Microchimica Acta (2024) 191:170
https://doi.org/10.1007/s00604-024-06250-2

ORIGINAL PAPER

In vitro drug screening models derived from different PC12 cell lines for exploring Parkinson's disease based on electrochemical signals of catecholamine neurotransmitters

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Received: 9 November 2023 / Accepted: 5 February 2024 / Published online: 1 March 2024

Abstract

Gold nanostructures and a Nafion modified screen-printed carbon electrode (Nafion/AuNS/SPCE) were developed to assess the cell viability of Parkinson's disease (PD) cell models. The electrochemical measurement of cell viability was reflected by catecholamine neurotransmitter (represented by dopamine) secretion capacity, followed by a traditional tetrazolium-based colorimetric assay for confirmation. Due to the capacity to synthesize, store, and release catecholamines as well as their unlimited homogeneous proliferation, and ease of manipulation, pheochromocytoma (PC12) cells were used for PD cell modeling. Commercial low-differentiated and highly-differentiated PC12 cells, and home-made nerve growth factor (NGF) induced low-differentiated PC12 cells (NGF-differentiated PC12 cells) were included in the modeling. This approach achieved sensitive and rapid determination of cellular modeling and intervention states. Notably, among the three cell lines, NGF-differentiated PC12 cells displayed the enhanced neurotransmitter secretion level accompanied with attenuated growth rate, incremental dendrites in number and length that were highly resemble with neurons. Therefore, it was selected as the PD-tailorable modeling cell line. In short, the electrochemical sensor can be used to sensitively determine the biological function of neuron-like PC12 cells with negligible destruction and to explore the protective and regenerative impact of various substances on nerve cell model.

Keywords Parkinson's disease · Different PC12 cell lines · Catecholamine neurotransmitter · Pramipexole · Electrochemical sensor

Introduction

PC12 cells normally have features intermediate between those of typical chromaffin cells and sympathetic neurons, for instance, homogeneous propagation in the unlimited way, capacity to undergo neuronal differentiation in response to NGF, and exocytosis of catecholamines and acetylcholine [1]. PC12 cells are highly employed in the study of neuroendocrine secretion (monoamine biogenesis and secretory vesicle dynamics), neuronal differentiation, and neurotrophin action [2, 3]. In addition, PC12 cells have characteristics of catecholamine neurotransmitter synthesis, storage, recruit, and release resemble in sympathetic neurons [1–3]. Therefore, the established PC12 cell line represents a potentially useful cell model for investigating the pathogenesis and etiological treatment to manifold neurodegenerative diseases, involving PD.

The widely employed techniques for evaluating the construction and intervention processes of PD damage model are microplate tetrazolium assays, containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), cell counting kit-8 (CCK-8), and so on. Nevertheless, these colorimetric methods have been doubted that neither depicted the number of viable cells, nor illustrated the cell proliferation and drug toxin screening [4–6]. Tetrazolium reduction virtually reflects mitochondrial enzyme activity. Although it is generally assumed that tetrazolium reduction is intracellular and related to energy metabolism, the reduction also associates with cytoplasm and non-mitochondrial

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membrane such as endosome/lysosome compartment and plasma membrane [5]. Numerous factors, like metabolic and energy destabilization, changes in the activity of oxidoreductases, endo-/exocytosis, and intracellular trafficking, could potentially influence the colorimetric result [6]. Furthermore, quiescent or dormant cells that are viable are not always clearly distinguished from dead cells by this means [4–6]. Yang et al. observed that the cell viability (as indicated by MTT assay) was not significantly affected, while obvious neurite atrophy could be found, accompanied with vesicular neurotransmitter release fluctuated [7]. Thus, more accurate assessments for inherent physiological state of cells are in need.

PC12 cells with a well-defined endocrine cell phenotype have drawn the concerns of scientists for cellular physiological assessment, namely neurosecretion activity. PC12 cells principally contain dopamine (DA), norepinephrine (NE), epinephrine, and so forth [8]. Determination for PC12 cell biomarkers (catecholamine neurotransmitters, represented by DA) to evaluate cell viability and function of disease model and drug intervention arouses great interest among researchers [9, 10]. PD is characterized by a drastic loss (50–70%) of dopaminergic neurons in substantia nigra pars compacta, resulting in obvious depletion of catecholamine neurotransmitters [11]. Monitoring of catecholamine neurotransmitters can provide an in-depth understanding of biochemical process through quantitative and dynamic changes in the exocytotic machinery [12]. To date, various analytical tools have been developed for neurotransmitter determination, mainly mass spectrometry, fluorimetry, chemiluminescence, colorimetry, ultraviolet and visible absorption spectrometry, etc.[13–16]. However, the instrumental method suffers from drawbacks of requiring expensive apparatus, complicated operation, high cost, and long period [17]. Electrochemical sensors have the potential to achieve rapid, sensitive, and low-cost determination [18, 19]. Importantly, DA possesses redox-active property that is tailored to electrochemical measurement.

In this study, to imitate the striking loss of dopaminergic neurons and disturbance of intra-synaptic cross talk, we employed the purchased low and highly-differentiated PC12 cells and self-made NGF-differentiated PC12 cells to construct PD cell models induced by dopaminergic neurotoxin 1-methyl-4-phenyl-pyridine ion (MPP^+) in Scheme 1. A Nafion/AuNS/SPCE was fabricated to monitor the PD modeling and intervention processes by examining neurosecretion activity. PC12 cells were treated with various conditions which were named as blank control group, negative control group, MPP^+ group, pramipexole (PPX) intervention group, and bone marrow mesenchymal stem cells (BMSCs) intervention group, respectively. The cell viability of the aforementioned groups was assessed by this developed electrochemical sensor, supplemented with CCK-8 assay and cell morphology. Collectively, the use of electrochemical approach in neural cell modeling can provide a new angle of view to monitor cellular physiopathological condition.

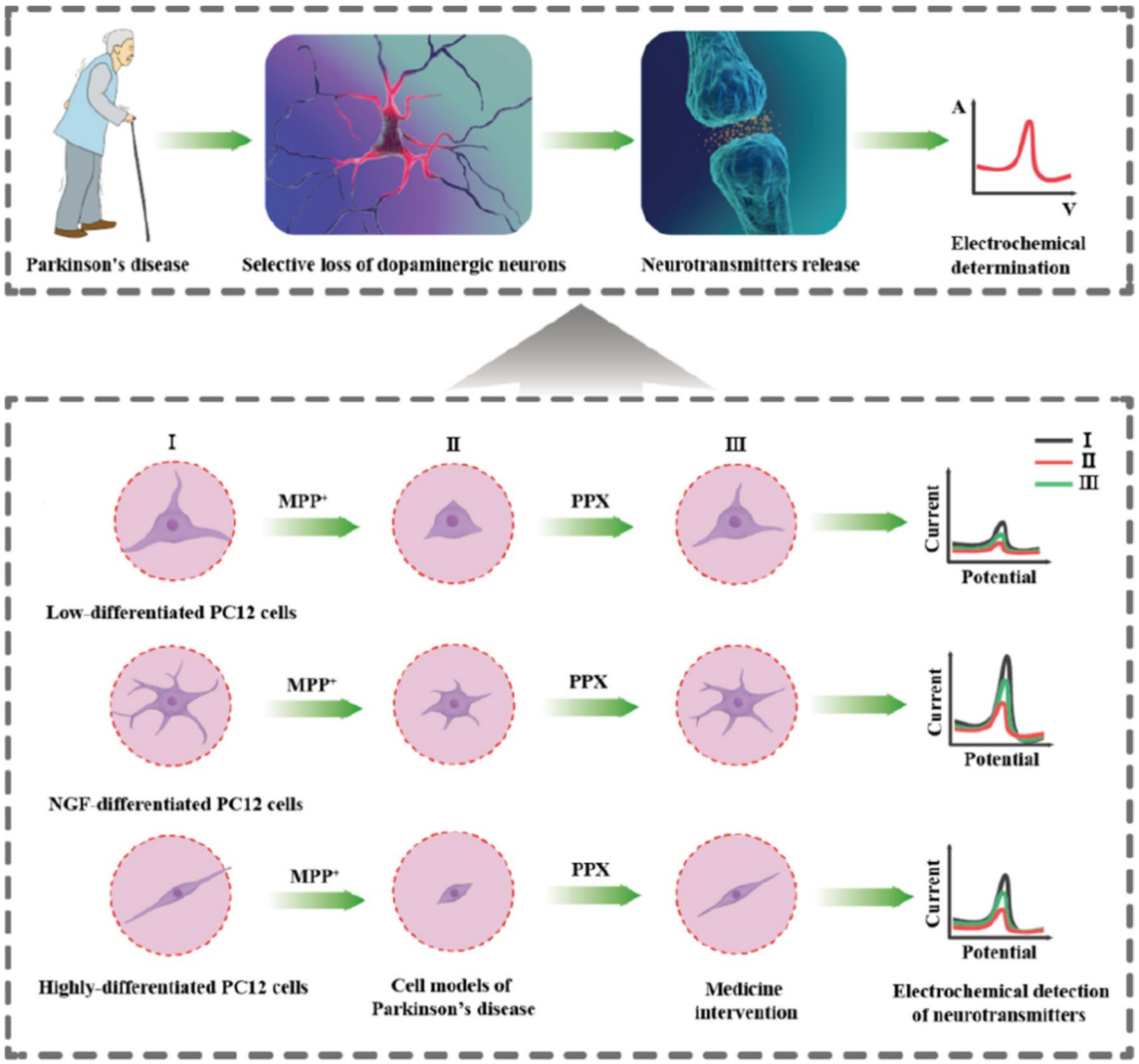
Material and methods

Materials, apparatus, construction, and characterization of Nafion/AuNS/SPCE, differentiation and characterization of different PC12 cell lines, and the construction and intervention of PD models can be referred in Supplementary Information.

Results and discussion

Cell differentiation and characterization

Since mature neurons are nondividing, a maximally typical neuronal model should be verstellbar between a condition in which it can replicate and a state in which it is nondividing as well as neuronally differentiated. The observations of PC12 cells treated with NGF highly resemble sympathetic phenotype involving extension of neurites, cessation of cell division, expression of neuronal markers, and improvement of electrical excitability [20]. Generally, neurite outgrowth including average neurite length and percentage of neurite-bearing cells was a routine index during the NGF differentiation period. As expected, low-differentiated PC12 cells without and with NGF treatment presented distinctly different morphology. Before the addition of NGF, low-differentiated PC12 cells had round or polygonal shapes (probably 20 μm in diameter obtained by ImageJ software) with a few short visible neurite networks (Fig. 1A and Fig. S1A). After 7 days of treatment with NGF in serum-free RPMI 1640 medium, cell multiplication ceased and the neuronal-like processes obtained that neurite lengths were substantially incremental (Fig. 1B and Fig. S1B). As shown in Fig. 1B, the significantly extended neurites of NGF-differentiated PC12 cells (50–100 μm in synaptic length) resemble those generated by cultural primary sympathetic neurons, such as, long, branched profusely and fascicled shape. Highly-differentiated PC12 cells showed elongated and fusiform shape with approximately 100 μm in length (Fig. 1C and Fig. S1C). The neurite number of low-differentiated and NGF-differentiated PC12 cells were distributed in the range of 0 to 5 and 0 to 9, respectively, illustrating that NGF had an effect on promoting sprout of neurites (Fig. 1D). And the neurites grew rapidly after exposure to NGF for several days, demonstrating the obvious NGF effect on extension of neurites (Fig. 1E). By 7 days, about 90% of the cells bore neurites, which is consistent with previous report [20].



Scheme 1 Schematic illustration of electrochemical sensors for monitoring catecholamine neurotransmitters released from different PD models in vitro

Apart from cell morphology, the exocytosis neurotransmitter function of PC12 cells was also assessed by electrochemical approach (Nafion/AuNS/SPCE), which directly reflects the cellular physiological activity. Note that freshly NGF-differentiated cells were chosen as target to determine neurotransmitters content due to reversibility of the NGF effects on PC12 cells. As displayed in Fig. 1F, the differential pulse voltammetry (DPV) responses of neurotransmitters generated from low-differentiated (curve b), NGF-differentiated (curve c), and highly differentiated (curve d) PC12 cells (about 5×10^5 cells in 5 mL phosphate buffer saline, PBS) treated with high concentration of potassium ion (K^+) were recorded. Blank PBS (pH 7.4, curve a) with addition of K^+ displayed negligible current. The obviously enhanced neurotransmitter release was observed after NGF treatment, due to the elaboration of an extensive network of neurites with pervasive synapses [20–23], certifying the neuron-like behavior of NGF-differentiated PC12 cells. And the result showed that the exocytosis neurotransmitters of highly-differentiated PC12 cells were slightly lower than that of NGF-differentiated PC12 cells. Moreover, the neurosecretion function of cells (*ca.* 2×10^6 cells) was also assessed by the competitive enzyme-linked immunosorbent assay (ELISA). The optical density (OD) was measured at 450 nm, and ΔOD value (the OD value of blank group without DA addition minus the OD value of sample group) represented the DA secretion level. The ELISA results were basically consistent with the electrochemical results (inset of Fig. 1F). Taking the cell morphology and neurotransmitter expression level into consideration, NGF-differentiated PC12 cells exhibited nerve characteristics containing neuron-like cell morphology and neurosecretion transmitter functions, which

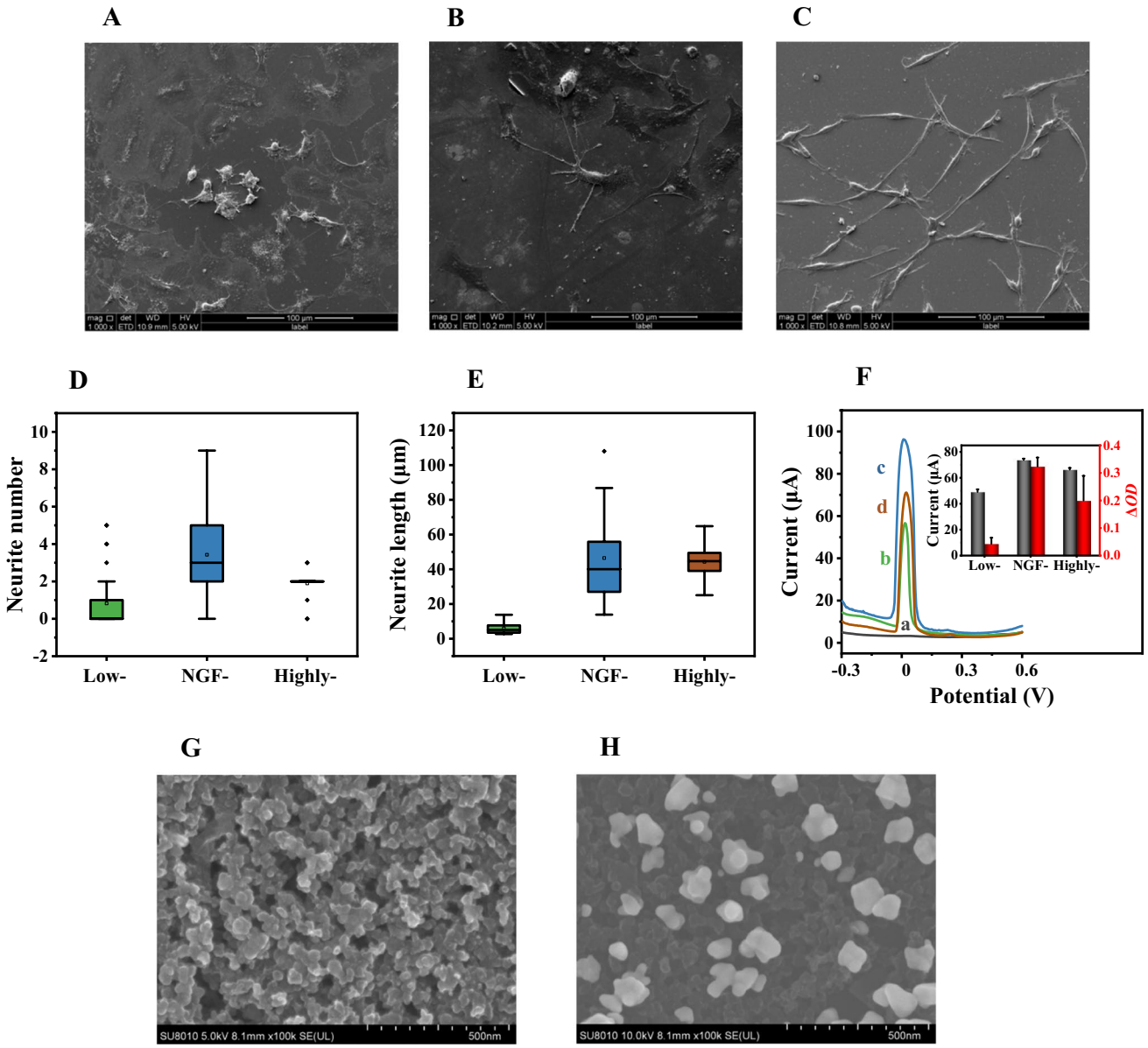


Fig. 1 Scanning electron microscopy images of low-differentiated (**A**), NGF-differentiated (**B**), and highly differentiated (**C**) PC12 cells (scale bar is 100 μm). Neurite number (**D**) and neurite length (**E**) of low/NGF/highly-differentiated PC12 cells. **F** DPV responses of catecholamine neurotransmitters released from PBS (curve a), low-differentiated (curve b), NGF-differentiated (curve c), and highly-differentiated (curve d) PC12 cells in PBS (pH 7.4) after K^+ stimulation. Inset: current values (black histogram) and ΔOD values (red histogram) of low/NGF/highly-differentiated PC12 cells after $K.^+$ stimulation. FE-SEM images of bare SPCE (**G**) and AuNS/SPCE (**H**)

was an outstanding modeling cell line for neurodegenerative disease.

Feasibility and application of the electrochemical sensor

The feasibility of the electrochemical sensor was explored with respect to electrochemical behavior and analytical performance in Supporting Information.

Au nanostructures (AuNS) were loaded onto the surface of cleaned SPCE by chronoamperometry at − 0.2 V for 400 s. The morphology of bare SPCE and the modified SPCE was characterized by field emission scanning electron microscopy (FE-SEM). In Fig. 1G, the unmodified electrode performed a homogeneous carbon powder microstructure. After electro-deposition, the AuNS did not form a continuous layer but organized in clusters in a flower-like hierarchical shape of Fig. 1H, providing a higher effective surface area for the loading of targeted analyte than the bare counterpart.

To demonstrate the application of the proposed sensor in quantifying neurotransmitters generated from PC12 cells, DPV measurement was conducted. As displayed in Fig. S4A, DPV responses generated from low/NGF/highly-differentiated PC12 cells (*ca.* 5×10^5 cells, severally) in 5 mL PBS solution were recorded. As control, the current signals of the blank PBS and cell-contacting PBS containing K^+ were measured. There was negligible current signal appeared at 0.08 V in black curves and blue curves, indicating that the current in red curves was caused by neurotransmitters. The current response was different among the three groups, reflecting the variation of neurotransmitters in the amount for the abovementioned PC12 cells. In addition, it could be calculated that the neurotransmitters secreted by each low-differentiated, NGF-differentiated, and highly differentiated PC12 cell were approximately 0.614 ± 0.13, 25.2 ± 0.98, and 21.2 ± 1.2 fmol (Table 1). Furthermore, exocytosis neurotransmitter capacity among three types of PC12 cells varied statistically significant (Fig. S4B). Recovery studies were performed by standard addition method in PBS contacted with PC12 cells (Fig. S4C). Recoveries all lied in the range of 93.9–113% (Table S2), illustrating the developed sensor's reliability.

Construction and intervention of cell damage models

MPP^+, as dopaminergic neurotoxin, gives rise to progressively pathological changes resemble with those happened in PD, that is, mitochondrial dysfunction, neurosecretory disorder, formation of reactive oxygen species (ROS), and degenerative death of dopaminergic neurons [24]. PPX, as the dopamine receptor agonist, has been widely used for the symptomatic treatment of PD. In this part, we investigated the effect of PPX/MPP^+-treated low/NGF/highly-differentiated PC12 cells and explored the optimal MPP^+ modeling condition via cell morphology and cell viability. Cell morphology was applied to visually represent the cell adhesion and growth, which was discussed in Supporting Information. Cell viability, obtained by CCK-8 assay and electrochemical technique, tended to reflect mitochondrial function and neurotransmitters secretion level of the PC12 cells, respectively.

Cell viability obtained by CCK-8 assay and electrochemical sensor after treatment of MPP^+

The cell viability of PC12 cells after exposure to various concentrations of MPP^+ was assessed by traditional CCK-8 assay firstly. In Fig. 2A–C, exposure to MPP^+ for 48 h (blue curves) induced an acute damage to cells. The results were identical with previous papers that the decreased cell viability appeared to commence within 24 h and reached a significant level (generally about 40–60%) by 24–48 h [25]. Three PC12 cell lines responded MPP^+ in a similar tendency as follows: negligible effect on cells was observed at low concentration (≤ 50 μM) and sharply decreased viability occurred at the range of 100–400 μM MPP^+, and then reached a platform phrase (600–2000 μM). Exposure to 200, 800, and 400 μM MPP^+, respectively, reduced cell viability to $53.86 \pm 0.07\%$, $51.49 \pm 0.05\%$, and $51.50 \pm 0.02\%$ for low/NGF/highly-differentiated PC12 cells. Therefore, these concentrations were deemed as half maximal inhibitory concentrations (IC_{50}). The results were basically consistent with the literature [4]. By comparison, the trend of cell toleration to MPP^+ (low- < highly < NGF-differentiated PC12 cells) might be relative to the levels of dopamine transport density on the cells owing to affinity between dopamine transporters and MPP^+ [9]. The results remind us that PC12 cells for construction of PD model should be selected according to research purposes.

The modeling concentrations of MPP^+ were further examined using the developed electrochemical sensor. Cell viability was calculated by dividing the neurotransmitter level of the sample group by that of the control group. Fig. S7 and Fig. S8 displayed the morphology of low/NGF/highly-differentiated PC12 cells and DPV responses of neurotransmitters in a concentration dependent manner. In Fig. 2A–C (purple curves), the curve dropped more dramatically, compared with blue curves, indicating that the neurosecretory function was more severely impaired than mitochondrial function. The serious damage of neurosecretion function mimicked the pathological features of dopaminergic neuron loss in PD. Specifically, 207.1, 380.9, and 231.7 μM were the IC_{50} values of low/NGF/highly-differentiated PC12 cells, which was determined by the electrochemical sensor. While treated with MPP^+ at the IC_{50} concentrations examined via CCK-8 assay, cell activity measured by the electrochemical

Table 1 Analysis for catecholamine neurotransmitters secreted from low/NGF/highly-differentiated PC12 cells at Nafion/AuNS/SPCE ($n = 3$)

PC12 cell type	Cell number	Average current (μA)	Catecholamine neurotransmitters released per cell (fmol)
Low-differentiated	*ca.* 5×10^5	49.1 ± 2.1	0.614 ± 0.13
NGF-differentiated	*ca.* 5×10^5	73.8 ± 1.1	25.2 ± 0.98
Highly differentiated	*ca.* 5×10^5	66.3 ± 1.4	21.2 ± 1.2

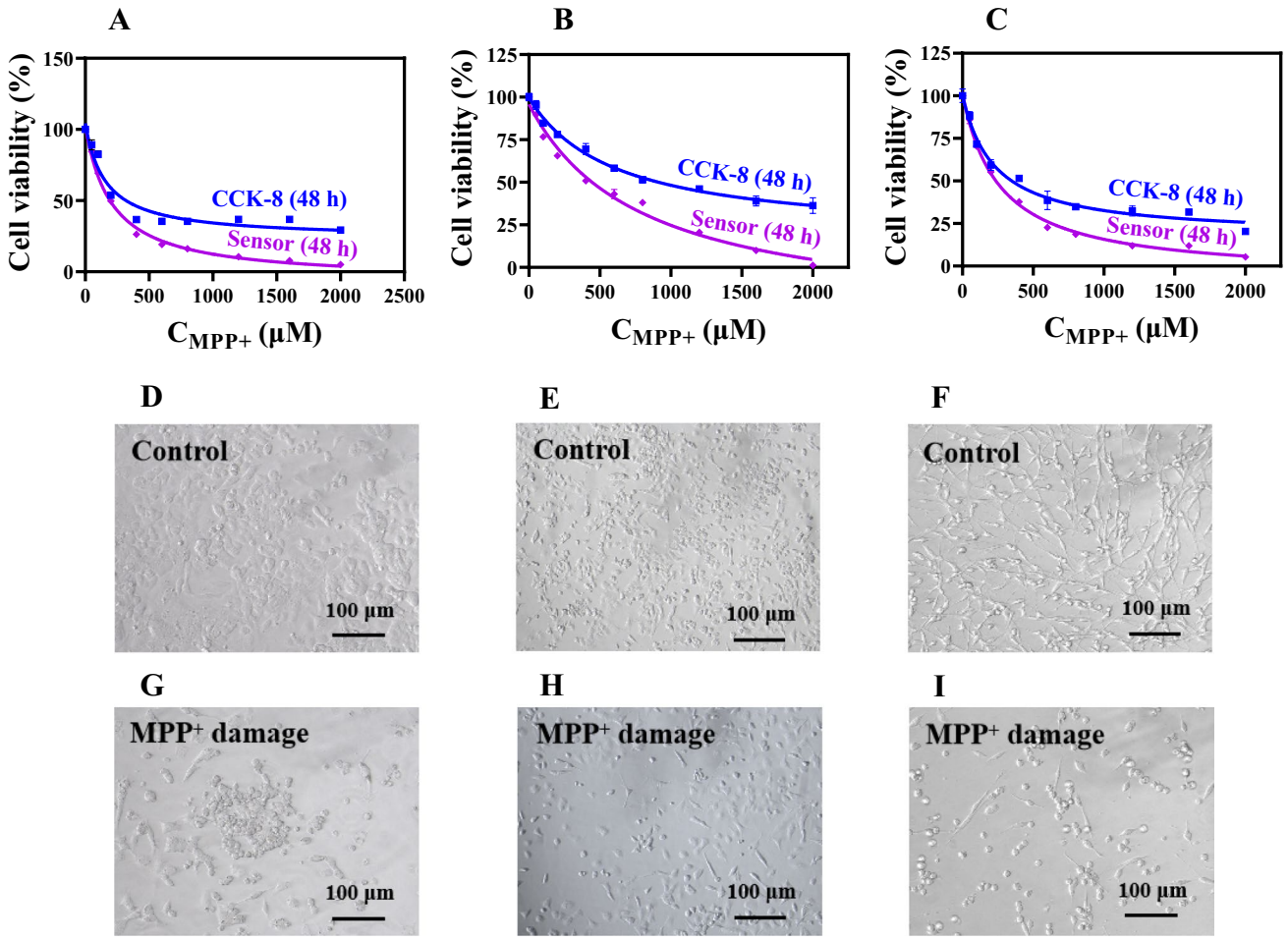


Fig. 2 Effects of MPP^+ on cell viability measured by CCK-8 assay, electrochemical sensor, and optical microscope. **A–C** The viability of low/NGF/highly-differentiated PC12 cells measured by CCK-8 assay (blue curves) and electrochemical sensor (purple curves) after treatment with MPP^+ for 48 h. Morphological characterization of low/NGF/highly-differentiated PC12 cells before (**D**, **E**, **F**) and after (**G**, **H**, **I**) treatment with the MPP^+ with IC_{50} concentrations obtained by CCK-8 assay

method declined to 49.64 ± 1.58%, 38.08 ± 0.79%, and 37.78 ± 1.04% for low/NGF/highly-differentiated PC12 cells (the correspondingly morphological changes shown in Fig. 2G–I). Obviously, these results were distinctly different from CCK-8 assay except for low-differentiated PC12 cells. For low-differentiated PC12 cells, similar IC_{50} results may be related to the minor morphological changes (Fig. 2D and G), which was caused by inherent property of the cells like the few dendrites in number and length. Nevertheless, for NGF-differentiated and highly-differentiated PC12 cells, when mitochondrial function was reduced by half, the neurosecretion function was more severely impaired that may be ascribed to serious neurite atrophy (Fig. 2E, F, H, and I), which made the CCK-8 assay prone to false negative results of cell viability. In this regard, the similar discrepancy was also reported previously. Yang et al. [8] observed that the cell viability (as indicated by MTT assay) was not significantly affected, while axonal length decreased and vesicular glutamate release changed, evidently. Different result of two approaches demonstrated that cell viability obtained by CCK-8 assay was closely linked to enzyme activity in mitochondrion but not fully embodied the biological function of cells, which was not suitable for monitoring of neural models alone. In general, electrochemical monitoring of neurotransmitters can provide an accurate biological function measurement during PD modeling process.

Cell viability obtained by CCK-8 assay and electrochemical sensor after treatment of PPX

PPX had been verified to protect human neuroblastoma SHSY-5Y cells and PC12 cells [26], against the toxicity of various toxins, like MPP^+, 6-hydroxydopamine (6-OHDA), rotenone, dieldrin, and paraquat. Safety evaluation of PPX was discussed in Fig. S10. The optimum concentration of PPX was researched by pre-incubating with PC12 cells. PPX

pre-incubation did not reduce the uptake of MPP^+ by cells, but might enhanced antioxidative capacity, supplied endogenous cellular defenses, and possessed some neurotrophic action [27]. For low-differentiated and NGF-differentiated PC12 cells, the cell viability gradually enhanced with the increase of PPX concentration (Fig. 3A and B, Fig. S10A and Fig. S10B). Considering the toxic effect, economic cost, and improvement of cell viability, 300 μM was chosen as the optimal concentration for following experiments. For highly-differentiated PC12 cells, the cell viability increased with the enhancement of PPX concentration at first, reached a peak at 100 μM, then decreased with the

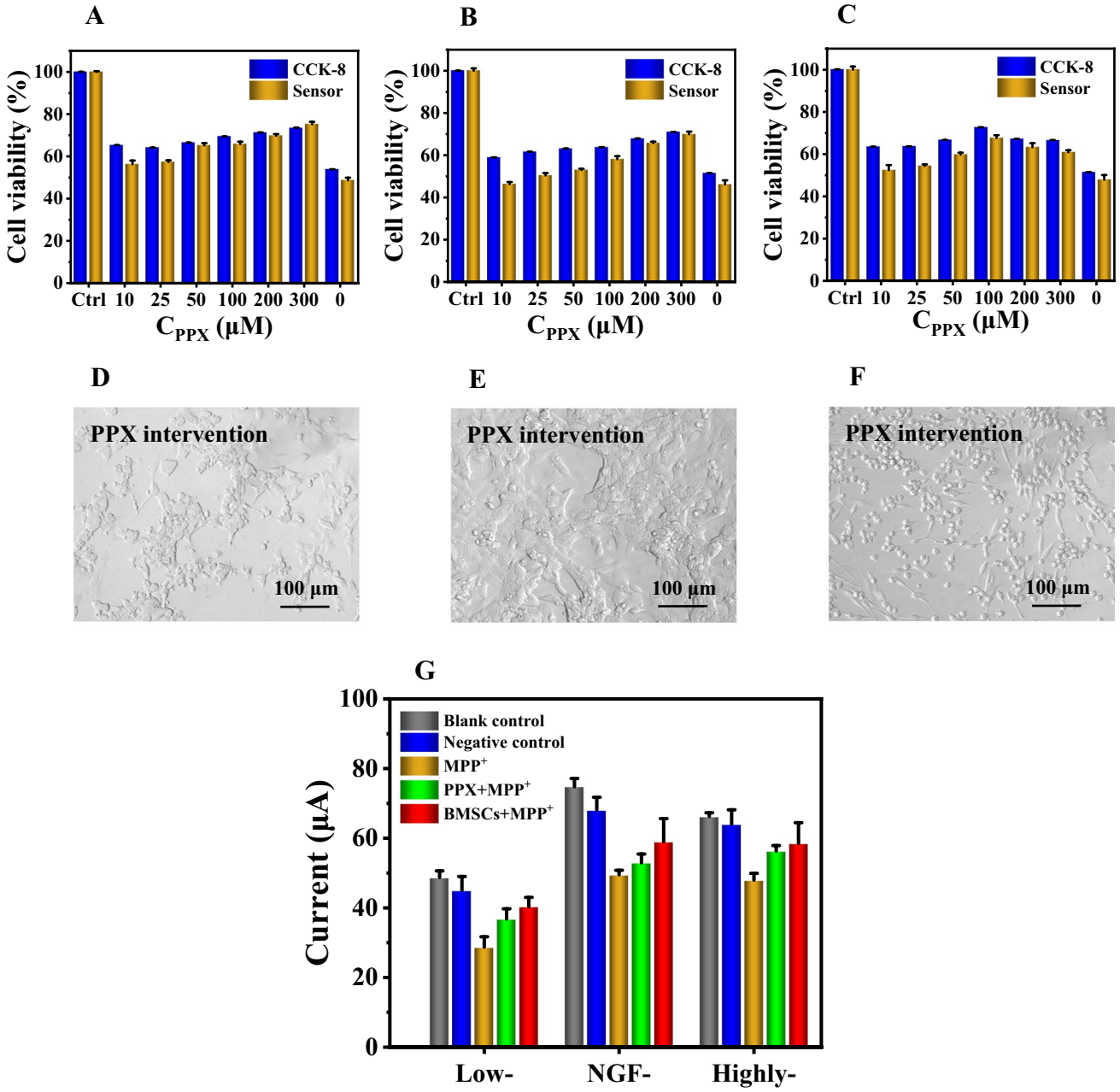


Fig. 3 Effects of PPX on cell viability measured by CCK-8 assay, electrochemical sensor, and optical microscope. **A**–**C** The viability of low/NGF/highly-differentiated PC12 cells determined by CCK-8 assay and electrochemical sensor. **D**–**F** Morphological characterization of low/NGF/highly-differentiated PC12 cells after PPX intervention. **G** The DPV peak current values of catecholamine neurotransmitters generated from low/NGF/highly-differentiated PC12 cells in various conditions: culturing with RPMI 1640 medium (blank control group), culturing with serum-free RPMI 1640 medium (negative control group), culturing with MPP^+ for 48 h (MPP^+ group), culturing with MPP^+ for 48 h after incubating with PPX for 24 h (PPX + MPP^+ group), and co-culturing with BMSCs for 24 h then culturing with MPP^+ for further 48 h (BMSCs + $MPP.^+$ group)

increasing concentration of PPX (Fig. 3C and Fig. S10C). Thus, 100 μM PPX was selected as the best condition. The morphological characterization also verified the effect of PPX intervention (Fig. 3D–F). Furthermore, cell viability variation tendency of electrochemical method was consistent with that of CCK-8 assay, indicating its applicability in drug intervention determination. However, different cell sensitivity to PPX at specific concentration was observed. This phenomenon also suggested that mitochondrial function of cells could not entirely reflect neurotransmitter secretion level of cells. Additionally, highly-differentiated PC12 cells presented the highest sensitivity to PPX among the three cell lines, followed by low/NGF-differentiated PC12 cells. The various drug sensitivity of the three PC12 cell lines proved that researchers should be cautious when selecting PD pathological model used for drug screening, diagnosis, and prognosis investigation.

Evaluation of cell viability intervened with various substances using electrochemical sensor

The cells responding to MPP^+, PPX, and BMSCs were recorded to evaluate drug sensitivity of the three PC12 cell lines. Cell morphology is related to cell structure, function, and their circumstances. Thus, morphological changes as visualized index and cell viability as biological function indicator were applied to measure cell injury. Three PC12 cell lines maintained in 12-well plates were divided into five groups, including blank control group, negative control group, MPP^+ group, PPX + MPP^+ group, and BMSCs + MPP^+ group. Cells were maintained in RPMI 1640 medium supplemented with 10% FBS for 72 h in blank control group, while cells were cultivated in serum-free medium for 72 h in negative control group. Compared with blank control groups, cells in serum-deficient medium appeared to be feeble, embodying in reduced attachment, weakened proliferation, and abated cell–cell interaction (Fig. S11). Similarly, the peak value of negative control group was smaller than blank control group, indicating that neurosecretion activity was weakened and serum-deficient medium is in favor of the construction of PD cell model (Fig. 3G and Table 2). The cells in blank control group were cultured in medium containing FBS, which involving various plasma proteins, peptides, fats, carbohydrates, growth factors, hormones and inorganic substances, etc. These substances probably disturb pathological damage of MPP^+ to PC12 cells, leading to modeling failure. After MPP^+ treatment in serum-free medium, the adverse impact exacerbated, especially significantly reduced cell number (Fig. S11). For highly-differentiated PC12 cells, neurite length was greatly shortened with damage of MPP^+, accompanied with the cleavage of neurites (Fig. S11B). Meanwhile, neurotransmitter release was negatively affected by the neurotoxicity of MPP^+ and decreased, implying all the three cell lines suffered serve neurosecretory dysfunction (Fig. 3G). These characteristics highly resemble with PD features involving the loss of dopaminergic neurons and decline in neurotransmitter level [28]. However, preconditioned with PPX, cellular state was markedly reversed, such as, enhanced cell number, elongated neurite, and stereoscopic cell body, certifying the cytoprotective effect of PPX (Fig. S11). Correspondingly, the peak current of neurotransmitters boosted in various extent, indicating that the suppressed neurosecretion function was evidently attenuated (Fig. 3G). The elevated neurotransmitter level verified that PPX did play the role of dopamine receptor agonists. BMSCs have been reported to promote neurological functional recovery, improve angiogenesis, increase cell proliferation, and decrease cell apoptosis, which may be related to the powerful paracrine capability of BMSCs [29]. With intervention of BMSCs, neurotransmitter expression was elevated distinctly compared with MPP^+ group, which was coincident with the report. Additionally, numerical calculation was carried out, and the amount of neurotransmitters released per cell under different condition is shown in Table 2. Notably, as a result of manual cell counting, the calculated single-cell neurotransmitter secretion levels fluctuated. Moreover, the enhanced neurotransmitter peak current in BMSCs + MPP^+ group manifested BMSCs cocultivation had better competence to repair neurological function than PPX, thanks to neuroprotective effects of exosome as well as neurotrophins released from BMSCs. The positive result for the use of BMSCs provided a potential direction for the therapy of neurodegenerative disorders.

Table 2 Comparison of catecholamine neurotransmitter release per cell in various groups ($n = 3$). Compared with MPP^+ group, $^{****}P < 0.0001$, $^{***}P < 0.001$, $^{*}P < 0.05$

PC12 cell type	Blank control (fmol)	Negative control (fmol)	MPP^+ (fmol)	PPX + MPP^+ (fmol)	BMSCs + MPP^+ (fmol)
Low-differentiated	0.614 ± 0.13	0.0917 ± 0.039	0.0530 ± 0.0070	$0.0721 \pm 0.0070^{***}$	$0.0809 \pm 0.0062^{****}$
NGF-differentiated	25.2 ± 0.98	0.292 ± 0.0037	0.191 ± 0.0069	$0.210 \pm 0.014^{*}$	$0.243 \pm 0.036^{****}$
Highly differentiated	21.2 ± 1.2	0.217 ± 0.0041	0.152 ± 0.0061	$0.184 \pm 0.0065^{***}$	$0.194 \pm 0.025^{****}$

Conclusion

In this work, an electrochemical sensing technique was used to determine cellular biological function (neurosecretion activity), to closely reflect cellular dysfunction and intervention processes, on the premise that dead cells are deprived of endocrine functions. Compared with other electrochemical sensors for detecting neurotransmitters released from living cells, the established sensor possessed a relatively wide linear range and low detection limit (Table S3). However, this sensor still has some shortcomings, such as incapability of in situ exocytosis detection and relatively poor selectivity. In summary, the developed sensor is expected to provide scientific experimental basis and technical support for the construction of neurodegenerative disease models, as well as the research and development of new drugs, drug screening, and disease course tracking.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00604-024-06250-2.

Acknowledgements The authors gratefully acknowledge the financial support of the National Natural Science Foundation of China (82373838) and Joint Funds for the Innovation of Science and Technology, Fujian Province (2020Y9009).

Author contribution Yu Zhong: investigation, data curation, writing—original draft, writing—review and editing; Meng-Meng Liu: investigation, writing—original draft; Ji-Cheng Li, Tai-Cheng Lu, Xia Cao, and Yuan-Jie Yang: investigation; Yun Lei and Ai-Lin Liu: conceptualization, supervision, project administration, funding acquisition, writing—review and editing.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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