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Research Article

Microelectrode Fabrication for Quantitative and Qualitative Analysis of Dopamine Level

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Abstract

In this study, low-cost, biocompatible intra-brain sensors were fabricated. Fabrication of the produced brain sensors consists of three stages. These are, respectively, the production of microelectrodes by photolithographic methods, packaging and coating with suitable chemical barriers. After the mask design, the production were carried out by applying the determined photolithographic steps to produce the microelectrode. In order to prevent the produced microelectrodes from being affected by environmental noise, only the recording zones and bonding zones were covered with an insulating layer. The production process was completed by slicing the microelectrodes produced collectively on substrate. Each microelectrode was packaged so that it can be connected to the test device (Voltammetry). In the packaging process, firstly the microelectrode was glued on the PCB and the connection areas of the microelectrode affixed on the PCB and the paths on the PCB are connected to each other with the help of gold wires using a wire-bonding device. Firstly, calibration tests of the produced sensors were performed, and then the microelectrodes that were successful in the calibration test were subjected to in vitro testing. In the calibration test, microelectrodes with R^2 value close to the desired level 1 and LOD (Limit of Detection) values between 0.3-0.5 were deemed successful. It was observed that the microelectrodes that passed the calibration test did not respond to AA (Ascorbic Acid) in the in vitro test, but responded to dopamine step by step. It was seen that the microelectrodes produced as a result of the measurements are suitable for intra-brain neurotransmitter measurement.

Keywords: Microelectrode, biosensor, neurotransmitter measurement

Dopamin Seviyesinin Kantitatif ve Kalitatif Analizi için Mikroelektrot Üretimi

Öz

Bu çalışmada, düşük maliyetli, biyouyumlu beyin içi sensörler üretildi. Üretilen beyin sensörlerinin imalatı üç aşamadan oluşmaktadır. Bunlar sırasıyla fotolitografik yöntemlerle mikroelektrotların üretimi, paketleme ve uygun kimyasal bariyerlerle kaplanmasıdır. Maske tasarımından sonra mikroelektrotu üretmek için belirlenen fotolitografik adımlar uygulanarak üretim gerçekleştirildi. Üretilen mikroelektrotların çevresel gürültüden etkilenmemesi için sadece kayıt bölgeleri ve bağlama bölgeleri bir yalıtım tabakası ile kaplandı. Üretim süreci toplu olarak üretilen mikroelektrotların alttaş üzerinde dilimlenmesiyle tamamlandı. Her mikroelektrot, test cihazına (Voltametri) bağlanabilmesi için paketlendi. Paketleme işleminde öncelikle mikroelektrot PCB üzerine yapıştırılır ve PCB üzerine yapıştırılan mikroelektrotun bağlantı alanları ile PCB üzerindeki yollar altın teller yardımı ile bir tel bağlama cihazı kullanılarak birbirine bağlandı. Üretilen sensörlerin ilk kalibrasyon testleri gerçekleştirildi, ardından kalibrasyon testinde başarılı olan elektrotlar in vitro testlere tabi tutuldu. Kalibrasyon testinde R^2 değeri istenilen seviye 1'e yakın ve LOD (Algılama Limiti) değerleri 0.3-0.5 arasında olan mikroelektrotlar başarılı olarak kabul edildi. Kalibrasyon testini geçen mikroelektrotların in vitro testte AA'ya

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(Askorbik Asit) yanıt vermediği, ancak dopamine adım adım yanıt verdiği görüldü. Yapılan ölçümler sonucunda üretilen mikroelektrotların beyin içi nörotransmiter ölçümü için uygun olduğu görüldü.

Anahtar Kelimeler: Mikroelektrot, biyosensör, nörotransmiter ölçümü

1. Introduction

The cause of the majority of brain diseases is associated with neurotransmitters working in synaptic spaces. Dopamine in Parkinson's disease, Huntington's glutamate, Epilepsy's GABA, Major Depression serotonin and norepinephrine, Schizophrenia, dopamine and serotonin, Alzheimer's disease acetylcholine and glutamate, Hepatic encephalopathy, Amyotrophic neuropathy and Chronic neuronal sclerosis, changes in the levels of the synchronic neuropathy and Chronic neuropathy.

It has been possible to measure the neurotransmitter activities in the Central Nervous System (CNS) with micro electrodes. Ceramic-based microelectrodes have been stated to be more suitable for in vivo experiments than silicon-based electrodes. Electrochemical measurements of dopamine and hydrogen peroxide were made using microelectrodes. It has been shown that the sensitivity, selectivity and response time characteristics of ceramic-based microelectrodes are better than silicon-based electrodes (Burmeister et al., 2000; Burmeister and Gerhardt, 2001). Microelectrodes have been used in different biological applications by applying selective enzyme coating suitable for the analyte desired to be detected in freely moving mice and human brain tissues (Burmeister et al., 2002; Burmeister et al., 2003). Microelectrodes continued to be developed using different manufacturing techniques. (Musallam et al. 2009; Frey et al., 2010) Produced electrodes using dry etching method suitable for neural recording applications. In another study conducted in 2009, a new fabrication technology for silicon-based nerve probes was presented. In addition to 2-dimensional microprobes, 3-dimensional microprobes were designed for neural recording (Herwik et al., 2009). In another study conducted in 2013, nickel-based multiple microelectrodes were used with low-priced nickel electroplating method. It has been found to be mechanically harder than silicone based. (Marton et al., 2013), microelectrodes produced by deep reactive ion etching method provided good mechanical stability in in vivo measurements on mice, and the opportunity to be placed in the desired area of the brain region precisely (Marton et al., 2013). High quality signal has been obtained in different regions of the rodent brain. Highly reliable devices have been developed in the microelectronics industry with complex fabrication methods (Voskerician et al., 2003). In parallel with this, especially the use of polymers and polymer-based micro fabrication technologies have also improved (Abgrall et al., 2007). In recent years, a lot of progress has been made in flexible-based microelectrodes and different clinical applications have been performed comfortably (Shi et al., 2020); Xiang, et al., 2016). SU-8, which is a negative photoresist, has been used frequently in biomedical applications due to its biocompatibility. This epoxy-based material has very good properties such as high mechanical strength, good adhesion with most substrate materials and high chemical stability. Due to these properties, SU-8 is used as an insulation material in fabrication due to its low fracture rate and negative resistance, especially in subcutaneous applications. In addition, SU-8 has been approved as a biocompatible material by the American Drug and Food Administration (FDA) (Chaudhri et al., 2010; Nemani et al., 2013).

In this study, low-cost and biocompatible brain sensor fabricated and calibration tests of the sensor were performed. The sensors that passed calibration test were coated with enzyme for

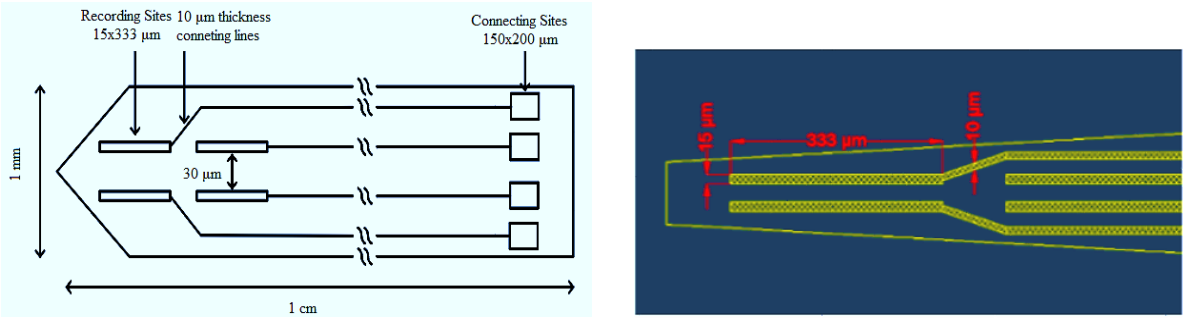
selectivity. After all, in-vitro tests have been done to examine the response of the sensors to dopamine.

2. Material and Methods

Fabrication of Microelectrodes

Outer dimensions of microelectrodes designed in this study was chosen as 1 mm x 1 cm. Also with the aim of providing convenience in the *in vivo* experiments, it was paid attention to choose outer sheath end as triangular shape (pointed) (**Fig. 1a**). The most important part that determines perception of microelectrodes is recording regions (**Fig. 1b**). The factor to be paid attention at the design of recording regions is geometric shape and surface area of the region. In this study, the screen shot of microelectrodes that have 4 record regions in

L-Edit program is seen at **Fig. 1a.** As it is seen from the figure, dimensions of recording regions are chosen as 15x333 µm.



a) b)

Figure 1. a) Microelectrode schematic picture b) Screen shot of recommended microelectrodes in L-Edit program

Enzyme-based multiple channel microelectrodes are fabricated with photolithographic method. This method allows for the fabrication of 5-10 µm recording surfaces. Besides, many microelectrodes may be fabricated on a single substrate during the same fabrication. Therefore, advantages will have been provided in terms of time and cost**.** In this study, alumina ceramic at the thickness of 125 µm was used as substrate. The fabrication steps are in **Fig. 2.** Cleaned substrate surface is coated with photoresist at the thickness of 200 nm with the help of spinner device. As photoresist AZ5214 positive photoresist was selected. After photoresist coating procedure, pre-heating is done at 110 °C for 55 sec. Under the UV light that was aligned to substrate coated with photoresist, microelectrode patterns at the mask drawn on the glass are transferred. In the meanwhile, as negative mask is used, the photoresist regions where record regions, connection paths and connection points are available will have been exposed to light. Then lift up of the regions that have been exposed to UV light will be provided with the help of developer. After that stage, metal coating procedure is applied. Before metal coating procedure, titanium (Ti) layer at the thickness of 800Å coated on the substrate with the aim of increasing adhesion of the Pt to the ceramic surface. 1500Å Pt layered substrate is put inside the acetone and photoresist at other regions except for recording sites, connection paths and connection points and undesirable metals available on these regions are lifted up.

As the final stage, the microelectrode surface except for recording sites and connection regions are coated with insulation layer with SU-8 using a second mask for forming insulation layer (**Fig. 3**).

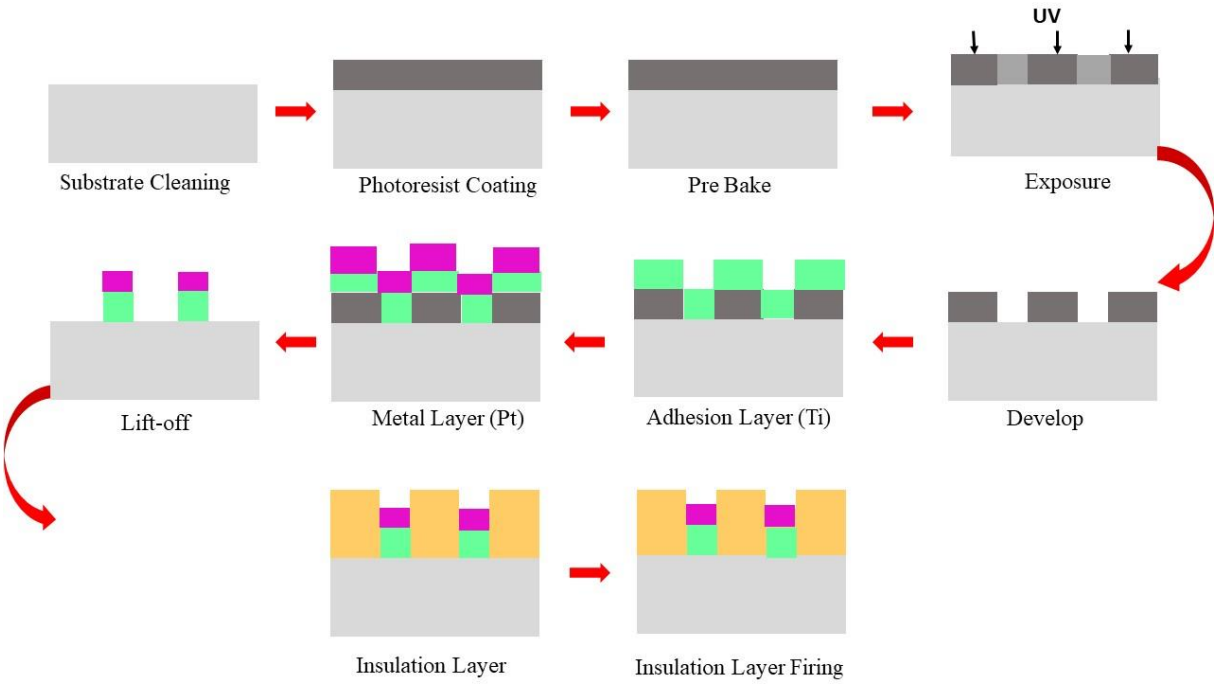
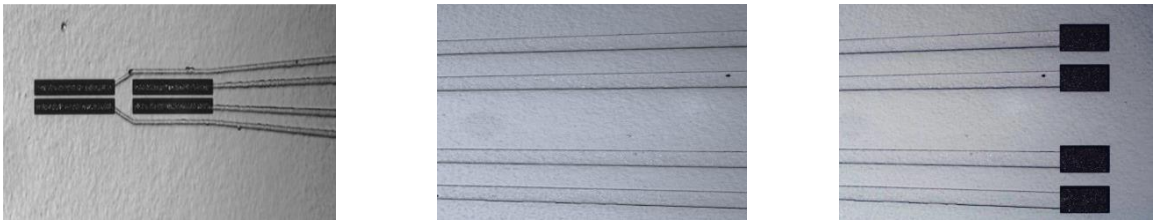


Figure 2. Schematic diagram for the fabrication steps of microelectrode

After fabrication of microelectrodes has been completed, it is required to pack them with the aim of using these in the in vitro and in vivo experiments. At the packing stage, microelectrodes were diced as not to damage the brain tissues and then they were combined by wire bonding with PCB. With the aim of strengthening the connection point and

isolating wires of the substrate, epoxy was used (Acar, 2014). The new electrodes made ready for the test after completing the fabrication and packing are seen at **Fig. 4**.



a) b) c)

Figure 3. Microscope image of new microelectrodes : a) Recording sites of microelectrodes b) Paths c) Connection regions

Calibration of Microelectodes

A typical dopamine release and uptake curve obtained in our studies is schematically given below. Some critical values are plotted on this curve (**Fig. 5**). The calibration tests of fabricated microelectrodes are done with the help of FAST-16 device (Fast16 System, 2015). During the calibration test, two important parameters are measured. These parameters are detection limit (LOD) and linearity (R^2). LOD value among these parameters is the indicator of that sensor may make perception for which concentration values. Therefore, low LOD values are a reason for preference at the selection of microelectrodes. Linearity parameter R^2 indicates proportion of the current amount that passes over the sensor to the hydrogen peroxide amount available at the environment. R^2 value used at the detection of relative effectiveness of this adjusted curve changes between 0 and 1 and the desired situation is being close to 1. The calibration test is done as follows. Firstly, phosphate tampon of which PH value is adjusted to seven is prepared and end part of the recommended microelectrode is sunk in this solution. Then start information are entered to FAST-16 record device and calibration procedure is started by applying +0.7 Volt to microelectrode record region. After calibration procedure is started, it is waited for that current will reach a constant subgrade level (approximately 5-10 minutes). After current value has decreased to subgrade level, 8.8 µmol hydrogen peroxide (H_2O_2) is added to the solution at certain intervals and response to microelectrodes to hydrogen peroxide is examined. As a result of the test, LOD and R^2 values are automatically calculated by FAST-16 device (**Table 1**).

Table-1 LOD and R^2 results of sensor insulated with SU-8 on ceramic-based microelectrode

	Channel-1	**Channel-2**	**Channel-3**	**Channel-4**
LOD	0.3838	0.5782	0.3961	0.4092
R^2	0.9985	0.9957	0.9981	0.9972

Figure 4. Image of new microelectrodes that were made ready for test after completing the fabrication.

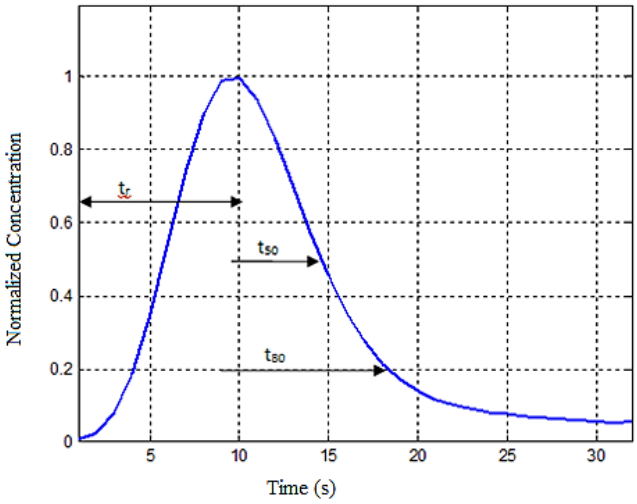


Figure 5. Typical dopamine curve. The times t_r, t_{50} and t_{80} are shown on the graph.

Chemical Coating of Electrodes

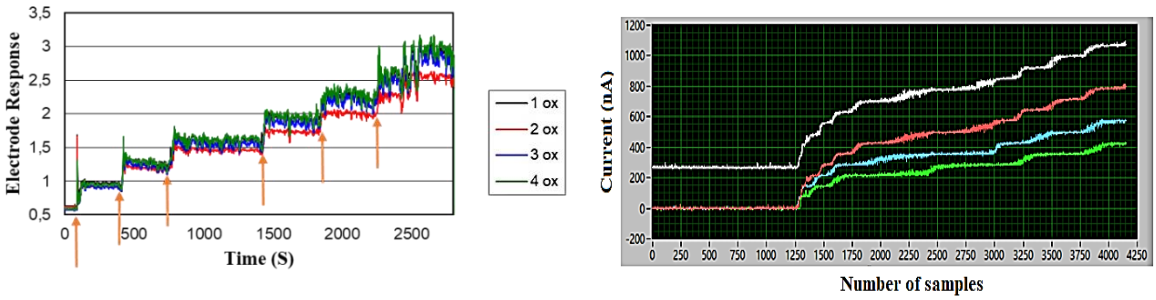
Barrier coating is applied after successful cleaning. It is applied to block and minimize unwanted active substances such as AA (ascorbic acid) or DOPAC found in high levels in the MSS. With this blocking, the microelectrode becomes more selective for the analyte of interest. The planar geometry of multi-channel microelectrodes often determines how recording regions come into contact with the material. The barrier and enzyme coating can inhibit diffusion of the compounds to the microelectrode surface by creating a diffusion barrier. This inhibition may delay the response time of the microelectrode. Therefore, electrode coating parameters need to be done in the optimal procedure for enzyme and barrier coating. Nafion is an anionic derivative of teflon and is widely used to increase the selectivity of voltammetric recordings in CNS tissues. Nafion (5%) is placed in 300 microliter centrifuge tubes. The tip of the microelectrode is immersed in the nafion and one turn is 1 s. The microelectrode is removed from the nafion electrode. It is put into the oven at 165–175 ºC for 4 minutes. Nafion dries at the tip of the electrode. After the nafion has hardened, it is allowed to dry at room temperature for 30 minutes before enzyme coating.

3. Findings

At this part, the test results belonging to the new microelectrodes that were insulated by using SU-8 among fabricated microelectrodes. At Fig. 6a, there are current time calibration graphics (response of microelectrodes to hydrogen peroxide) of two microelectrodes insulated with SU-8 The red arrows at the figure indicate the hydrogen peroxide times that were dripped to the solution. When these figures are examined, it is seen that every record regions of the microelectrodes react as in the shape of steps as expected when hydrogen peroxide is added to the solution. The linearity belonging to these microelectrodes (R^2) and LOD values are seen at Table 1. It is seen from Table 1 that R^2 value at every record region is close to 1 value (0.999) which is the desired level and LOD values are within the desired level (0.1-0.5). R^2 being close to each other shows that step response of microelectrodes to added peroxide is good. LOD values being at the within range shows that effect of the noise on the current was decreased at the improved microelectrodes and more constant results were obtained.

4. Results and Discusssion

In this study, new microelectrodes to use in the diagnosis or treatment of brain diseases were fabricated. These electrodes that were fabricated are ceramic-based and have four record regions. The test procedure of which fabrication and packing procedures were completed was done with the help of FAST-16 device. Current time graphics of the microelectrodes (response of microelectrodes against hydrogen peroxide were obtained in the test procedure and it was observed that these were at the shape of stages (**Fig. 6a**). Also LOD and R^2 values of microelectrodes deemed as quality parameters were examined and it was observed that these values were at desired ranges. Enzyme coating was applied to microelectrodes that successfully passed the calibration test and were subjected to in vitro test. Microelectrodes did not respond to AA (Ascorbic Acid), but responded stepwise to dopamine (**Fig. 6b**). With the aim of using the microelectrodes fabricated later on this study at the measurement of neurotransmitters such as dopamine which is the active substance of brain diseases, it is required that record regions of microelectrodes will be covered with the enzymes that will provide selectivity for neurotransmitters.



a) b)

Figure 6. a) Calibration test response of the sensor insulated with SU-8 on ceramic-based microelectrode b) Sensor response after Z1PaGR enzyme coating for dopamine.

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