Therapeutic effect of microencapsulated porcine retinal pigmented epithelial cells transplantation on rat model of Parkinson's disease

Hou-Liang ZHANG, Jian-Jun WU, Hui-Min REN, Jian WANG, Ya-Ru SU, Yu-Ping JIANG

Department of Neurology, Huashan Hospital, Fudan University, Shanghai 200040, China

Abstract: **Object** To investigate the therapeutic effect of microencapsulated porcine retinal pigmented epithelial cells (RPE-M) transplantation on rat model of Parkinson's disease (PD). **Methods** Primary porcine RPE cells were harvested by enzyme digestion and expanded in culture medium. Determine the levels of dopamine (DA) and homovanillic acid (HVA) by high performance liquid chromatography electrochemical (HPLC) assay, and the levels of brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) were detected by ELISA. Alginate-polylysine-alginate (APA) microencapsulated cells were produced by using a high voltage electrostatic system. PD rat model was established by unilateral injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB). After that, the RPE-M was transplanted into the corpus striatum of PD rat, and then the rotation test scores were recorded and biochemical changes of the corpus striatum were tested. **Results** The levels of DA, HVA, BDNF and GDNF secreted by RPE were stable in the RPE culture supernatant and were not changed by the microencapsulation. Eighty-three percent rats developed PD by unilateral lesion of 6-OHDA in the MFB. The RPE-M transplantation had therapeutic effect on 33% PD rats. **Conclusion** Porcine RPE cells grow actively *in vitro* and could secrete DA, HVA, BDNF, and GDNF constantly, which does not be affected by the passage culture and the APA microencapsulation. RPE-M transplantation of may be a curative therapy for PD.

Keywords: retinal pigment epithelium; dopamine; microencapsulations; transplantation; Parkinson's disease

1 Introduction

Parkinson's disease (PD) is characterized by degeneration of the dopamine (DA) neuron in the substantia nigra (SN) and reduction of the DA release in the corpus striatum. Implanting DA-secreting cells into the corpus striatum is a promising treatment for PD. One of the rare DA-secreting cells is the retinal pigment epithelial (RPE) cells, which contain tyrosine hydroxylase (TH) and could secrete DA^[1]. Besides, the brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) secreted by RPE cells may be of benefit to the transplantation cells^[2]. In this research, the therapeutic effect of microencapsulated RPE on PD rats was studied.

2 Material and methods

2.1 Animal model establishment^[7] and grouping A total of 30 male Sprague-Dawley rats (provided by Agricultural Science Academy of China) weighing 200–225 g were used in this work. The rats were maintained in a temperature-controlled environment under a 12-h light/dark cycle with free access to food and water before the experiments. After being anesthetized with 10% chloral hydrate (0.3 mL/100 g, i.p.), the animals were placed into a stereotaxic frame with the mouth bar set at 2.4 mm (flat skull). The 6-hydroxydopamine (6-OHDA, 16 µg; Sigma) dissolved in the isotonic NaCl containing 0.02% vitamin C were injected into the right medial forebrain bundle (MFB) of rat using a 10-µL syringe following the coordinate (with reference to bregma and dura) AP –4.4 mm, LP 1.2 mm, and DV 7.8 mm. The syringe was left in place for an additional 5 min before being slowly retracted. The injection speed was 1 µL/min.

The rotational behavior induced by apomorphine (0.6 mg/kg, i.p.; Sigma) was monitored over 30 min at the first week and the third week after lesion. The turning rates were

Corresponding author: Yu-Ping JIANG
Tel: 86-21-62490808
E-mail: napianghai518@hotmail.com
Article ID: 1673-7067 (2007) 03-0137-08
CLC number: R742.5
Document code: A
Received date: 2006-11-21

recorded as turns/min. Only those rats exhibiting a rotation rate of more than seven full body turns/min ipsilateral to the lesion side were included in the study. The eligible rats were divided into three groups as following: microencapsulated RPE cells group ($n = 9$), saline group ($n = 6$) and empty microcapsule group ($n = 6$). Additional 8 normal rats were used to identify the normal contents of DA, homovanillic acid (HVA), and 3,4-dihydroxyphenylacetic acid (DOPAC) in rat striatum by high performance liquid chromatograph (HPLC) and TH immunohistochemistry.

2.2 Culture of porcine RPE (pRPE) cells^[3] RPE cell sheets were isolated from the freshly enucleated globes of pig (age range 1–2 weeks). The globes were taken out at half an hour after animal death, and maintained in cold storage for 0.5 h prior to plating. The eyes were washed with saline solution containing 0.1 mg/mL of streptomycin and 100 U/mL of penicillin (Gibco, USA). The anterior segment was removed from the hemisection of the globe at the ora serrata. The retina was then detached from the pigment epithelium and dissected away from the optic nerve. After rinsing with D-hank's saline, the eyecup was placed at the bottom of a 50-mL centrifuge tube with the scleral side down and immersed in the dispersal solution [0.25% (W/V) trypsin, 0.1% (W/V) hyaluronidase and 2% (W/V) fetal calf serum in physiologic saline] for 30 min at 37 °C. The RPE cells were removed from the Bruch's membrane of the eyecup by gentle pipetting and then transferred into a tube containing 3 mL of culture medium [Dulbecco's modified Eagle's medium (DMEM) and Nutrient Mixture F12, 1:1 mixture; and 20% fetal bovine serum (Gibco)]. After being centrifuged at 800 g, the cells were resuspended in 10 mL of culture medium and plated in two 25-cm² tissue culture flasks for incubating 1 h at 37 °C in 5% CO₂. The pRPE cells were purified by removal of the culture medium and transferred into a new tissue culture flask (adherent fibroblastic cells were discarded). Repeat this process again to obtain the highly purified RPE cells.

Primary cultures were at 37 °C in 5% CO₂ for 3 d. pRPE cells were routinely passaged by 0.05% (W/V) trypsin and 0.02% (W/V) ethylenediaminetetraacetic acid (EDTA) in physiologic saline, followed by replating at a split ratio of 1:3 to 1:6. The passage 2 pRPE cells were plated on 8-well chamber slides at a density of 1×10^5 cells/cm² and grown for 3 d in serum-contained medium and then used for immunocytochemistry with cytokeratin monoclonal antibody (Lab Vision Co., USA), S-100 monoclonal antibody (Lab Vision

Co., USA) and anti-TH antibody (Sigma)^[4].

The medium was replaced every 5 d. The culture medium for the passage 2 RPE was collected at 2, 4, 8, 12, 24, 36, 48, 60, 72, and 96 h after the passage and the culture medium for each RPE passage was collected at 48 h after seeding.

DA and HVA concentrations in the medium was measured by HPLC^[5]. The analytical system was consisted of a 1049A electrochemical detector, G1311A pump, G1322A degasser, and a G1313A autosampler. A Hypersil BDS C18 column (150 × 2.1 mm I.D., Agilent, USA) was employed for chromatography. The HPLC detector potential was set at 0.65 V. The mobile phase (citric acid 30.2 mmol/L, sodium acetate 76.2 mmol/L, sodium heptanesulfonate 200 mg/L, EDTA 50 mg/L, natrium aceticum 76.2 mmol/L, NaCl 5.28 mmol/L, and 15% methanol in HPLC grade water, final pH 4.8) was delivered at a flow rate of 0.25 mL/min at 38 °C. Aliquots (20 µL) were injected by an autosampler with the cooling module set at 4 °C.

GDNF and BDNF were measured with ELISA (Promega) and recorded with timetable^[2].

2.3 Encapsulation^[6] Briefly, a mixture of pRPE cells and 2.4% sodium alginate (Sigma) were extruded into a ice-cold 1.1% CaCl₂ solution by a high-tension electrostatic droplet generator (University of Shanghai for Science and Technology). After ionotropic gelation (10 min), the droplets were coated with low-relative-molecular-mass poly-L-lysine (PLL) (Mr, less than 10 000; Sigma) by incubated in 0.05% PLL solution for 3 min at room temperature. The coated particles were mixed with 0.24% sodium alginate again for 6 min, and then with 0.1 mmol/L citrate sodium for 3 min. These "composite" microcapsules were approximately 200–300 µm in diameter (approximately 83 cells per microsphere). Microencapsulated RPE cells were cultured in DMEM at 37 °C in 5% CO₂.

2.4 Intrastratial transplantation of pRPE cells^[8] The microcapsules containing pRPE cells, microcapsules only or physiologic saline were implanted into the right striatum of PD rats in different groups. Stereotaxic injections were performed using a 20-µL syringe following the coordinate (from bregma): AP +0.8 mm, LP –2.8 mm, DV –5.6 mm, and AP –0.8 mm, LP –4.5 mm, DV –5.6 mm. The injection rate was 1 µL/min. The needle was left in place for an additional 2 min to prevent reflux before being slowly withdrawn.

2.5 Assessment of the effect Rotation test was performed at the first, second, fourth, eighth, and twelfth week after

the cell transplantation. The therapeutic effect was assessed according to the percent of reduction in rotation rate.

TH immunohistochemistry was used to detect the survival RPE cells. Twelve weeks after the lesion, rats were deeply anesthetized with 10% chloral hydrate (0.35 mL/100 g, i.p.) and perfused through the ascending aorta with 50 mL of 1% PBS (pH 7.4), followed by 120 mL of 10% formalin solution. The brains were removed and dehydrated in 20% sucrose solution for 1 d and then in 30% sucrose solution for 2 d, till the brain sank to the bottom of the bottle. Coronal sections were cut at 15-mm thickness on a freezing microtome and processed for TH immunohistochemistry^[9].

The concentrations of DA, HVA and DOPAC in rat brain were determined by HPLC. Rats were deeply anesthetized and the brains were removed rapidly. The striatum were dissected quickly, frozen and stored at -20°C . The tissue samples were weighed and placed in 1.5-mL plastic tubes containing ice-cooled perchloric acid (300 μL , 0.4 mol/L), homogenized for 10 s using ultrasound and centrifuged at 15 000 g for 20 min at 4°C . The supernatant was kept at -20°C until HPLC analysis was done^[5].

2.7 Statistical analysis Data were analyzed by *t*-test. All data were expressed as mean $\pm$ SD. $P < 0.05$ was considered statistically significant.

3 Results

3.1 PD models Rate of the rotation induced by intraperitoneal injection of 0.6 mg/kg of apomorphine reached a plateau 3 weeks after lesion. Among 30 rats, 25 of them rotated at more than 7 cycles/min, i.e. 83 percent of rats developed PD after the unilateral lesion in MFB by 6-OHDA. The TH immunochemistry showed that the TH-positive

Tab. 1 Contents of DA, HVA and DOPAC detected in the PD rat

	Left side of striatum	Right side of striatum
DA ($\mu\text{mol/L}$)	1.631 $\pm$ 0.044	0.201 $\pm$ 0.022*
HVA ($\mu\text{g}/\mu\text{L}$)	0.113 $\pm$ 0.042	0.070 $\pm$ 0.034
DOPAC ($\mu\text{g}/\mu\text{L}$)	0.071 $\pm$ 0.023	0.063 $\pm$ 0.021

* $P < 0.05$ vs the left (uninjured) striatum

cells and fibers in the SN and ventral tegmental area (VTA) of uninjured side (left side) were more than those in the lesion side (right side), and DA secretion in the right striatum was significantly lower than the left ($P < 0.05$).

3.2 Cell culture For the primary culture, outgrowth of RPE cells from the RPE isolates was visible under the light microscope from day 1 after seeding. The outgrowing cells showed weaker and weaker pigmentation as they grew from the original RPE sheet.

For the secondary cultures, the passages 1-4 cells reached confluence after approximately 5 d and appeared a regular cobblestone pattern, monolayer cells. The passages 5 and 6 cells showed large variations in cell size and shape, and reached confluence after approximately 2 weeks. The cells in passage 7 did not reach confluence and showed loss of hexagonal shape, multilayer and large variations in cells size and shape. Cells in each passage developed pigmentation visible under the microscope. (Fig. 1)

RPE cells were immunopositive for cytokeratin and S-100 antibodies (showing brown sedimentum in intracytoplasm), and TH (showing buffy in intracytoplasm). Melanin granules were very clear (Fig. 2).

The passage 2 RPE cells grew rapidly on day 1-4 after the seeding, and entering the platform period on day 5 (Fig. 3).

3.3 DA secretion The capacity of RPE to secrete DA had

Tab. 2 Levels of DA in the culture supernatant of different passages RPE at 48 h after seeding (ng/10⁴ cells)

	Passage 2	Passage 3	Passage 4	Passage 5	Passage 6
DA	0.209 $\pm$ 0.016	0.182 $\pm$ 0.008	0.202 $\pm$ 0.003	0.183 $\pm$ 0.023	0.193 $\pm$ 0.019

Tab. 3 Levels of DA, HVA, GDNF and BDNF in the culture supernatant of passage 2 RPE (ng/10⁴ cells)

	2 h	4 h	8 h	12 h	24 h	36 h	48 h	60 h	72 h	96 h
DA	0.143 $\pm$ 0.021	0.258 $\pm$ 0.018	0.261 $\pm$ 0.026	0.267 $\pm$ 0.031	0.229 $\pm$ 0.030	0.234 $\pm$ 0.027	0.209 $\pm$ 0.034	0.203 $\pm$ 0.025	0.215 $\pm$ 0.032	0.217 $\pm$ 0.029
HVA	2.521 $\pm$ 0.431	6.888 $\pm$ 0.662	6.074 $\pm$ 0.723	5.546 $\pm$ 0.703	4.492 $\pm$ 0.644	4.474 $\pm$ 0.612	3.799 $\pm$ 0.459	3.535 $\pm$ 0.501	3.621 $\pm$ 0.493	3.467 $\pm$ 0.415
GDNF	0.030 $\pm$ 0.005	0.034 $\pm$ 0.004	0.030 $\pm$ 0.004	0.028 $\pm$ 0.003	0.030 $\pm$ 0.005	0.031 $\pm$ 0.003	0.032 $\pm$ 0.002	0.033 $\pm$ 0.004	0.301 $\pm$ 0.005	0.033 $\pm$ 0.005
BDNF	0.014 $\pm$ 0.002	0.019 $\pm$ 0.003	0.021 $\pm$ 0.002	0.021 $\pm$ 0.003	0.023 $\pm$ 0.003	0.021 $\pm$ 0.005	0.025 $\pm$ 0.004	0.020 $\pm$ 0.004	0.020 $\pm$ 0.003	0.020 $\pm$ 0.003

Fig. 1 Cultured RPE cells. A: passage 2 cells exhibited an acanthoid shape with dense pigmentation; B: passage 7 cells did not reach confluence and showed large variations in cell size and shape. Scale bar, 80 μm in A, 40 μm in B.

Fig. 2 Result of S-100 immunohistochemical stain, (A) cytokeratin immuno-histochemical stain (B) and TH immunohistochemical stain (C) of RPE. The brown sedimentum in A and B and the buffy sedimentum in C showed the S-100, cytokeratin and TH immunopositive cells, respectively. Scale bar, 30 μm in A and B, 60 μm in C.

Fig. 3 Growth curve of the passage 2 RPE. RPE cells grew rapidly within 90 h (approximately day 1-4) after the seeding, and entering the platform period afterwards.

no significant differences among different passages ($P > 0.05$, Tab. 2). The secreted DA, HVA, GDNF and BDNF in the culture solution of the passage 2 RPE cells could be detected at 2 h after the seeding, up to peak at 4 h, and then declined to a stable state at 24 h. (Fig. 4, Tab. 3)

3.4 The result of RPE cells encapsulation Alginate-polylysine-alginate (APA) microcapsule showed globular, uniform size [diameter (245 ± 23) μm] and smooth surface under the light microscope even after repeatedly suction with a 5-mL injection syringe (Fig. 5A). Cells in microcapsule survived well, but non-division (Fig. 5B). The number of cells was counted after the microcapsule was de-

Fig. 4 DA, HVA (A), BDNF and GDNF (B) secretions of passage 2 RPE cells at different time points after the seeding.

Fig. 5 Encapsulation of RPE cells. A: Empty microcapsule. APA microcapsule showed globular, uniform size and smooth surface. B: Microencapsulated RPE cells. Sporadic RPE cells in microcapsule survived well. Scale bar, 100 μm in A, 50 μm in B.

Fig. 6 Concentrations of DA in the culture supernatant of the passage 2 RPE cells at different times after microencapsulation.

stroyed with 1 mol/L NaOH, and the average number of cells in a microscope was 81 ± 6 . The DA secretion capacity of encapsulated RPE cells was shown in Fig. 6.

3.5 Assessment of the therapeutic effect The lesioned

Fig. 7 Rotation test in different groups after RPE cells transplantation.

Fig. 8 TH immunochemical staining of microencapsulated (A) and microcapsule disintegrated (B) RPE cells in the striatum of rat. Black arrow indicates the microencapsulated RPE; red arrow indicates the vacant space where microcapsule shed when staining. The open arrow in B indicates the broken microcapsule. Scale bar, 40 μm in A, 80 μm in B.

Tab. 4 Result of DA, HVA and DOPAC detection in rat's striatum after RPE transplantation

	Normal group (<i>n</i> = 8)	Microencapsulated RPE group		Empty microcapsule group (<i>n</i> = 6)	Physiologic saline group (<i>n</i> = 6)
		Effective (<i>n</i> = 3)	Inefficient (<i>n</i> = 6)		
DA (μmol/L)	1.631±0.044	1.460±0.621*	0.382±0.023	0.192±0.012	0.202±0.012
HVA (μg/μL)	0.113±0.042	0.081±0.003	0.070±0.012	0.065±0.020	0.071±0.013
DOPAC (μg/μL)	0.071±0.023	0.052±0.003	0.051±0.014	0.050±0.012	0.060±0.024

**P* < 0.05 vs the inefficient rats of the microencapsulated RPE cells group, the rats of the empty microcapsule group or the rats of the physiologic saline group

animals of saline group had no alteration in the rate of apomorphine-induced rotation. Empty microcapsule alone was found only produce a transient decrease in the rotation rate, and this effect lasted less than 2 weeks. After cells were implanted in the striatum of PD rats, three of them showed a long-term reduction in apomorphine-induced rotation rate, but the others (*n* = 6) showed no difference with the saline group and the empty microcapsule group, consistent with the view that cells adhering to microcapsules exhibit long-term survival time (Fig. 7). Inspection of these data illustrates an experimental effect of the implant in 33% rats. Statistical analyses showed highly therapeutic effect of microcapsule-adhered cells on the PD rats (*P* < 0.01).

TH immunohistochemistry showed that microencapsulated RPE cells survived well in the rats whose symptoms were improved (Fig. 8A), and no RPE cells but some broken microcapsules were observed in those rats whose symptoms were not improved (Fig. 8B). The concentrations of DA, HVA and DOPAC in rat brain detected with HPLC were shown in Tab. 4. *t*-Test showed that the DA secretion of effective rats in microencapsulated RPE group increased significantly compared with the inefficient rats of microencapsulated RPE cells group, rats of empty microcapsule group and saline group (*P* < 0.05), similar to the rats of normal group (*P* > 0.05). The secretion of HVA and DOPAC had no significant difference among groups (*P* > 0.05).

4 Discussion

PD is characterized by degeneration of the dopaminergic neurons in the SN. The pathophysiology of PD has been attributed to the dramatic drop in DA content within the striatum, particularly in the postcommisural putamen, secondary to the degeneration of SN neurons. Pharmacological therapeutic strategies for PD are currently centered on replacement of DA in the brain by exogenous supplementation of DA precursors (levodopa), DA agonists, catechol-O-methyltransferase (COMT) inhibitors, or the

drugs that modulate glutamate- or acetylcholine-mediated neurotransmission.

Considering disabling side effects occurred in majority PD patients produced by these medicines, cell transplantation is one of the methods to continuously release levodopa without these complications occurrence. RPE cells have the advantages of easily obtainable large quantities, DA secretion, and high survival after transplantation^[1]. RPE cells also have been reported to produce GDNF and BDNF, which may be beneficial to the transplantation cell. These advantages make RPE cells proper for transplantation. Our method of primary culture yielded the cells that could propagate for seven passages with well preserved morphological features, but would not breed anymore after seven passages. We found that the cells tend to deteriorate in morphology after several weeks in culture. The over time deterioration may be due to a constant high rate of cell turnover that the apoptosis of cells was replaced by continuing proliferation, or may be due to some structural or functional defects in the cultured cells. It was found that the bovine RPE cells had the DA secretion property that was not changed by microencapsulation, which is important for cell transplantation^[2]. Because of the closer genetic homogeneity between pig and human being, we chose pig as the cell donor to avoid the potential prion virus infection and expected this method could be helpful for PD patients. The DA, HVA, GDNF and BDNF secretions of RPE cells could be detected at 2 h after seeding, up to peak at 4 h, and then declined to a stable state at 24 h. We presume that the original peak is a response to stimulation, and the later stable state is the normal secretion state.

The key point of cell transplantation is to avoid immunological rejection. APA microencapsulated cells may get the point and prolong RPE cells life span. The microcapsule has the selective permeability to permit the micromolecules such as DA release and to preclude the immunocyte (lymphocyte and macrophage) and immunoelement

(immunoglobulin, antibody, complement, etc) from getting in^[10]. The cells were spherical, aggregated together or scattered, and suspended in the microencapsule with no adherence. The cells did not proliferate during the four surveying weeks, but the DA, HVA, BDNF and GDNF secretions of RPE cells did not change after encapsulation. This may be due to the loss of normal culture condition for adherent growth, so RPE cells could not breed, but the secretion capability and the metabolic activity were not affected.

Animal model of PD was normally established by peritoneal injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP) or 6-OHDA stereotaxis impairing SNc, but the stability and the success rate for model establishment was not ideal. By injecting 6-OHDA in the MFB, 83% rats successfully developed PD with positive rotation test just one week after the surgery in our research. TH immunochemical staining showed more DA neurons and fibers in the SN and VTA of the uninjured side (left side) than in the lesion side (right side).

Subramanian T *et al.* transplanted the human RPE cells attached to microcapsules into the striatum of PD rats^[2,11]. They found that the rotation test of the models was improved with an average of 41%. They surveyed for 18 weeks, but did not detect the secretion of DA, HVA and DOPAC. In the present study, we found that the effective rats in the microencapsulated RPE group secreted much more DA than the inefficient ones, the rats in empty microcapsule group or the rats in physiologic saline group ($P < 0.05$). However, the HVA and DOPAC secretion had no significant difference among groups, which may be due to the accumulation of HVA and DOPAC attenuating the differences. We also proved that rotation test was not improved apparently if just only a few cells survived. Symptoms were improved significantly just until the survival cells were up to a certain degree.

In conclusion, the transplantation of microencapsulated RPE cells has a certain therapeutic effect on part PD rats with, but not all. Efforts need to focus on how to enhance microcapsule stability without changing other features, e.g. permeable for nutrient substance, to decrease the implantation injury and improve the therapeutic effect.

Acknowledgements: This work was supported by the

Shanghai Science Committee Foundation (No. 04ZR14021) and the Special Scientific Research Foundation for Doctoral Discipline Area of the Institution of Higher Learning (No.20040246035).

References:

- [1] Smith SB, Zhou BK, Orlow SJ. Expression of tyrosinase and the tyrosinase related proteins in the Mitf^{vit} (vitiligo) mouse eye: implications for the function of the microphthalmia transcription factor. *Exp Eye Res* 1998, 66: 403-410.
- [2] Subramanian T, Marchionini D, Potter EM, Cornfeldt ML. Striatal xenotransplantation of human retinal pigment epithelial cells attached to microcarriers in hemiparkinsonian rats ameliorates behavioral deficits without provoking a host immune response. *Cell Transplant* 2002, 11: 207-214.
- [3] Wu Z, Rogers B, Kachi S, Hackett SF, Sick A, Campochiaro PA. Reduction of p66Shc suppresses oxidative damage in retinal pigmented epithelial cells and retina. *J Cell Physiol* 2006, 209: 996-1005.
- [4] Vinore SA, Henderer JD, Mahlow J, Chiu C, Derevjani NL, Larochelle W, *et al.* Isoforms of platelet-derived growth factor and its receptors in epiretinal membranes: immunolocalization to retinal pigmented epithelial cells. *Exp Eye Res* 1995, 60: 607-619.
- [5] Hollenbach E, Schulz C, Lehnert H. Rapid and sensitive determination of catecholamines and the metabolite 3-methoxy-4-hydroxyphen-ethyleneglycol using HPLC following novel extraction procedures. *Life Sci* 1998, 63: 737-750.
- [6] Sun Y, Ma X, Zhou D, Vacek I, Sun AM. Normalization of diabetes in spontaneously diabetic cynomolgus monkeys by xenografts of microencapsulated porcine islets without immunosuppression. *J Clin Invest* 1996, 98: 1417-1422.
- [7] Yuan H, Sarre S, Ebinger G, Michotte Y. Histological, behavioural and neurochemical evaluation of medial forebrain bundle and striatal 6-OHDA lesions as rat models of Parkinson's disease. *J Neurosci Methods* 2005, 144: 35-45.
- [8] Stover NP, Bakay RA, Subramanian T, Raiser CD, Cornfeldt ML, Schweikert AW, *et al.* Intrastriatal implantation of human retinal pigment epithelial cells attached to microcarriers in advanced Parkinson disease. *Arch Neurol* 2005, 62: 1833-1837.
- [9] Nishino S, Honda K, Riehl J, Okura M, Mignot E. Neuronal activity in the cholinceptive basal forebrain of freely moving narcoleptic dobermans. *Neuroreport* 1998, 9: 3653-3661.
- [10] Liu ZC, Chang TM. Transdifferentiation of bioencapsulated bone marrow cells into hepatocyte-like cells in the 90% hepatectomized rat model. *Liver Transpl* 2006, 12: 566-572.
- [11] Subramanian T. Cell transplantation for the treatment of Parkinson's disease. *Semin Neurol* 2001, 21: 103-115.

微囊化猪视网膜色素上皮细胞移植治疗帕金森病的实验研究

张厚亮, 邬剑军, 任惠民, 王坚, 苏雅茹, 蒋雨平

复旦大学附属华山医院神经内科, 上海 200040

摘要: **目的** 研究微囊化后的猪视网膜色素上皮细胞(retinal pigment epithelial, RPE)对帕金森病大鼠模型的移植疗效。**方法** 原代培养 RPE 并传代, 高效液相色谱法测定培养液上清中多巴胺(dopamine, DA)和高香草酸(homovanillic acid, HVA)的含量, ELISA法检测脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)和胶质细胞源性神经营养因子(glial-derived neurotrophic factor, GDNF)的含量。用高压静电成囊装置制备海藻酸钠-多聚赖氨酸-海藻酸钠微囊化细胞。6-羟基多巴胺(6-hydroxydopamine, 6-OHDA)毁损内侧前脑束 (medial fore-brain bundle, MFB)建立 SD 大鼠帕金森病模型。立体定向移植 RPE+ 微囊, 检验旋转实验、免疫组化和脑内生化的变化。**结果** RPE 培养上清液中 DA、HVA、BDNF、GDNF 的含量稳定, 微囊化后细胞长期存活, 活性没有明显变化。6-OHDA 毁损 MFB 建立大鼠帕金森病模型的成模率为 83%。移植微囊化的 RPE 后有效率为 33%。**结论** 猪 RPE 体外培养生长旺盛, 持续分泌 DA、BDNF 和 GDNF, 微囊化不影响其分泌功能。RPE 移植对帕金森病大鼠有一定的治疗作用。

关键词: 猪视网膜色素上皮细胞; 多巴胺; 微囊化; 移植