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Research Article

Dopamine Detection from PC12 Cells with a Carbon-fiber Microelectrode Controlled by a Homemade System

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Abstract

Dopamine (DA) is a neurotransmitter that is naturally produced in the body. It provides nerve conduction in the central nervous system and plays an important role in the regulation and control of movements, motivation and cognitive development. Imbalance in the amount of DA can cause many diseases ranging from Parkinson's disease to depression. In this study, a homemade system that can locally detect DA released from a PC12 cell cluster after chemical stimulation with K^+ ions was developed. First, the production of a needle-tipped carbon-fiber microdisk electrode (CF-MDE) was carried out by micropulling method. CF-MDEs are frequently used for effective and high sensitivity detection of DA. Afterwards, a cell cluster of PC12 cells was obtained and the change in the amount of DA release by the PC12 cells in the cluster was electrochemically measurement. For the measurement process, the homemade system comprised of a microcontroller unit integrated to an inverted microscope and a potentiostat was used to precisely position the needle-tipped electrode and carry out the electrochemical measurement. Results clearly showed that DA release following chemical stimulation with K^+ ions was successfully measured. The homemade system is thought to have the potential to be used in the evaluation of dopaminergic cells and the effects of dopaminergic drugs.

Keywords: Carbon-fiber Microelectrode, Dopamine, PC12 cells, Electrochemical Detection, Local Analysis.

Ev Yapımı Bir Sistem Tarafından Kontrol Edilen Karbon Fiber Mikroelektrot ile PC12 Hücrelerinden Dopamin Tespiti

Öz

Dopamin (DA), vücutta doğal olarak üretilen bir nörotransmiterdir. DA, merkezi sinir sisteminde sinir iletimini sağlar ve hareketlerin düzenlenmesi ve kontrolünde, motivasyonda ve bilişsel gelişimde önemli bir rol oynar. DA miktarındaki dengesizlik, Parkinson hastalığından depresyona kadar birçok hastalığa neden olabilir. Bu çalışmada, bir PC12 hücre kümesinden K^+ iyonları ile kimyasal uyarımı sonrasında DA'yı lokal olarak tespit edebilen ev yapımı bir sistem geliştirilmiştir. İlk olarak mikro çekme yöntemi kullanılarak iğne uçlu karbon fiber mikrodiskelektrotların (KF-MDE) üretimi gerçekleştirilmiştir. KF-MDE'ler, DA'nın etkili ve yüksek hassasiyetli tespiti için sıklıkla kullanılmaktadır. Daha sonra PC12 hücrelerinden oluşan bir hücre kümesi elde edilmiş ve kümedeki PC12 hücrelerinin DA salınım miktarındaki değişim elektrokimyasal olarak ölçülmüştür. Ölçüm işlemi için, ters mikroskoba entegre edilmiş bir mikrokontrol ünitesi ve bir potansiyostattan oluşan ev yapımı sistem, iğne uçlu elektrodu hassas bir şekilde konumlandırmak ve elektrokimyasal ölçümü gerçekleştirmek için kullanılmıştır. Sonuçlar, K^+ iyonlarıyla kimyasal uyarım sonrasında DA salınımının başarıyla ölçüldüğünü açıkça göstermektedir. Ev yapımı sistem, dopaminerjik hücrelerin ve dopaminerjik ilaçların etkilerinin değerlendirilmesinde kullanılma potansiyeline sahip olduğu düşünülmektedir.

Anahtar Kelimeler: Karbon fiber Mikroelektrot, Dopamin, PC12 hücreleri, Elektrokimyasal Tespit, Lokal Analiz.

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1. Introduction

Dopamine (DA), also known as 3,4-dihydroxy phenethylamine, is a catecholamine neurotransmitter naturally produced in the body (Güzel vd., 2019). It provides neurotransmission in the central nervous system and plays an important role in regulation and control of movements, motivation and cognitive development (Latif vd., 2021). The normal level of DA in human blood is in the range of 0.01 to 1 μM (Jiang vd., 2015). Abnormal levels of DA have been associated with various conditions. While high levels of DA results in cardiotoxicity causing rapid heartbeat, hypertension and heart failure (Feng vd., 2018), low levels of DA levels can cause Parkinson's disease, Alzheimer's disease, stress and depression (Begieneman vd., 2016; Pan vd., 2019). Detecting quick changes in DA level could provide means for early diagnosis and control of related diseases (Balestrino vd., 2020). Hence, it is of paramount importance to develop systems for the measurement of DA in order to understand its physiological functions and evaluate dopaminergic cells and drugs (Whitton vd., 2020). Considering some of the advantages such as high sensitivity, fast response, real-time analysis, good stability, reproducibility, simplicity of application and low cost, electrochemical methods are frequently preferred in measurement of catecholamines including DA. The applications of various electroanalytical methods including cyclic voltammetry (CV), chronoamperometry, differential pulse voltammetry, fast scan CV for high detection of DA detection (Ding vd., 2018; Şen vd., 2022, 2023; Seven, Gölcez, & Şen, 2020; Yang vd., 2021) have been successfully demonstrated. Among these methods, chronoamperometry provides high sensitivity due to reduced non-Faradaic current and real-time analysis. Measuring the current over time allows evaluation of the rate of compound arrival at the electrode, diffusion processes and the rate of electrochemical reaction (Habekost, 2019).

Dopaminergic drugs affect the dopaminergic system by altering DA levels or by acting on DA receptors (Miller vd., 1988). Dopaminergic drugs are used to increase DA levels in the brain, and are often used in the treatment of many diseases caused by low levels of DA. These drugs are often used to control the symptoms of Parkinson's disease and compensate by stimulating DA receptors (Olanow vd., 2006). Dopaminergic drugs are used in different combinations and doses according to the patient's condition. However, dopaminergic drugs can cause some side effects and their dosage should be carefully adjusted (Cummings, 1991). An *in vitro* model system can contribute to the trial and development of dopaminergic drugs in different aspects, such as cost and convenience. The system can be used to monitor DA release and to monitor the effect of dopaminergic drugs under controlled conditions *in vitro*. In many studies, rat pheochromocytoma cells, known as PC12, have been used as a mature dopaminergic neuron (source of DA) due to their nerve cell-like structure and their ability to release neurotransmitters, particularly DA and norepinephrine (Pozzan vd., 1984). PC12 cells are preferred mainly because of their extreme versatility, ease of culture, and large amount of background information about proliferation and differentiation. PC12 cells can synthesize and store DA. Therefore, PC12 cells are an appropriate model system for use in *in vitro* catecholaminergic neurotoxicity studies (Giordano vd., 2011). Increased extracellular K^+ level causes depolarization of PC12 cells and induces exocytosis (Qin vd., 2020). Depolarization occurs in the plasma membrane and triggers Ca^{2+} channels and Ca^{2+} ions to enter the cell. The increase in Ca^{2+} ions in the cell causes neurotransmitter release (Shinohara vd., 2013).

Here, a homemade system with a microcontroller unit was set up to monitor the release of DA from an adherent PC12 cell cluster using a closely positioned carbon-fiber microdisk electrode (CF-MDE). The physical and chemical properties of CF-MDE are favorable for DA detection (Swamy vd., 2007). The electrode has high-speed electron transfer kinetics, allowing real-time monitoring of neurotransmitter release (Tian vd., 2023) and good biocompatibility for use in biological media (Zhou vd., 2019). Also considering their size, CF-MDE might not cause significant harm to living cells during interaction. First, a CF-MDE was fabricated using a micropuller and then successfully applied for the real-time monitoring of DA release from PC12 cells following stimulation with K^+ ions. The integrated system has great potential to be used in the evaluation of dopaminergic cells and the effects of dopaminergic drugs.

2. Material and Method

2.1. Materials

DA (Sigma-Aldrich, USA), ferrocenemethanol ($FcCH_2OH$) (Sigma-Aldrich, USA), collagen (type IV, 0.5–2 mg/mL) (Sigma-Aldrich, USA), fetal bovine serum (FBS) (Sigma-Aldrich, USA), penicilin/streptomisin (Sigma-Aldrich, USA), RPMI 1640 (Sigma-Aldrich, USA), potassium chlorate ($KClO_3$) (Sigma-Aldrich, USA), Borosilicate Glass (World Presicion Instruments Inc., USA), Conductive Silver Paste (Sigma-Aldrich, USA), 2K Fast Hardener and Varnish (Akripol, Turkey).

2.2. Fabrication of CF-MDE

First, an individual carbon-fiber was cut to an appropriate length and connected to a copper wire using a conductive Ag paste. The Ag paste was solidified on a hot plate at 120°C for 10-15 minutes to strongly link the carbon-fiber to the wire. Subsequently, the individual carbon-fiber was placed in a glass capillary tubes with the help of the copper wires. The copper wire was rounded around the tip of the glass capillary tube and fixed with a piece of macaron to ensure the electrical connection and fixation of the carbon-fibers. The glass tube with the carbon-fiber inside was pulled using a micro-puller (PC-10, Narishige Inc. Japan) to seal the carbon-fiber with glass (Aydin vd., 2017; Aykaç vd., 2021; Şen, 2019). Next, the protruding carbon-fiber at the electrode tip was removed using a microforge (MF-800, Narishige Inc. Japan) and the electrode was made water tight by dipping it into a varnish. The varnish was left to dry and hence solidify at room temperature for 4 h. Lastly, the tip of the electrode was ground with a micro grinder (EG-400, Narishige Inc. Japan) to obtain a microdisk electrode.

2.3. Characterization of CF-MDE

First, the CF-MDE was visualized under an inverted microscope to ensure that the electrode is in the shape of a disk. Electrochemical behavior of the electrode was checked using CV, where the potential applied to the electrode was swept between 0 and +0.5 V (vs. Ag/AgCl) in 1 mM $FcCH_2OH$ containing PBS solution at a scan rate of 50 mV/s. A CV curve was also obtained in DA at 200 µM by sweeping the potential between 0 and +0.4 V (vs. Ag/AgCl). Chronoamperometry was used to detect DA at varying concentrations to determine limit of detection (LOD). Briefly, a constant potential of +0.4 V (vs. Ag/AgCl) was applied for 100 seconds in DA solutions at different concentrations (5, 10, 25, 50, 100, and 200 µM) and the final current was used to obtain a calibration curve.

2.4. Cell culture

PC12 cells were purchased from German Collection of Microorganisms and Cell Cultures GmbH (DSMZ). Considering the fact that the cells were inherently semi-suspended, the surface of the culture flasks was coated with collagen type IV. The cells were cultured in RPMI 1640 (10% DHS + 1% FBS + 1% L-Glutamine + 0.1% Gentamycin) (Seven, Gölcez, Yaralı, vd., 2020). To obtain cell clusters, cells were seeded into a 60 mm petri dish coated with collagen and left to proliferate a few days in an incubator (% 5 CO_2) at 37 °C.

2.5. Real-time measurement of DA release from PC12 cells

The measurement of DA released from a PC12 cell cluster was performed in PBS via the homemade system. The system was composed of an inverted microscope with a camera, a three axis (x-, y- and z-) microcontroller unit and a potentiostat. The CF-MDE was positioned 20 µm above the cell cluster and the release of DA was detected in real time following stimulation with K^+ ions. The whole process was monitored with the camera of the microscope. The data was recorded using the potentiostat (Autolab PGSTAT204, Metrohm, Switzerland). Briefly, +0.4 V (vs. Ag/AgCl) was applied to CF-MDE and once the current reached a steady state, 0.1 mM K^+ ions were added to stimulate DA release by exocytosis.

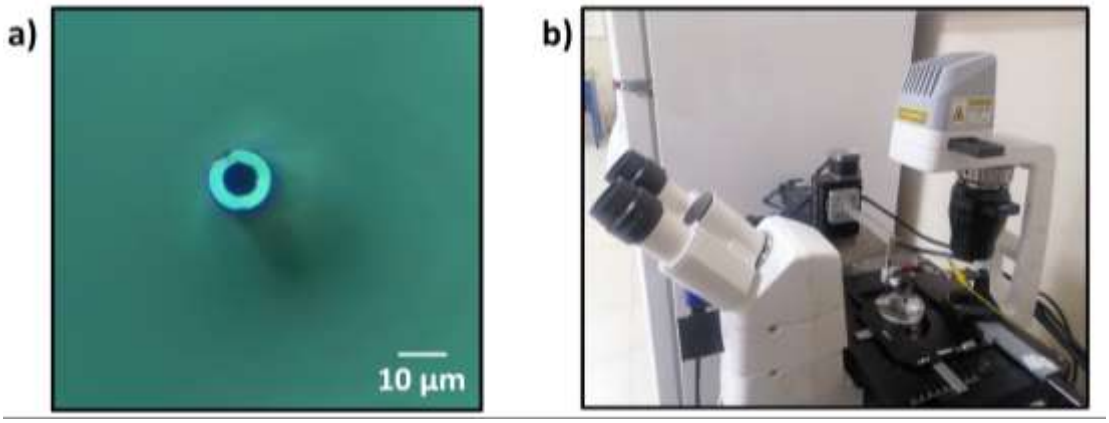

Figure 1. Optical images of the tip of a CF-MDE (a) and the homemade system that can control the position of the microelectrode in x-, y- and z-axis.

3. Results and Discussion

The adopted strategy allowed successful fabrication of CF-MDE as can be seen in Figure 1a. The electrode had a diameter of~8 µm. Considering its small size, the microelectrode potentially gives no or minimal damage to cells or tissues, when used in *in vitro* or *in vivo* studies. The CV curve obtained in $FcCH_2OH$ confirmed that the electrode displayed a reasonable microelectrode behavior (Figure 2a). A CV curve was also obtained in DA in which case the electrode displayed a peak-to-peak potential separation of 68.6 mV, proving that the electrode displayed a high electron transfer efficiency and can be used for quantitative detection of DA (Figure 2b). Basically, DA is an electroactive molecule and converted to o-dopaminequinone electrochemically by giving two electrons to an electrode in aqueous solution (Alipour vd., 2013). Next, chronoamperometry measurements were taken in PBS containing DA at varying concentrations (0, 5, 10, 25, 50, 100 and 200 µM). As can be seen in Figure 2c, the level of DA oxidation current increased linearly with increasing DA level. The current values of each concentration level at 60 sec was used to obtain a calibration curve, which basically demonstrates the relationship between DA and the electric current signal. Figure 2d clearly shows that the relationship was linear in the range of 5 to 200 µM of DA with an R^2 value of 0.996. The LOD of the CF-MDE was calculated as 0.88 µM using the formula of LOD = 3.3 × σ/slope. The low LOD value proves that the microelectrode is suitable for monitoring DA release from cells or tissues. The electrode displayed an excellent sensitivity of 42,116.2 $\mu A \cdot mM^{-1} \cdot cm^{-2}$.

Next, the homemade system integrating a microcontroller unit to an inverted microscope was used for real-time measurement of DA release from PC12 cells after chemical stimulation with K^+ ions. The microcontroller system can be automatically controlled with a precision close to 1 µm in the x-, y- and z-axis via a user friendly interface. The length and speed of the microcontroller system steps have been optimized for cell analysis. The motion of the electrode can be monitored continuously through the camera of the inverted microscope. Considering the low level of current that needs to be detected, the noise of the system was drastically reduced by grounding both the microcontroller unit and the microscope components.

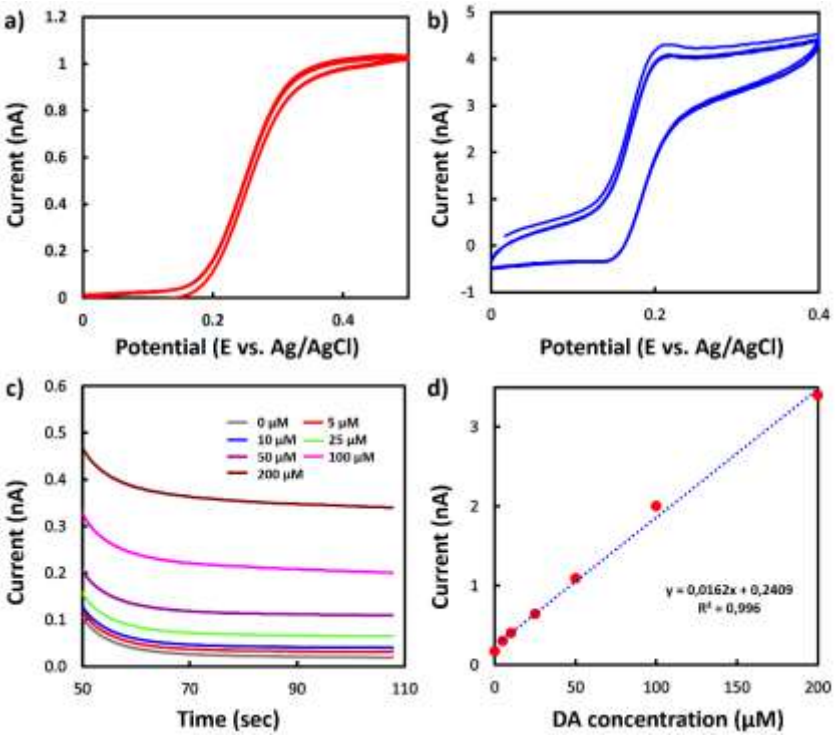

Figure 2. CV curves of a CF-MDE obtained in 1 mM $FcCH_2OH$ (a) and 200 µM DA (b). Chronoamperometry curves obtained in PBS containing varying concentrations of DA (c) along with a calibration curve (d).

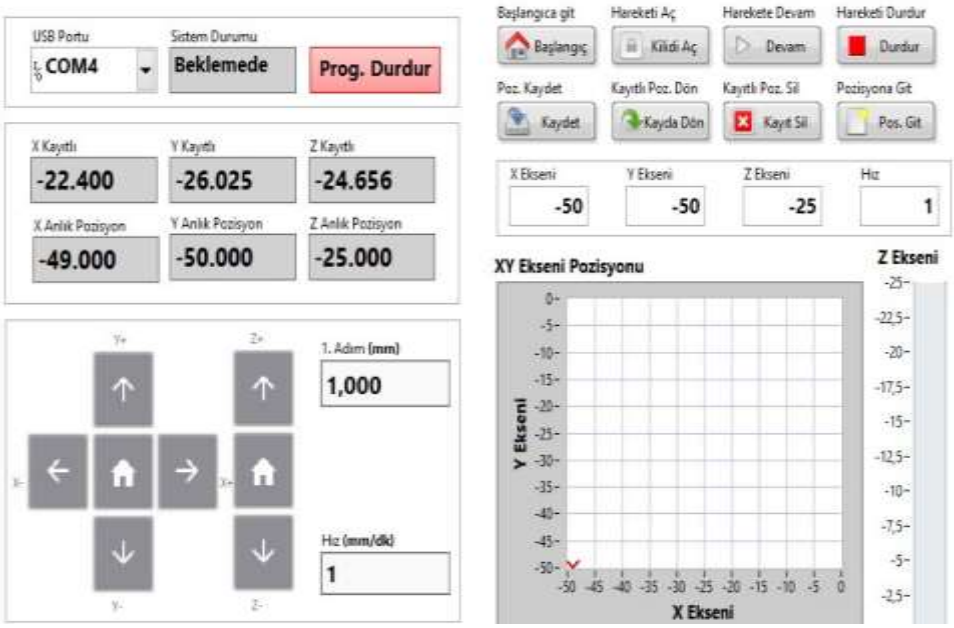

Figure 3. An image of the homemade system's user interface for controlling the position of the CF-MDE in x-, y- and z-axis.

It is known that extracellular stimulation by K^+ ions induces membrane depolarization, which in turn causes Ca^{2+} channels to open. The influx of intracellular Ca^{2+} through these channels stimulates PC12 cells to rapidly release DA stored in vesicles. Here, the homemade system was tested for real time measurement of DA release from a cell cluster following stimulation with a high level of K^+ ions. First, PC12 cells were first seeded into a collagen-coated petri dish and cultured a few days in an incubator to obtain a cell cluster. The size of the cluster was daily checked to make sure it reaches to a substantial size (around 100 µm). On day 3, the cells were used for testing. Prior to the measurement, the cell medium was replaced with warm PBS minimize any damage to the cluster. The CF-MDE was slowly approached to a spot where no cell was present at 1 µm/sec with the help of the microcontroller unit of the system where the current at +0.4 V (vs. Ag/AgCl) was monitored. As the probe approached to the empty surface, the current slowly decreased and reached a plateau as the diffusion of molecules was hindered by the empty surface. After reaching the plateau, the monition of the microcontroller unit was stopped and the CF-MDE was retracted 20 µm. Next, the electrode was moved to a position right above the cells. Prior to stimulating DA release from PC12 cell clusters, the electrical current was first allowed to attain a steady state at +0.4 V (vs. Ag/AgCl) and then K^+ ions were injected into the petri dish four times in a row with a micropipette. As can be clearly seen in Figure 4, the DA oxidation current increased every time K^+ ions were introduced. As mentioned above, K^+ ions induced membrane

depolarization and eventually caused DA release from the cells. Stimulation with high level of K^+ ions did not cause any observable damage to the cells according to the optical images and the release DA was successfully and reasonably detected in real time by the electrode. It is believed that all cells collectively contributed to the current response.

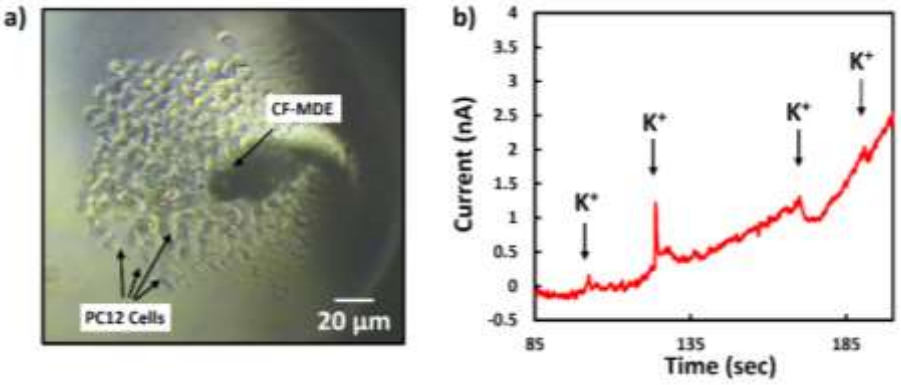

Figure 4. An optical image of a CF-MDE 20 µm above a PC12 cell cluster (a). A chronoamperometry curve showing the real-time change in DA release as a result of chemical stimulation of the cell cluster with K^+ ions (b).

4. Conclusions and Recommendations

DA functions as a neurotransmitter in the central nervous system and plays a key role in cell communication. Some diseases of the nervous system are related to an imbalance in DA levels. Dopaminergic drugs that increase DA level are frequently used to treat such diseases. An *in vitro* model system has the potential to contribute to the trial and development of such drugs in terms of cost and convenience. Here, a homemade model system was developed for real time measurement of DA release from a PC12 cell cluster that is exposed to a chemical stimulus. The system helped precisely control the motion of the CF-MDE. Results confirmed that the electrode exhibited a high sensitivity in the measurement of DA and was successfully applied to local and real time measurement of the neurotransmitter released from a cell cluster following stimulation by high level of K^+ ions. The homemade system has great potential to be applied to dopaminergic cell studies and screening of dopaminergic drugs.

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References

Alipour, E., Majidi, M. R., Saadatirad, A., mahdi Golabi, S., & Alizadeh, A. M. (2013). Simultaneous determination of dopamine and uric acid in biological samples on the pretreated pencil graphite electrode. *Electrochimica Acta*, *91*, 36–42.

Aydin, V. K., & Şen, M. (2017). A facile method for fabricating carbon fiber-based gold ultramicroelectrodes with different shapes using flame etching and electrochemical deposition. *Journal of Electroanalytical Chemistry*, *799*, 525–530.

Aykaç, A., Tunç, I. D., Güneş, F., Erol, M., & Şen, M. (2021). Sensitive pH measurement using EGFET pH-microsensor based on ZnO nanowire functionalized carbon-fibers. *Nanotechnology*, *32*(36), 365501.

Balestrino, R., & Schapira, A. H. V. (2020). Parkinson disease. *European journal of neurology*, *27*(1), 27–42.

Begieneman, M. P. V, Ter Horst, E. N., Rijvers, L., Meinster, E., Leen, R., Pankras, J. E., Fritz, J., Kubat, B., Musters, R. J. P., & van Kuilenburg, A. B. P. (2016). Dopamine induces lipid accumulation, NADPH oxidase-related oxidative stress, and a proinflammatory status of the plasma membrane in H9c2 cells. *American Journal of Physiology-Heart and Circulatory Physiology*, *311*(5), H1097--H1107.

Cummings, J. L. (1991). Behavioral complications of drug treatment of Parkinson's disease. *Journal of the American Geriatrics Society*, *39*(7), 708–716.

Ding, A., Wang, B., Zheng, J., Weng, B., & Li, C. (2018). Sensitive dopamine sensor based on three dimensional and macroporous carbon aerogel microelectrode. *Int. J. Electrochem. Sci*, *13*, 4379–4389.

Feng, P., Chen, Y., Zhang, L., Qian, C.-G., Xiao, X., Han, X., & Shen, Q.-D. (2018). Near-infrared fluorescent Nanoprobes for revealing the role of dopamine in drug addiction. *ACS applied materials & interfaces*, *10*(5), 4359–4368.

Giordano, G., & Costa, L. G. (2011). Primary neurons in culture and neuronal cell lines for in vitro neurotoxicological studies. *In Vitro Neurotoxicology: Methods and Protocols*, 13–27.

Güzel, Ö. Ü. D., Tanyeli, Ö. Ü. A., Kalfa, Z., & Yalım, H. N. (2019). Dopamin ve Fizyolojik Özellikleri. Içinde Academic Researches in Health Sciences.

Jiang, G., Jiang, T., Zhou, H., Yao, J., & Kong, X. (2015). Preparation of N-doped carbon quantum dots for highly sensitive detection of dopamine by an electrochemical method. *RSC Advances*, *5*(12), 9064–9068.

Latif, S., Jahangeer, M., Razia, D. M., Ashiq, M., Ghaffar, A., Akram, M., El Allam, A., Bouyahya, A., Garipova, L., & Shariati, M. A. (2021). Dopamine in Parkinson's disease. *Clinica chimica acta*, *522*, 114–126.

Miller, D. D., Harroid, M., Wallace, R. A., Wallace, L. J., & Uretsky, N. J. (1988). Dopaminergic drugs in the cationic form interact with D2 dopamine receptors. *Trends in Pharmacological Sciences*, *9*(8), 282–284.

Olanow, C. W., Obeso, J. A., & Stocchi, F. (2006). Continuous dopamine-receptor treatment of Parkinson's disease: scientific rationale and clinical implications. *The Lancet Neurology*, *5*(8), 677–687.

Pan, X., Kaminga, A. C., Wen, S. W., Wu, X., Acheampong, K., & Liu, A. (2019). Dopamine and dopamine receptors in Alzheimer's disease: A systematic review and network meta-analysis. *Frontiers in aging neuroscience*, *11*, 175.

Pozzan, T., Gatti, G., Dozio, N., Vicentini, L. M., & Meldolesi, J. (1984). Ca^{2+}-dependent and-independent release of neurotransmitters from PC12 cells: a role for protein kinase C activation? *The Journal of cell biology*, *99*(2), 628–638.

Qin, X., Li, Z.-Q., Zhou, Y., Pan, J.-B., Li, J., Wang, K., Xu, J.-J., & Xia, X.-H. (2020). Fabrication of high-density and superuniform gold nanoelectrode arrays for electrochemical fluorescence imaging. *Analytical Chemistry*, *92*(19), 13493–13499.

Şen, M. (2019). Using electropolymerization-based doping for the electroaddressable functionalization of a multi-electrode array probe for nucleic acid detection. *Analytical Sciences*, *35*(5), 565–569.

Şen, M., & Avcı, İ. (2023). A simple microfluidic redox cycling-based device for high-sensitive detection of dopamine released from PC12 cells. *Journal of Electroanalytical Chemistry*, *939*, 117473.

Şen, M., Azizi, E., Avcı, İ., Aykaç, A., Ensarioğlu, K., Ok, İ., Yavuz, G. F., & Güneş, F. (2022). Screen printed carbon electrodes modified with 3D nanostructured materials for bioanalysis. *Electroanalysis*, *34*(9), 1463–1471.

Seven, F., Gölcez, T., & Şen, M. (2020). Nanoporous carbon-fiber microelectrodes for sensitive detection of H_2O_2 and dopamine. *Journal of Electroanalytical Chemistry*, *864*, 114104.

Seven, F., Gölcez, T., Yaralı, Z. B., Onak, G., Karaman, O., & Şen, M. (2020). Guiding neural extensions of PC12 cells on carbon nanotube tracks dielectrophoretically formed in poly (ethylene glycol) dimethacrylate. *RSC advances*, *10*(44), 26120–26125.

Shinohara, H., Sakai, Y., & Mir, T. A. (2013). Real-time monitoring of intracellular signal transduction in PC12 cells by two-dimensional surface plasmon resonance imager. *Analytical biochemistry*, *441*(2), 185–189.

Swamy, B. E. K., & Venton, B. J. (2007). Carbon nanotube-modified microelectrodes for simultaneous detection of dopamine and serotonin in vivo. *Analyst*, *132*(9), 876–884.

Tian, Z., Qin, X., Shao, F., Li, X., Wang, Z., Liu, S., & Wu, Y. (2023). Electrofluorochromic imaging analysis of dopamine release from living PC12 cells with bipolar nanoelectrodes array. *Chinese Chemical Letters*, *34*(1), 107656.

Whitton, A. E., Reinen, J. M., Slifstein, M., Ang, Y.-S., McGrath, P. J., Iosifescu, D. V, Abi-Dargham, A., Pizzagalli, D. A., & Schneier, F. R. (2020). Baseline reward processing and ventrostriatal dopamine function are associated with pramipexole response in depression. *Brain*, *143*(2), 701–710.

Yang, L., Wang, J., Lü, H., & Hui, N. (2021). Electrochemical sensor based on Prussian blue/multi-walled carbon nanotubes functionalized polypyrrole nanowire arrays for hydrogen peroxide and microRNA detection. *Microchimica Acta*, *188*(1), 25.

Zhou, L., Hou, H., Wei, H., Yao, L., Sun, L., Yu, P., Su, B., & Mao, L. (2019). In vivo monitoring of oxygen in rat brain by carbon fiber microelectrode modified with antifouling nanoporous membrane. *Analytical chemistry*, *91*(5), 3645–3651.