



Fabrication of Ultra-micro Carbon Fiber Electrode Probes for Detection of O₂ and H₂O₂

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Abstract: Carbon fiber electrodes (CFEs) are commonly used in detection of neurotransmitters like dopamine. Besides, modification of these electrodes with enzymes enables development of biosensors capable of local analysis. Here, CFEs were fabricated using glass capillary tubes. Basically carbon fibers were inserted into glass capillary tubes and then the tubes were pulled using a micro-puller to insulate carbon fibers. Subsequently, the electrode surface was modified with Pt nanoparticles to evaluate the potential of these electrodes in detection of O_2 and H_2O_2 . In the future, these electrodes will be used for construction of biosensors for detection of local ATP.

Keywords: Carbon fiber electrode, H_2O_2 , biosensor, electrochemical analysis.

1. Introduction

In general, biosensors are analytical devices with a biological detection unit capable of sensing physiologically important molecules with the desired precision and converting them into numerical data. Enzymes, antibodies, microorganisms, cells, nucleic acids and sometimes tissues are used as biological detection units in biosensors [1-4]. These types of devices are designed mostly in the form of microchips or probes, while being completely unique to the investigator. In recent years, research has concentrated on making biosensors more efficient and cheaper, with higher sensitivity. The method used in the detection may be optical, mechanical or electrochemical. Although most of the biosensors developed so far are optically based, there are some disadvantages to this technique, such as fluctuations due to quenching or emission from non-target molecules, the shielding effect by the turbid solution and the need to mark the target molecule with a specific marker [5, 6]. Moreover, the devices used in optical detection are large and heavy, limiting the practical use of them. As an alternative method, electrochemical detection is used in biosensors. In general, electrochemical methods are thought to be more advantageous than optical methods due to reasons such as rapid response, cheap production, simple and easy to use, microsuitability for biosensor production and low cost equipment for signal conversion [7-9]. Using a simple electrode, the oxygen consumption of the cells, the activity of some released proteins, or the analysis of cell proteins can easily be done [10, 11]. The ability of developing the technology to produce ultrafine and

Received on: 19.01.2017 Accepted on: 13.03.2017 nano-level electrodes makes it possible to use these electrodes in micro and nano-level analyzes [12-14]. Unlike millimeter-sized electrodes, micro- or nanoelectrodes have features that enhance sensitivity, such as low ohmic potential drop, low double layer charging current and high molecular transport [15, 16]. In addition, small size electrodes enable analysis in small volume media.

Carbon fibers are highly conductive and due to their small dimensions, it is possible to produce micro and nano probes with them, which offers certain advantages such as the use of these electrodes in single cell analysis. Carbon fiber based microelectrodes are commonly used for local and highly sensitive detection of neurotransmitters such as dopamine or recording of neuronal action potentials known spikes, enabling electrochemical monitoring of as neurochemical activity of brain [17-20]. Carbon fiber electrodes (CFEs) can allow for electrochemical monitoring in short-time domains and recording of neuronal activity in real time [21, 22]. Tissue damage during monitoring with CFEs is quite limited compared to other tools such as microdialysis probes. Brain has a very complex structure. and tools like CFEs have helped scientists gain a substantial amount of knowledge regarding function in a very easy manner. In addition to being used in such measurements, CFEs also have potential for detection of biologically important molecules with great sensitivity and selectivity by simply modifying the surface of the electrode with a biorecognition molecule [23-25]. In a recent study, Salazar and his colleagues modified CFEs with Prussian Blue to realize a glutamate microbiosensor for neuroscience applications [26]. They first modified the surface of CFEs with Prussian Blue using electrodeposition. Next, they coated the surface with poly-o-phenylenediamine (PoPD) for higher stability and polyethyleneimine (PEI) as a glucose oxidase (GOx)

immobilization agent. Using this microbiosensor, they successfully detected glutamate at a significantly low concentration (<50 µM). In another study, Lee and coworkers used CFEs for detection of glucose [27]. They first treated carbon fiber electrodes with KOH for activation, a process that yielded improved adsorption of GOx, then used urea treatment to improve the conductivity of the electrodes. According to the results, the sensitivity value of the modified electrodes was two to three times higher than untreated electrodes. These are some of the many studies showing the benefits of using CFEs for bio-sensing applications. In this study, the surface of CFEs were modified with Pt nanoparticles to detect O₂ and H₂O₂. Detection of cells' O₂ consumption is highly desirable as it plays a vital role in respiration and cell metabolism. It is also crucial for a variety of practical applications including steel-making and food preparation [28]. Detection of H_2O_2 is also important in a variety of fields ranging from food preparation to environmental monitoring [29, 30]. Moreover, the excess presence of H_2O_2 in cells causes oxidative stress and hereby leading to various diseases including cancer. Usually enzymes such as horse radish peroxidase are used to make H₂O₂ biosensors, however, complex fabrication procedures, low reproducibility and enzyme instability limits large scale application of these biosensors [5, 31]. Recently, more focus has been given to constructing enzymeless H₂O₂ biosensors and their practical applications. Basically, in this study, production of CFE probes was carried out by pulling carbon fiber inserted glass capillary tubes using a micropuller to seal single carbon fibers with a thin layer of glass. Subsequently, the electrode surface was ground to expose the disk electrode which then was modified with platinum nanoparticles. Lastly, the ability of these probes in oxygen and hydrogen peroxide detection was analyzed.

2. Materials and Methods

2.1. Materials

Patch clamp glass (OD/ID: 1.65/1.1 mm) (World Precision Instruments, USA), silver paste (Sigma-Aldrich, USA), chloroplatinic acid – H₂PtCl₆ (Sigma-Aldrich, USA), hydrogen peroxide solution, 30 % (w/w) in H₂O (Sigma-Aldrich, USA), phosphate buffered saline (PBS) (Sigma-Aldrich, USA), hydrogen chloride (HCl) (Sigma-Aldrich, USA), ferrocenemethanol (FMA) 97% (Sigma-Aldrich, USA).

Carbon fibers with a diameter of $\sim 6 \ \mu m$ were kindly provided by Dr. Mustafa Erol (Izmir Katip Celebi University, Turkey)

2.2. CFE Fabrication and Characterization

First, carbon fibers were attached to copper wires that are only exposed at the ends using a silver (Ag) paste and then the connection was made permanent by baking and solidifying the silver paste at 180 °C. The carbon fibers attached to the copper wires were placed in the glass capillary tubes with the help of the copper wires, and the copper wires were fixed on the glass tubes with heatshrinking rollers to prevent any damage to the carbon fibers in the subsequent operation. In the next process, a thin layer of glass was formed on carbon fibers by pulling the glass capillary tubes using micropulling method to produce CFEs (Figure 1A). PC-10 micropulling machine (Narishige, Japan) was used for micropulling. The pulling parameters were optimized prior to CFE fabrication. The parameters used for micropulling are as follows; option: 1 and heating level: 65. As the last step of the probe production, the tip of the CFEs were ground to expose the disk electrodes using a machine called microgrinder (EG-401, Narishige, Japan) (Figure 1B, C_I).

Following the production of CFEs, the electrochemical behavior of these probes was analyzed with a PBS solution containing electroactive ferrocenemethanol (FMA). PBS solution containing 1 mM FMA was prepared for the electrochemical analysis. Cyclic voltammetry (CV) of the CFEs were obtained in this solution using a potentiostat (Autolab PGSTAT204, Metrohm, Switzerland). At this point, the current was measured by sweeping the potential of the working electrode between 0 and +0.5 V (vs Ag / AgCl) at a scan rate of 50 mV / s.



Figure 1. Fabrication of carbon fiber electrode (CFE) probes by micropulling carbon fiber inserted glass capillaries (A). Optical images of CFEs with disk (C_1) and cylinder (C_{II}) tips.



Figure 2. CV curves of a platinum-modified (Pt-CFE) and a bare CFE in 1 mM FMA + PBS.

2.3. Electrode surface Modification and Characterization

Bare CFEs were cleaned in acetone and milli-Q water prior to surface modification with Pt nanoparticles. Chronoamperometry was used for electrochemical modification. Basically, CFEs were placed in a solution containing 2 mM $H_2PtCl_6 + 0.1M$ HCl and the potential was held at 0 V (vs. Ag / AgCl) for about 70 s. point, CV curves for H_2O_2 at different concentration levels were obtained. The potential of the modified electrodes were swept between 0 V and +1.4 V (vs. Ag / AgCl) at a scan rate of 100 mV / s to obtain the respective CV curves. Chronoamperometry was used to determine the lowest concentration level that the Pt-CFE could measure. Basically, the Pt-CFE was placed in a PBS solution and during which the potential of the modified electrode was kept constant at +0.8 V (vs. Ag / AgCl). Once the current gained a steady state, concentrated solutions of H_2O_2 were added to realize first 1µM and then 10µM of H_2O_2 in PBS.

3. Results and Discussion

The leak-proof CFEs whose electrochemical behavior can be quantitatively analyzed, can be easily produced by the production method proposed and developed in this study. Briefly, carbon fibers were first connected to coated copper wires that are only exposed at the ends, because of several reasons such as carbon fibers need to be inserted into glass capillary tubes and thereafter connected to the electrochemical system. Then, carbon fibers fixed to copper wires were inserted into glass capillaries, which were then pulled using a micropuller to seal carbon fibers with a thin layer of glass. In the final step, the tip of the CFEs were ground to realize microdisk electrodes at the tip. The



Figure 3. Modification of Pt-CFE with platinum nanoparticles in 0.2 mM H_2 PtCl₆ + 0.1 M HCl at 0 V (vs. Ag/AgCl) (A) and comparison of the oxygen reduction potential of these electrodes with bare CFEs in PBS solution using CV (B).

Afterward, electrochemical behavior of Pt nanoparticle modified CFEs (Pt-CFEs) were analyzed in 1 mM FMA + PBS solution using CV, for which the potential of the working electrode was swept between 0 and +0.5 V (vs Ag / AgCl) at 50 mV / s.

2.4. Electrochemical Detection

CV was used for the electrochemical detection (reduction) of oxygen. Basically, bare and Pt-CFEs were placed in a PBS solution, respectively and the CV curves were obtained, at which time current was measured by sweeping the potential of the working electrode between +0.4 V and -0.6 V (vs. Ag / AgCl).

Next, hydrogen peroxide (H_2O_2) was electrochemically detected with Pt-CFEs and the detection limit of these probes was determined. At this optical microscopy image of a CFE was taken after grinding its tip (Figure 1B, C_I). As can be understood from the pictures taken, the probe tip was completely flattened and the disk electrode was successfully produced. It is also possible to produce cone-shaped CFEs of various sizes with another machine called "microforge" without using the micro-grinding machine (Figure 1C_{II}). Basically, flame etching is used to give carbon fiber at the tip of the CFE a cone-shape. In this study, only disk shaped electrodes were used for electrochemical detection. Following the production of CFEs, the electrochemical behavior of these probes was analyzed with a PBS solution containing electroactive FMA. PBS solution containing 1 mM FMA was prepared for the electrochemical analysis. CV of the CFEs were obtained in this solution using a potentiostat (Metrohm-Autolab PGSTAT204) (Figure 2).



Figure 4. CV was used for electrochemical hydrogen peroxide detection with Pt-CFE (0, 1, 10, 25, 50 and 100 μ M) (A). The smallest concentration level (limit of detection - LOD) that these electrodes can measure is determined by chronoamperometry (B). H₂O₂ calibration curve whose data were retrieved from respective CV curves (C).

At this point, the current was measured by sweeping the potential of the working electrode between 0 and +0.5 V (vs Ag / AgCl) at a scan rate of 50 mV / s. The maximum current observed in CV was around 1.5 nA, which is close to the theoretically calculated value. In the theoretical maximum current calculation, the formula "I = 4nFDCr" was used for the microdisk electrode (I: current, n: number of electrons (FMA = 1); F: Faraday constant (96485.329 s / mole); D: diffusion constant (D_{FMA} = 6.7x10-6 cm²s⁻¹) C: analyte concentration (FMA = 1 mM); r: electrode radius (1um). This finding has led to the conclusion that the produced CFEs can be used for quantitative electrochemical detection. The surfaces of the CFEs in the next stage were electrochemically modified with platinum nanoparticles. Chronoamperometry was used for electrochemical modification (Figure 3A). During the process, the potential of Pt-CFE was held at 0 V (vs. Ag / AgCl). As can be understood from Figure 3A, the reduction current increased in negative direction during the electrochemical modification process as expected. It was then determined whether the modification process was successful by comparing the performance of a modified Pt-CFE with that of a normal CFE in both oxidizing FMA and reducing oxygen. First, a Pt-CFE was immersed in a solution containing 1 mM electroactive FMA + PBS in which case the potential of the electrode was swept from 0 V to 0.5 V vs. Ag/AgCl at a scan rate of 50 mV / s to obtain a CV curve. When compared with that of bare CFE, the peak current of Pt-CFE was significantly high

in FMA solution (Figure 2). Higher current response indicates that the modification of CFEs with Pt nanoparticles increases the electrode surface area. The increase in the surface area of the electrode contributes positively to the sensitivity of such electrodes. Second, the performance of Pt-CFE in reducing of O₂ was determined and compared with that of bare CFE. Platinum has a high catalytic activity in the reduction of oxygen. CV was used for the electrochemical reduction of oxygen, during which the current was measured by sweeping the potential of the working electrode (carbon fiber and platinum modified carbon fiber electrodes) between +0.4 V and -0.6 V (vs. Ag / AgCl) at a scan rate of 50 mV / s. The reduction current obtained from platinum-modified electrodes was observed to be much higher than that of bare CFEs, indicating the successful modification of electrode surface with platinum nanoparticles (Figure 3B).

Next, the performance of Pt-CFEs in electrochemical detection of hydrogen peroxide (H₂O₂) was determined. At this point, the detection limit was also investigated. As can be understood from Figure 4A, the indicated H₂O₂ concentration levels (10, 25, 50, 100 μ M) were successfully modified measured with electrodes. the Chronoamperometry was used to determine the lowest concentration level that the Pt-CFE could measure, and during this process the potential of the modified electrode was kept constant at +0.8 V (vs. Ag / AgCl). As can be concluded from these results, H₂O₂ can be measured at 1 µM with Pt-CFEs (Figure 4B). Considering the possibility of high potential damage to the electrode, +0.8 V (vs. Ag / AgCl) was used instead of +1.2 V (vs. Ag / AgCl). In the

last step, a calibration curve was generated by using 3 different data for the respective concentration levels. According to the results, the Pt-CFEs showed a linear response between 0 and 100 μ M H₂O₂ concentration levels (Figure 4C).

5. Conclusions

In this study, low-cost and highly sensitive CFE based O_2 and H_2O_2 sensing probes were fabricated. To the best of my knowledge, this is the first study where probe type ultra-micro CFEs were modified with Pt nanoparticles for such an application. Briefly, CFE probes were produced by micropulling carbon fiber inserted glass capillaries. After grinding the tip of the probe to realize microdisk electrode at the tip, the surface was electrochemically modified with Pt nanoparticles. Results indicate that O2 and H2O2 were successfully detected using the probes. The introduced fabrication method is quite simple and cost efficient as carbon fibers are a lot cheaper than noble metals such as Pt or Au. In addition, the produced Pt-CFE probes have high potential for local analysis because of their small size and development of various microbiosensors with small surface modifications (e.g., glucose biosensor). Future work is planned for the use of Pt-CFEs in the development of a very sensitive ATP biosensor.

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6. References

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