Fabrication of Highly Effective Enzyme-Embedded Electrochemical Sensing Platform for Urea Detection

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ABSTRACT

In a recent investigation, we have modified glassy carbon electrodes (GCE) with amine groups ($-NH_2$) to produce aminated GCE that effectively detects current changes during urea decomposition reactions. The silk fibroin (SF) scaffolds are used to place urease near the surface of the aminated GCE (Urs/SF/aminated GCE). The as-synthesized electrode is employed for electrochemical urea sensing that could be monitored via cyclic voltammetry (CV) and amperometric techniques. The fabricated sensing platform displays rapid detection response (~1 min) and sensitivity (112.3 μA mM^{-1} cm^{-2}) with linear correlation (0.3–8.4 mM) between the current and urea concentrations. Moreover, the analogous sensing responses obtained via SF scaffold discs (generated and functionalized) in the urease/SF/aminated GCE assure the suitable platform for the urea sensing application devices.

Keywords: Biosensor, Electrochemical, Enzymatic, Sensing, Urea.

INTRODUCTION

It is well known that, biosensing technology originates from the synergistic coupling between electronics and biotechnology. Whereas, the biosensors involve the integration of bioactive matrices (e.g. enzymes) with suitable detectors equipped with sophisticated reference transducers. Of note, electrochemical biosensors are analytical gadgets comprising biologically active moieties immobilized on the suitable transducers that is capable of transforming the biochemical signals into electric signals.

As urea is an important constituent of blood and serum, it is vital to keep its levels within a controlled normal range. That is why, the recommended concentration of urea in the serum is fixed in the range of 1.7–8.3 mM. Mostly, the accurate estimation of urea concentration is thus of great importance for the clinical diagnosis. Various new strategies for the fabrication of biosensors for *in-situ* measurement of urea levels have been recently reported. In this study, we have focused on the electrochemical biosensing approach for urea detection because of its easy handling, miniaturization and efficiencies.

OBJECTIVES

To fabricate electrochemical biosensor for urea detection.

To improve sensitivity of enzymatic urea sensor.

To ensure reliability of detection results from the sensor.

METHODOLOGY

It consists of four steps;

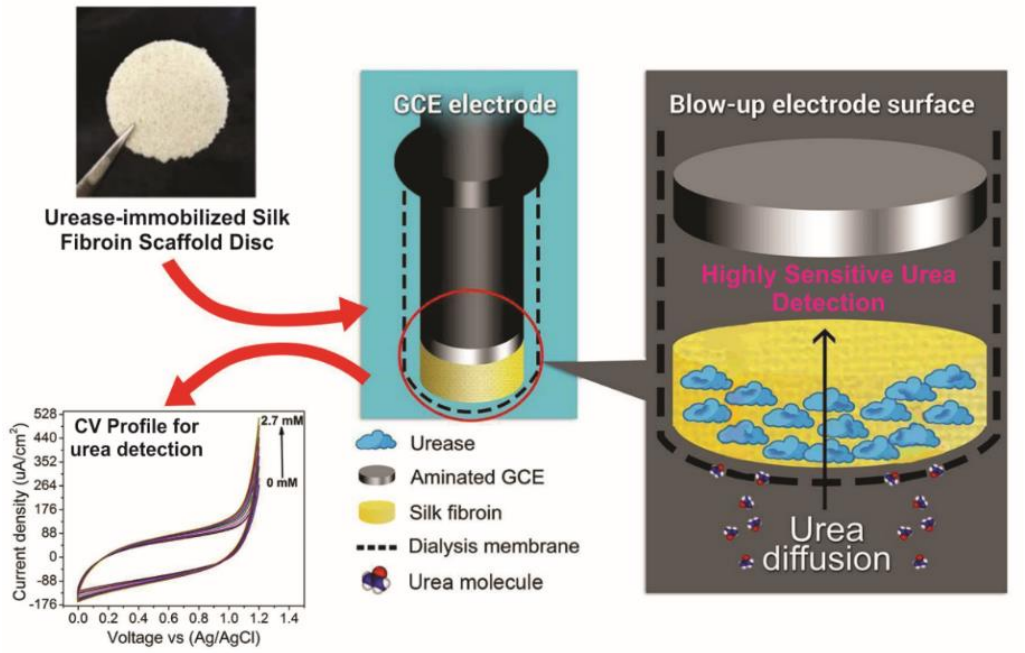
Amination of GCE: The surface of glassy carbon electrode is functionalized with amine groups in accordance with an already reported procedure. Amination is performed through cyclic voltammetry in a three-electrode

cell setup using 10 mL of ammonium carbamate aqueous solution (pH ~9).

Direct immobilization of urease on the aminated GCE: Aminated glassy carbon electrode is overnight immersed in the acidified mixture of 2.5% glutaraldehyde (v/v) and 12.5 mg/mL urease solutions. Glutaraldehyde acts as a crosslinking agent and it binds urease with amine groups present on the surface of aminated glassy carbon electrode. The final electrode is represented as Urs/aminated GCE.

Preparation of silk fibroin scaffolds: Porous silk fibroin scaffolds are prepared by adding a mixture of salt/sucrose particles to silk fibroin polymer solution. Resultant mixture is stirred gently which is poured into a silicone mold and heated at 60 °C for 3 h. After cooling it down to room temperature, solidified mixture is immersed in DW and ethanol along with mold to leach out unnecessary particles, leaving behind residual pores in the silk fibroin polymer matrix. The SF scaffold is made ready for application and activities.

Fabrication of Urs/SF/aminated GCE: For the purpose to further enhance the efficiency, the porous SF scaffold is immersed in 10% (v/v) glutaraldehyde solution. After rinsing with phosphate buffer, excess glutaraldehyde is removed and SF scaffold is transferred to 4 mg/mL urease solution for 2 h at ambient temperature. At this stage, SF scaffold is again rinsed with phosphate buffer to remove excess enzyme moieties from the surface. Finally, the urease-immobilized SF scaffold (Urs/SF) is divided into discs of 3 mm thickness and 8 mm diameter. To fabricate the working electrode, as synthesized Urs/SF discs are placed over surface of glassy carbon electrode.



CONCLUSIONS

Glassy carbon electrode was successfully functionalized via electrooxidation of carbamic acid. For direct enzyme immobilization, glutaraldehyde was used as crosslinking agent. Alternatively, enzyme-immobilized silk fibroin scaffold discs were employed to place urease near the surface of GCE. The fabricated electrodes were used for detecting urea concentration in solution by CV and amperometry. It has been concluded that, the sensing platform was found to be highly sensitive that could enhance rapid detection. Moreover, this scientific approach has ensured the reliability of results by replacement of enzyme-immobilized silk fibroin scaffold discs.

REFERENCES

1. Rafiq, Khezina, *et al*. "Fabrication of a highly effective electrochemical urea sensing platform based on urease-immobilized silk fibroin scaffold and aminated glassy carbon electrode." Sensors and Actuators B: Chemical 251 (2017): 472-480.
2. Mai, Hien Duy, Gun Yong Sung, and Hyojong Yoo. "Fabrication of nickel oxide nanostructures with high surface area and application for urease-based biosensor for urea detection." RSC advances 5.96 (2015): 78807-78814.
3. Moon, Bo Mi, *et al*. "Fabrication and characterization of three-dimensional silk fibroin scaffolds using a mixture of salt/sucrose." Macromolecular Research 22.12 (2014): 1268-1274.
4. Wang X, Uchiyama S. Amperometric glucose sensor fabricated by combining glucose oxidase micelle membrane and aminated glassy carbon electrode. Analytical letters. 2008 Jun 13;41(7):1173-83.
5. Wang, Xiuyun, *et al*. "Amperometric urea biosensor using aminated glassy carbon electrode covered with urease immobilized carbon sheet, based on the electrode oxidation of carbamic acid." Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis 19.12 (2007): 1300-1306.