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NOVEL STRATEGIES FOR NEUROTRANSMITTER DETECTION AND MEASUREMENT USING ADVANCED MATERIALS

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Abstract. Several laboratories have recently published the findings indicating that nanomaterial-based biosensors could be very effective in detection of neurotransmitters in biological samples. Most of the research has been focused on monoamine neurotransmitters such as dopamine, serotonin and norepinephrine. These novel approaches have mostly been tested in *in vitro* conditions, however, there are indications that they might be also safely and effectively used in living organisms. In this concise review, we focus on the recent publications regarding the detection and measurement of monoamine neurotransmitters using nano-based sensors. We cover the research done during the past 5 years in the fields nanotechnology and nanomedicine. Finally, we discuss potential future approaches and trends in these research areas, as well as issues and challenges that scientists are faced with, when performing experimental or clinical work with nanomaterials.

1. INTRODUCTION

Neurotransmitters are biomolecules that modulate signal transmission between the neurons across a synapse. The signals are in most cases action potentials which are electrical events occurring on a cell (neuron, muscle fiber) membrane. In human brain, there are many classes of neurotransmitters, such as amino acids (glutamate, glycine, aspartate), peptides (i.e. substance P), and monoamines (dopamine, norepinephrine, serotonin).

Monoamine neurotransmitters play a vital role in arousing and regulating emotions and mood [1]. Many research studies in the field of neurosciences have shown a relationship between emotional processes and change in level of neurotransmitters such as dopamine, norepinephrine, serotonin, and adrenaline [2]. Dopamine is essential for reward processing, it regulates emotional states, it has impact on human motivation, and is related to goal-directed behavior [3]. Research also shows that there is a link between serotonin and stress [4]. Additionally, serotonin regulates other processes such as learning, memory body temperature, sleep, appetite. There is also a connection between the decrease in the density of serotonergic receptors and aging and Alzheimer's disease [5]. The reduction of the nigral neurons in the Parkinson disease

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Table 1. Examples of nano-based systems for neurotransmitter detection.

Neurotransmitter	Nanoparticle system	Reference
Dopamine	DNA, o-phenylenediamine, and gold nanoparticle bioimprinted polymer electrochemical sensor	Biosens Bioelectron. 2015 Apr 15;66:490-6
	Graphene quantum dots and TiO_2 nanocomposites	Anal Chim Acta. 2015 Jan 1;853:258-64
	Nafion-modified gold nanoparticle-sheathed glass capillary nanoelectrode	Bioelectron. 2015 Jan 15;63:262-8
	Microelectrodes with gold nanoparticles and self-assembled monolayers	Analyst. 2012 Jun 21; 137(12):2813-20
	Ferrocene-capped gold nanoparticle/streptavidin conjugates	Biosens Bioelectron. 2013 Mar 15;41:730-5
Serotonin	Platinum electrode modified with carbon nanotubes/polypyrrole/silver nanoparticles nanohybrid	Mater Sci Eng C Mater Biol Appl. 2014 Jul 1;40:49-54
	PEDOP/MWCNTs-Pd nanoparticle modified glassy carbon electrode (for multiple neurotransmitters)	J Nanosci Nanotechnol. 2012 Mar;12(3):1903-9
Norepinephrine	Au-nanoparticles/poly(2-amino-2-hydroxymethyl-propane-1,3-diol) film modified glassy carbon electrode	Colloids Surf B Biointerfaces. 2014 Nov 1;123:23-32
	Molecularly imprinted polymer-coated PdNPs	Biosens Bioelectron. 2014 Oct 14;65C:366-374

is related with altered vesicular storage of dopamine [6]. Some eating disorders, such as bulimia and anorexia nervosa are connected with disruption in serotoninergic and noradrenergic systems [7]. Some results show that alterations in level of monoamine neurotransmitters may be connected with depression and aggression [8].

Detection and measurement of neurotransmitter activity in biological samples is of fundamental importance both in basic neurophysiology, and clinical neurology research. In recent years, various nanomaterial-based biosensors have been developed and applied to serve this purpose [9,10]. Nanoparticles, due to their small size, have some unique physical and chemical characteristics which enable them to pass through biological barriers, and reach the target tissue, without exhibiting significant toxicity [11-14]. Nanomaterials can also be conjugated with various medications, contrast agents and other biological substances which can be useful in design of more efficient bioassays [15-21]. Several laboratories have recently published the findings indicating that these nano-based bioassays could perhaps be very effective in detection of dopamine, serotonin and norepinephrine. These novel approaches for monitoring the level of neurotransmitters have mostly been tested in *in vitro* conditions, however, there are indications that they might be also safely and effectively used in living organisms, such as animal experimental models, or even in a clinical setting.

In this concise review, we focus on the recent publications regarding the detection and measurement of neurotransmitters using nano-based sensors. We cover the research done during the past 5 years in the fields nanotechnology and nanomedicine, see, e.g. Table 1. Finally, we discuss potential future approaches and trends in these research areas, as well as issues and challenges that scientists are faced with when performing experimental or clinical work with nanomaterials.

2. DOPAMINE AND NANOMATERIALS

Dopamine is an important neurotransmitter in limbic system, and it is at least partially responsible for physiological regulation of mood, memory and perception. The increased dopaminergic activity may result in errors in perception such as hallucinations, and is a characteristic of certain psychiatric diseases such as schizophrenia. On the other hand, the reduction of dopaminergic neurons is often seen in some neurological/motor diseases such as Parkinson's disease.

In recent years there have been numerous efforts to design a nano-based biosensor for dopam-

ine detection. For example, Rezaei et al. (2015) designed a simple methodology involving DNA, o-phenylenediamine, and gold nanoparticle bioimprinted polymer electrochemical sensor. A special pencil graphite electrode was constructed with electrochemical entrapment of Au nanoparticles and DNA at the bioimprinted film. The authors tested the overall quality of the method by determining stability, sensitivity, and reproducibility of the biosystem [22].

Ultrasensitive determination of dopamine was proved to be possible by constructing a visible light photoelectrochemical sensor based on graphene quantum dots and TiO_2 nanocomposites [23]. This method is relatively simple and inexpensive, and although done in in vitro conditions could be potentially applicable in biological samples.

The example of a study for nano-based dopamine detection system in laboratory animals (in vivo) is the publication of Liu et al. (2015) where the authors demonstrated that Nafion-modified gold nanoparticle-sheathed glass capillary nanoelectrode (Au/GCNE) can be applied successfully in the striatum of anesthetic rats [24]. Amperometricall monitoring of dopamine in subcortical structures (i.e. basal ganglia) could become a potentially significant method for evaluation of psychiatric symptoms, as well as potential effects of psychoactive substances in animal models.

Another study in rat brains is the research of Mu et al. (2014) where the authors stated that catecholamines such as dopamine, norepinephrine and epinephrine can augment the chemiluminescence signal of the on-line gold nanoparticle-catalyzed luminol system. This system was shown to be effective even in non-purified brain samples indicating its quality and applicability in neurophysiology research [25].

Liu et al. (2013) showed that ferrocene-capped gold nanoparticle/streptavidin conjugates can be extremely useful in Amplified voltammetric detection of dopamine. The study was done in artificial cerebrospinal fluid (comprising of NaCl, KCl, $CaCl_2$, $MgCl_2$ and sodium phosphate), and the biosystem demonstrated relatively good analytical qualities [26].

Finally, it should be mentioned that *in vivo* recording of subcortical brain dopamine was also proved to be feasible using microelectrodes with gold nanoparticles and self-assembled monolayers [27]. Tsai et al. (2012) demonstrated that this approach can be used to modify platinum microelectrodes and improve the sensitivity and specificity of dopamine detection in animal model of Parkinson's disease.

3. SEROTONIN

Serotonin (5-hydroxytryptamine) is an important neurotransmitter in brainstem and reticular formation, and serotonergic neurons reach many regions of the central nervous system. Serotonergic transmission was found to have a major role in regulation of physiological mood. It is thought that depression as a psychiatric illness is at least partially result of the lowered serotonergic activity in the limbic system and other brain areas. In many forms of depression, selective serotonin reuptake inhibitors (SSRIs) are often the first line medications during treatment. Any novel method capable of measuring the serotonin activity in brain and other biological samples, would possibly in the future have a significant impact on the diagnosis and treatment of not only mood disorders, but also a variety of other psychiatric conditions where serotonin has a role, such as anxious disorders, stress disorders etc.

Liu et al. (2013) demonstrated a method based on polydopamine functionalized multiwalled carbon nanotubes that was proved capable of serotonin detection (portion of the study performed in rat brains). Also, using the mentioned nanosystem and capillary electrophoresis-amperometric technique, the authors were able to simultaneously detect other monoamines, such as epinephrine and norepinephrine [28]. If the reproducibility of this method is confirmed in the future research, it could be highly applicable in brain serotonin measurement in both physiological and pathological conditions.

Most of the other studies on nanosytem-based serotonin detection were focused on *in vitro* methods with potential for biological sample analysis. Cesarino et al. (2014) described a sensor based on a multi-walled carbon nanotubes, polypyrrole and colloidal silver nanoparticles. These compounds have been electrodisposed on surface of a platinum electrode [29]. The authors also tested the reproducibility and repeatability of the sensor and confirmed its potential applicability in the field of biomedicine.

Simultaneous electrochemical detection of serotonin and dopamine was also proved feasible with palladium-functionalized, multi-walled carbon nanotubes, glassy carbon electrode and poly 3,4-ethylenedioxy pyrrole [30]. Kim & Jeon (2012) investigated lowest detection limits of such system, as well as the linear ranges for both monoamines.

Finally, serotonin and its metabolites, such as 5-hydroxyindoleacetic acid can be determined in urine using gold-nanoparticle-assisted laser desorp-

tion/ionization time-of-flight mass spectrometry [31]. Kuo et al. (2011) successfully tested this method as a way to quantify urinary biomarkers of carcinoid tumors, however, there is a possibility that in the future the method could be useful as a supplemental diagnostic tool in many neuropsychiatric disorders where monoamine urine quantification is performed.

4. NOREPINEPHRINE

Norepinephrine in the central nervous system has an important role in the physiological regulation of mood, will, consciousness and other psychic functions. Pathological changes in norepinephrine transmission in the brain are a significant factor influencing the development of many mental illnesses, such as depression and a range of anxiety disorders. Some novel medications for treatment of depression are selective reuptake inhibitors of both serotonin and norepinephrine.

In 2014, Chen et al. described an advanced nanosystem consisting of imprinted polymer-coated palladium nanoparticles synthetized using sol-gel method. The system used norepinephrine as a template and tetramethoxysilane was also added as a crosslinker. The authors tested the technique on injection and urine samples, and determined that the sensor exhibited high sensitivity and selectivity for norepinephrine molecule. The linear ranges and detection limits were also successfully determined [32].

Palladium nanoparticles synthetized on glassy carbon and indium tin oxide electrodes were also proved to be capable biosensors of norepinephrine, but also of other catecholamines (epinephrine, dopamine) as well [33]. In the presence of ascorbic acid, the system could be able to efficiently detect the presence of the compounds in injection solutions, which would be useful in many clinical disciplines. The advantage of this biosensor, apart from its high sensitivity and reproducibility is the fact that it doesn't require a lot of material resources and expertise to manufacture, which further increases its applicability in fundamental and applied biological sciences.

Another method of norepinephrine (and other catecholamine) detection in urine was described by Jiang et al. (2014). The authors used aminophenylboronic acid functionalized magnetic nanoparticles extraction, which was followed by high-performance liquid chromatography. As in the previous study, the linear ranges, detection limits, and other parameters of the method quality and sensitivity were tested [34].

Taei and Ramazani (2014) were able to simultaneously detect norepinephrine, acetaminophen and tyrosine with glassy carbon electrode modified by gold nanoparticles/poly 2-amino-2-hydroxymethyl-propane-1,3-diol. The authors showed that the nano-based (differential pulse voltammetry) method could be applied for norepinephrine sensing in both pharmaceutical and biological samples [35].

5. CONCLUDING REMARKS

Due to the rapid development of nanotechnology during the past decade, many new methods have been designed in order to detect neurotransmitters in chemical and biological samples. Most of these techniques have been focused on detection of monoamine compounds such as dopamine, norepinephrine, and serotonin in *in vitro* conditions. Biosensors based on gold, platinum, palladium, and other metallic nanoparticles were found to have good sensitivity, reproducibility and relatively low detection limits.

Before these strategies can be applied *in vivo,* several important issues need to be addressed. First, toxicity of many nanomaterials has not yet been properly investigated. It is still unclear if these compounds have detrimental effects on the normal function of liver and kidneys. Second, interaction between most nanomaterials and the immune system is also unknown. Any immunomodulatory effects of nanoparticles would pose an obstacle in potential clinical application of the biosensor. Finally, although many nano-sensors presented in this article had relatively good sensitivity and specificity for monoamine detection, it does not necessarily mean that the same effectiveness will be demonstrated in vivo, where many other confounding factors are present.

In the future, it also remains to be seen if these nano-based systems can be successfully applied in experimental animal models for imaging techniques or other potential diagnostic procedures. Conjugation of nanoparticles with various dyes and contrast agents offers numerous possibilities for novel imaging assay development. Even the simple application such as identification of a neurotransmitter in a brain tissue sample for pathohistological analysis, would prove valuable for neurophysiology and other fundamental research. It goes without saying that if future research demonstrates that these nanosensors are useful in clinical medicine, they have a chance to revolutionize diagnosis and treat-

ment of many neurological and psychiatric disorders.

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