recorded.

The measurement principle of the biosensor is based on the oxidation of glucose in the presence of oxygen and the enzyme GOx to D-glucono-1,5-lactone and hydrogen peroxide. The latter is then oxidized at the carbon electrode at a constant applied potential of the working electrode, and the resulting current is monitored over time. For the amperometric measurements, different glucose concentrations from 0.5 to 4 mM were prepared using a 250 mM stock solution. Each glucose concentration was measured for 7 min under continuous stirring. To adjust an optimum working potential, measurement sequences with different applied potentials (500, 700, 900, 1100, and 1200 mV) to the thick-film biosensor cured with different curing profiles (50 °C/24 h, 80 °C/24 h, or 160 °C/1 h) were carried out. The recorded data were normalized to their values based on the 7 min region (where only PBS was measured) using the PSTrace software.

The long-term experiments were performed with three sensors of each curing profile. Biosensors with carbon electrodes cured at 50 °C for 24 h and at 80 °C for 24 h were measured daily from day 1 to day 5, as well as at days 12, 18, and 350; biosensors with carbon electrodes cured at 160 °C for 1 h were studied daily at days 1–5, and at days 13, 20, and 361. Between the measurements, the carbon working electrodes were stored in a closed container together with dry agent silica gel in the refrigerator at 4 °C. Cross-sensitivity studies were carried out with one sensor of the long-term experiment of each curing profile. Here, 50 nM noradrenaline, 50 nM adrenaline, 100 μ M ascorbic acid, and

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different glucose concentrations from 0.5 to 4 mM were titrated in the described order to the PBS.

3. Results and Discussion

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3.1. SEM Characterization of the Carbon Working Electrode onto the Fibroin Substrate

The thick-film carbon electrodes deposited onto the fibroin substrate and cured with different curing profiles were characterized by SEM (JEOL JSM-7800F, Freising, Germany) applying an acceleration voltage of 5.0 kV. The SEM characterization addressed two different regions (middle of the WE and crosssectional view of the WE): **Figure 2a–**c presents the respective sensing area of the carbon electrode for the different curing profiles of 50 °C/24 h, 80 °C/24 h, and 160 °C/1 h (left to right).

The surface of the carbon working electrode has a pronounced, uniform 3D structure without showing visible holes or cracks. The different 3D structures appear very similar, independent of the applied curing profile. In the cross-sectional view of the carbon electrode (see Figure 2d–f), one can clearly distinguish between the smoother fibroin substrate on the bottom and the rougher carbon working electrode on top.

3.2. Electrochemical Characterization of the Biocompatible Thick-Film Biosensor

To determine the plateau region of the prepared biocompatible thick-film biosensor for the subsequent amperometric measurements, CV curves were performed for each curing profile of the carbon working electrode. **Figure 3** exemplarily shows typical CV plots obtained from three different biocompatible thick-film biosensors with the carbon electrode cured at 50 °C (blue), 80 °C (black), and 160 °C (red).



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Figure 3. CV curves obtained from three different biocompatible thick-film biosensors, where the carbon working electrodes have been cured at 50 °C for 24 h (blue), 80 °C for 24 h (black), and 160 °C for 1 h (red), respectively.

The curves had visible differences between the three curing profiles. For the CV curve obtained from the thick-film biosensor cured at 50 °C for 24 h (blue curve), an anodic plateau can be defined in the potential range from around +0.2 to +0.8 V. However, the anodic plateau region of the biosensor cured at 80 °C for 24 h (black curve) is located in the range from -0.3to +1.2 V. In contrast, no distinct plateau in the CV curve recorded by the thick-film biosensor cured for 1 h at 160 °C (red curve) is visible, indicating the possibility of slight faradaic currents. For amperometric measurements, the working potential should be located in the anodic plateau region, also eliminating the influence of other electroactive species. Furthermore, the selected potential region is not only composed of the plateau in the CV curve, but also of other factors such as the reactionspecific energy, the material-dependent electrode overvoltage, and the electrolyte solution.^[31]

As the CVs did not provide a clear indication of the optimum potential to be applied to the carbon working electrode, a series of chronoamperometric measurements was performed. Here, the



Figure 2. SEM images of a–c) surface of the carbon working electrode (WE, magnification $\times 1000$) cured at temperatures of 50 °C for 24 h, 80 °C for 24 h, and 160 °C for 1 h, respectively, and d–f) cross-sectional view through the middle of the carbon working electrode (magnifications: (d) $\times 330$, (e) $\times 400$, and (f) $\times 500$).

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Figure 4. Dynamic amperometric response of the thick-film biosensor with immobilized GOx measured in PBS at pH 7.4, containing different glucose concentrations (0.5–4 mM). The carbon electrode has been cured at: a) $50 \degree C$ for 24 h, b) $80 \degree C$ for 24 h, and c) $160 \degree C$ for 1 h; different working potentials were set to the carbon electrodes for each curing profile: 500 mV black, 700 mV red, 900 mV green, 1100 mV blue, and 1200 mV violet. b,d,f) Corresponding calibration curves of the biosensor (0.5–2 mM) evaluated from (a,c,e). Note that all measurement curves in (a,c,e) were normalized to their initial starting value in PBS.

influence of an increasing potential (between 500 and 1200 mV) to the thick-film carbon-based working electrode for amperometric measurements was studied for glucose concentrations from 0.5 to 4 mM, as depicted in **Figure 4**.

At each curing profile (50 °C for 24 h (Figure 4a), 80 °C for 24 h (Figure 4b), and 160 °C for 1 h (Figure 4c), the applied potential of the biosensor varied from 500 to 1200 mV with potential steps of 200 mV for each measurement sequence. An applied potential at the carbon working electrode higher than 1200 mV damaged the thick-film electrode by detaching the glucose oxidase membrane from the carbon electrode. The corresponding calibration curves for Figure 4a,c,e are shown in Figure 4b,d,f.

In general, for applied potentials to the carbon working electrode of 500 (black curve) and 700 mV (red curve), no clear

signal changes could be recognized for increasing glucose concentrations from 0.5 to 4 mM, independently of the curing profile. This is also confirmed by the low sensitivity values determined in the glucose concentration range from 0.5 to 2 mM, as listed in **Table 1**. At an applied potential to the working electrode of 900 mV (green curve), sensor signal steps by titration of low glucose concentrations from 0.5 to 2 mM could be seen for all curing profiles, while higher glucose concentrations could not be distinguished from each other. A further increase of the applied potential (1100 mV, blue curve and 1200 mV, violet curve) resulted in distinct steps for all glucose concentrations, independent of the curing procedure. Biosensors fabricated with curing the carbon working electrode at 50 °C for 24 h (Figure 4a, violet curve) and 160 °C for 1 h (Figure 4e, violet curve) had

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Table 1. Glucose sensitivity values and their standard deviations with increasing applied potential to the carbon-based working electrode treated by different curing regimes.

Curing profile	Applied potential [mV]	Sensitivity [nA mM ⁻¹]
50 °C/24 h	500	$\textbf{0.1}\pm\textbf{0.02}$
	700	1.0 ± 0.2
	900	11.4 ± 1.4
	1100	117.4 ± 23.4
	1200	$\textbf{162.7} \pm \textbf{45.4}$
80 °C/24 h	500	$\textbf{0.2}\pm\textbf{0.04}$
	700	$\textbf{3.7}\pm\textbf{0.8}$
	900	$\textbf{31.3} \pm \textbf{4.0}$
	1100	$\textbf{80.6} \pm \textbf{18.0}$
	1200	185.7 ± 46.7
160 °C/1 h	500	$\textbf{1.0}\pm\textbf{0.08}$
	700	$\textbf{9.5}\pm\textbf{2.4}$
	900	$\textbf{27.4} \pm \textbf{3.8}$
	1100	113.2 ± 25.2
	1200	$\textbf{167.2} \pm \textbf{37.8}$

similar sensitivity values of $162.7 \pm 45.4 \text{ nA mM}^{-1}$ and $167.2 \pm 37.8 \text{ nA mM}^{-1}$ when applying a potential of 1200 mV. The highest glucose sensitivity of $185.7 \pm 46.7 \text{ nA mM}^{-1}$ could be reached with the thick-film biosensor where the carbon electrode was cured at 80 °C for 24 h (Figure 4c, violet curve) by applying a potential of 1200 mV. All glucose sensitivities were evaluated in the linear concentration range from 0.5 to 2 mM and are summarized in Table 1. As an applied potential of 1200 mV to the biocompatible thick-film carbon-based biosensor provides the highest sensitivity with the most pronounced steps, this potential is used for the subsequent long-term stability tests and cross-sensitivity experiments.

3.3. Long-Term Stability of the Thick-Film Carbon-Based Biosensor

For the long-term stability tests, three individual thick-film biosensors of each curing profile were first measured daily up to day 5, and then periodically with increasingly longer intervals up to day 350 or 361, respectively. **Figure 5** depicts the obtained glucose sensitivities of the studied biosensors treated with different curing profiles during thick-film fabrication of the carbon working electrode at the different measurement days. The sensitivity is normalized to the highest value of the three biosensors at the first day of measurement.

The slight increase in sensitivity of biosensor 1 (green bar) and biosensor 2 (orange bar) cured at 50 °C for 24 h (see Figure 5a) might be due to membrane conditioning for the repeated measurements within the first 5 days. After 350 days, the sensitivity of all biosensors has decreased to a similar level of about 35% compared to the highest value at the first day (biosensor 3).

The glucose biosensors cured at 80 °C for 24 h (see Figure 5b) had quite similar sensitivity values at day 1. Biosensor 1 (orange bar) and biosensor 3 (purple bar) strongly decreased over time.



At day 350, no longer sensor response to glucose titrations could be observed. In contrast, the obtained glucose sensitivity of biosensor 2 (green bar) is increasing up to day 5. At day 350, a similar decrease in sensitivity was found as for the former biosensors from Figure 5a.

The change in sensitivity for the thick-film biosensors with the carbon working electrode cured with a curing profile of 160 °C for 1 h is shown in Figure 5c. Biosensor 2 (green bar) showed quite similar normalized sensitivities over the first 20 days. The same is true for biosensor 1 (orange bar) but at a much lower sensitivity level. Both biosensors showed a strong decrease in sensitivity at day 361 compared to day 1. Biosensor 3 (purple bar) first had an increase in sensitivity up to day 3 and then decreased. At day 361, no signal change of the sensor was detectable when glucose was added to the buffer solution.

3.4. Cross-Sensitivity of the Biocompatible Thick-Film Biosensor

The sensitivity and selectivity of an amperometric glucose biosensor can be affected by other redox-active molecules in the analyte solution. Furthermore, each electroactive species will be oxidized or reduced at the electrode surface by applying a certain potential. Therefore, the biocompatible thick-film biosensors cured with different curing profiles are examined regarding their cross-sensitivity adding typical disturbing substances such as ascorbic acid, noradrenaline, and adrenaline. These components can falsify glucose measurements on Pt working electrodes (at an applied potential of +600 mV) and were also investigated for their influence on the developed carbon-based thick-film biosensor.^[29] Ascorbic acid is a key element in the human body for reduction and oxidation processes; the concentration in blood plasma varies from 20 to 110 µM.^[32] Likewise, catecholamines such as adrenaline and noradrenaline are present in blood with concentrations ranging from 0.3 to 1.6 nM.^[33,34]

Amperometric measurements and corresponding calibration curves of the cross-sensitivity study are depicted in **Figure 6**a,b with three exemplary carbon electrodes, cured at 50 °C for 24 h (blue curve), 80 °C for 24 h (black curve), and 160 °C for 1 h (red curve).

The measurements were performed in PBS solution, while different substitutes (50 nM noradrenalin, 50 nM adrenaline, and 100 µM ascorbic acid) were titrated to the analyte solution, one after the other. To compare possible cross-sensitivity influences on the biosensor performance, different glucose concentrations (0.5-4 mM) were spiked into the analyte solution after measuring ascorbic acid. Again, each analyte was measured for 7 min. The experiment clearly demonstrated that the signals of the used thick-film biosensors are not remarkably affected by the addition of noradrenaline, adrenaline, and ascorbic acid in the physiologically relevant concentration range at the applied working potential of 1200 mV. Even for ascorbic acid, the signal increase is about three times lower than for the lowest glucose concentration of interest. Furthermore, the sensitivity values achieved from the measurements of different glucose concentrations in the spiked PBS containing the substitutes (noradrenalin, adrenalin, and ascorbic acid) are similar in comparison to those,







Figure 5. Long-term stability measurements for glucose sensitivity obtained from three individual biosensors (n = 3), each cured at: a) 50 °C for 24 h, b) 80 °C for 24 h, and c) 160 °C for 1 h. The glucose sensitivity was evaluated in the concentration range from 0.5 to 2 mM. Orange represents biosensor 1, green biosensor 2, and purple biosensor 3 of each group. The measurements were performed in PBS at pH 7.4, 21.0 °C, with an applied potential of 1200 mV versus the Ag/AgCl reference electrode. All sensitivities are normalized to the highest value at the first day of measurement for each curing profile.



Figure 6. a) Dynamic amperometric response of thick-film glucose biosensors cured with different curing profiles (50 °C/24 h in blue, 80 °C/24 h in black, and 160 °C/1 h in red) measured in PBS (pH 7.4) proofing the cross-sensitivity by spiking sequentially 50 nM noradrenaline (NA), 50 nM adrenaline (AD), and 100 μ M ascorbic acid (AA), and different glucose concentration from 0.5 to 4 mM; b) corresponding calibration curves of the three biosensors in (a) with a linear fit from 0.5 to 2 mM. Note that all measurement curves in (a) were normalized to their initial starting value in PBS.

resulting from measurements in PBS with only titrated glucose concentrations, as shown in **Table 2**.

4. Conclusions

To solidify the carbon paste on a biodegradable silk-fibroin substrate, the influence of three different curing profiles (50 $^{\circ}C/24$ h, 80 °C/24 h, and 160 °C/1 h) on the morphological and electrochemical properties of the resulting thick-film biosensor with immobilized GOx was investigated. Glucose concentrations from 0.5 to 4 mM (which are part of the biomedically relevant range) were analyzed in PBS, at a physiological pH of 7.4, varying the applied potential of the working electrode between 500 and 1200 mV versus the Ag/AgCl reference electrode.^[35] An applied potential of 1200 mV gives the highest sensitivity and strongest www.advancedsciencenews.com

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Table 2. Obtained sensitivity values for glucose with carbon-based biosensors cured at different curing profiles: glucose sensitivity in PBS spiked with interfering substances (middle column) and in only PBS (right column).

Curing profile	Sensitivity in PBS with interfering species at day 350 resp. 361 [nA mM ⁻¹]	Sensitivity in PBS [nA mM ⁻¹]
50 °C/24 h	133 ± 30.3	135.9 ± 23.7
80 °C/24 h	45 ± 8.3	$\textbf{54.9} \pm \textbf{9.1}$
160 °C/1 h	$\textbf{24.1} \pm \textbf{5.1}$	24.5 ± 4.0

signal strengths of all investigated potentials. Even after curing at 160 °C for 1 h, the biodegradable fibroin substrate behaves flexible. Long-term studies did successfully proof the application of the biosensor. Future studies will focus to extend the detectable range of glucose concentrations to become compatible with glucose monitoring, e.g., in diabetes health care.

Typical interfering substances such as noradrenaline, adrenaline, and ascorbic acid were spiked into the PBS, indicating some slight influence of ascorbic acid to the biosensor signal. Here, either using a biodegradable mediator to lower the applied potential of the working electrode or a semipermeable membrane to limit the access of ascorbic acid to the carbon electrode might be straightforward strategies for further experiments.^[36]

Casted silk-fibroin membranes represent a highly promising material for possible clinical applications due to their unique properties such as high flexibility, high mechanical strength, and biodegradation under physiological conditions.^[29,37] The ability to process fibroin with various properties expands its use in, e.g., food industry and cosmetics.^[38,39] Due to the versatility of fibroin, a new field in the fabrication of biosensors can be established. This material also enables, for example, the combination of different electrode materials onto the same sensor chip: the capability should be further envisaged to prepare both the working electrode including the receptor layer and the counter-/(pseudo)reference electrode by applying biocompatible materials.^[40] Fibroin as a biodegradable and biocompatible material is highly attractive and beneficial for in vivo sensing and implantable devices, as well. For such a scenario, the presented setup should be further developed. One idea might be a triggered degradation system depending on, e.g., the degree of crystallization of the used silk structure. Furthermore, a point-of-care system as a lab-on-chip device would allow to detect relevant medical parameters such as lactate, typical tumor biomarkers, or inflammatory factors (e.g., reactive protein).

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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

biocompatible materials, biodegradable electronic devices, biosensors, carbon electrodes, glucose, screen-printing, silk fibroin

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