Research Article

Effect of Gold/ Fe_3O_4 Nanoparticles on Biocompatibility and Neural Differentiation of Rat Olfactory Bulb Neural Stem Cells

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Transplantation of stem cells is a potential clinical therapy for repair of central nervous system injury. However, transplanted cells are especially difficult to arrive at the targeted site because of poor survival rate and low efficiency. Recently, gold nanoparticles (NPs) and Iron oxide NPs, as novel nanoparticles, have been used as auxiliary strategy to investigate the nervous system diseases. The present study demonstrates the effect of Gold/ Fe_3O_4 NPs on biocompatibility and differentiated properties of rat olfactory bulb stem cells. Cell viability was assumed by MTT test and cytotoxicity was assessed by Hoechst 33342-PI stain. Cells were cultured at Gold/ Fe_3O_4 NPs concentration range of 40 to 200 $\mu\text{g}/10^4$ cells for 24 h. Differentiation was assessed by NSE (a neuronal marker) stain. Results showed that Gold/ Fe_3O_4 NPs at the concentrations of 40 $\mu\text{g}/10^4$ cells enhanced cell viability and decreased the cell death rate. Furthermore, the differentiation properties were detected by NSE marker. These findings suggest that Gold/ Fe_3O_4 NPs may thus be used as new nanotechnologies in stem-cell-based transplantation therapies for diagnosis and treatment of central nervous system diseases.

1. Introduction

With industrialization of society, central nervous system diseases are becoming an important public health problem [1]. The central nervous system, consisting of spinal cord and brain, is commonly affected by trauma, inflammation, infection, and tumor [2]. Disorders and diseases that injure the central nervous system may produce a variety of symptoms such as paraplegic, quadriplegic, and motor and sensory disorders, which has a significant impact on the quality of life, and the patients' health became increasingly frail [3]. Therefore, the development of new technologies to repair damaged central nervous system is important for the patients' quality of life.

Transplantation of stem cells is a potential clinical therapy for repair and regeneration of injured spinal cord or brain. Neural stem cells/neural precursor cells (NSCs/NPCs) derived from the subventricular zone (SVZ)/olfactory bulb (OB) of mammals persist and proliferate throughout life [4], serving as ideal sources of stem cells for subsequent central

nervous system transplantation [5]. However, transplanted cells are especially difficult to migrate in the targeted site because of poor survival rate and low efficiency [6]. Factors proposed for causing such low survival rate include immune reactions, limited trophic factors, and hypoxia [7], so it appears that developing new technologies to deliver stem cell into transplant site may provide insights into the stem-cell-based therapy.

Recently, the Gold/ Fe_3O_4 NPs become a new research focus because of their magnetic properties, huge surface areas and limited cytotoxicity. However, the study about their properties and applications in neural stem cells therapy is limited. In addition, Gold/ Fe_3O_4 nanoparticles (Gold/ Fe_3O_4 NPs), as magnetic nanoparticles, are being extensively investigated for use as drug carriers and in diagnosis of diseases [8]. Recently, there is accumulating evidence that nanoscale materials can facilitate tissue engineering and stem cell therapy [9]. Restoration of body function is the desire of people with nervous and bone injuries [10–14]. Region-specific cues are important in the neuronal differentiation of stem cells.

The process of NSCs toward certain differentiation may be promoted by nanoparticles [15]. Nanotechnology provides a broad range of opportunities to develop new methods for clinical therapy [16]. Therefore, the development of new nanotechnologies to enhance cell survival and differentiation is crucial to stem cell therapy.

In this study, we have introduced novel Gold/ Fe_3O_4 magnetic nanoparticles. The *in vitro* effects of Gold/ Fe_3O_4 nanoparticles on NPCs were investigated, and cell viability, cytotoxicity, and differentiation were studied.

2. Methods

2.1. Materials. The Gold/ Fe_3O_4 nanoparticles (50 nm, 5 mg/mL) in an aqueous suspension were obtained from GoldMag Nanobiotech Co. Ltd. (Xi'an, China) [17]. DMEM/ F^{12} , basic fibroblast growth factor-2 (bFGF-2), Epidermal growth factor (EGF), and trypsin were obtained from *Invitrogen* Corp. (CA, USA). Hoechst 33342 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Monoclonal mouse anti-Nestin, monoclonal mouse anti-NSE, rabbit secondary antibodies conjugated with the fluorescent dye Cy3, and total (rabbit polyclonal antibody) were purchased from Chemicon (USA). Streptomycin and penicillin were purchased from Amresco (USA). Other chemicals used in this study were obtained commercially and were of reagent grade.

2.2. Characterization and Fabrication. Gold/ Fe_3O_4 NPs were prepared as described earlier [17]. The synthesized Gold/ Fe_3O_4 NPs were characterized by scanning electron microscopy (SEM) (FE-SEM, Leon 1530 with EDS) as previously described methods [18–20].

2.3. Rat NPC Cultures and Characterization. NPCs were prepared from the olfactory bulbs of neonatal Sprague-Dawley rats. All animals used in the experiments were provided by the animal center of the Peking University. Procedures concerning animals reported in this study were approved by the Committee of Animal Use for Research and Education of Beihang University. All procedures were prepared as described in previously studies [21]. After mechanical dissociation of dissected and pooled olfactory bulbs and enzymatic digestion with 0.125% trypsin, the neurosphere populations were collected. All cells were seeded in DMEM/ F^{12} that was supplemented with streptomycin (50 $\mu\text{g}/\text{mL}$), penicillin (50 U/mL), bFGF (20 ng/mL), and EGF (20 ng/mL). Neural progenitor cells were obtained from at least two passages neurospheres. In experimental conditions (Gold/ Fe_3O_4 NPs at the concentrations of 0, 40, and 200 $\mu\text{g}/10^4$ cells), cells were cultured in these media for 24 hours. For the cell lineage analysis, the plates were fixed in 4% cold paraformaldehyde (15 min) and washed (three times, 5 min each) with PBS. The fixed cells were blocked for 30 min in PBS containing 5% normal goat serum and 0.25% Triton X-100, and then incubated overnight at 4°C with primary antibodies diluted in the PBS. Cell types were identified by monoclonal mouse

FIGURE 1: Scanning electron microscopy (SEM) photograph of Gold/ Fe_3O_4 NPs showed that Gold/ Fe_3O_4 NPs were spherical-like, well-dispersed, and uniform in size and shape, about 50 nm in diameter.

anti-Nestin. Cy3-conjugated secondary antibodies were used to detect the primary antibodies.

2.4. Cell Viability Assay. NPCs were prepared and placed in Gold/ Fe_3O_4 NPs at the concentrations of 0, 40, and 200 $\mu\text{g}/10^4$ cells for 24 h. Cell survival was determined by 3-(2,5-diphenyltetrazolium bromide (MTT) (Sigma, USA) assay as previously described methods [22]. Following the treatment, the treated and control NPCs were rinsed three times with PBS. The 200 μL aliquots of NPCs suspension ($10^5/\text{mL}$) were seeded to three 96-well plates in eight replicates, and 20 μL aliquots of MTT solution (5 mg/mL) were added to each well and incubated for 4 hr in a humidified 5% CO_2 incubator at 37°C. The supernatant culture medium was carefully aspirated after centrifuge, and 200 μL aliquots of DMSO were added to each well to dissolve the formazan crystals. ODs were read using 570 nm as a reference wavelength.

2.5. Cytotoxicity Assay. To distinguish between live and dead cells, a staining of nuclei with DNA dyes Hoechst 33342 and propidium iodide was applied as follows. After exposure, pretreated cells were trypsinized, washed with PBS, and stained with propidium iodide (5 $\mu\text{g}/\text{mL}$) and Hoechst 33342 (1 $\mu\text{g}/\text{mL}$) (Sigma, USA) for 10 min at RT. After rinsing in PBS, coverslips were examined using an Olympus BX60 fluorescence microscope equipped with a digital IX 71 camera (Olympus, Tokyo, Japan). Cells were counted, scoring at least 300 cells in 5 microscopic regions randomly selected on each coverslip. The experiments were performed in triplicate.

2.6. Immunocytochemistry. NPCs were prepared and placed in Gold/ Fe_3O_4 NPs at the concentrations of 0, 40, and 200 $\mu\text{g}/10^4$ cells for 24 h. Then treated neurospheres labeled by Hoechst 33342 were transferred to polyornithine-coated glass coverslips. The medium contained 10% fetal bovine serum. After being allowed to differentiate for seven days, the cells were verified by neuronal marker. Half of the medium was replaced every second day. The differentiated cells were fixed in 4% paraformaldehyde and incubated at 4°C overnight with monoclonal mouse anti-NSE.

FIGURE 2: Isolation and characterization of NPCs from neonatal rat OB. (a) Isolated NPCs developed into cell spheres after 7 days in culture; (b) immunocytochemistry analyses revealed that OB NPCs formed neurospheres and were stained by nestin. The scale bar corresponds to 100 μm .

FIGURE 3: Low concentration of Gold/ Fe_3O_4 NPs increases cell viability. The results of an MTT assay indicated that 40 $\mu\text{g}/10^4$ cells group enhanced the cell viability of NPCs. Cells cultured in 200 $\mu\text{g}/10^4$ cells group showed lower cell viability than other groups. * $P < 0.05$.

2.7. Statistical Analysis. The difference between groups was determined with one-way analysis of variance (ANOVA) followed by Tukey's test using Statistical Package for the Social Sciences (SPSS) 13.0 (SPSS Inc.) software. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Characterization of Gold/ Fe_3O_4 NPs. Figure 1 displays the scanning electron microscopy (SEM) photograph of Gold/ Fe_3O_4 NPs showing that Gold/ Fe_3O_4 NPs were spherical-like, well-dispersed, uniform in size and shape, and about 50 nm in diameter.

3.2. Isolation and Characterization of NPC. Newborn rat brains were removed and the olfactory bulbs were dissected. Neural precursor cells were obtained from olfactory bulb tissue. After 3–7 days in culture, the NPCs displayed rounded

spherical cells which were dividing and forming cell spheres or aggregates (Figure 2(a)). Cell types were identified by monoclonal mouse anti-Nestin, markers of neuroepithelial stem cells (Figure 2(b)).

3.3. Low Concentration of Gold/ Fe_3O_4 NPs Increases NPC Cell Viability. NPCs were prepared and placed in Gold/ Fe_3O_4 NPs at the concentrations of 0, 40, and 200 $\mu\text{g}/10^4$ cells for 24 h. The *in vitro* effect of 24 h of Gold/ Fe_3O_4 NPs on NPC viability was assessed. The results of an MTT assay indicated that 40 $\mu\text{g}/10^4$ cells group increased the cell viability of NPCs. Cells cultured in 200 $\mu\text{g}/10^4$ cells group showed lower cell viability than other groups (Figure 3).

The NPCs cultured in 200 $\mu\text{g}/10^4$ cells group resulted in a remarkable decrease in cell viability. But in the 40 $\mu\text{g}/10^4$ cells group, cell viability was higher. Therefore we presumed that the concentration of Gold/ Fe_3O_4 NPs might play an important role in cell survival and death of exogenous stem cells.

3.4. Low Concentration of Gold/ Fe_3O_4 NPs Decreases Cell Death Rate of NPC. In order to investigate the cytotoxicity properties of NPCs after 24 h exposure to Gold/ Fe_3O_4 NPs, staining with Hoechst 33342 and propidium iodide (PI) was used so that the number of dead cells (PI-positive) could be expressed as a percentage of total cells. Thus, the percentage of dead cells of the low concentration of Gold/ Fe_3O_4 NPs (at the concentrations of 40 $\mu\text{g}/10^4$ cells) was decreased compared with the control (Figures 4(a) and 4(b)). At the concentrations of 200 $\mu\text{g}/10^4$ cells group, an increase in the cell death rate. Thus, concentration range of external Gold/ Fe_3O_4 NPs affect the cell survival and death.

3.5. Low Concentration of Gold/ Fe_3O_4 NPs Enhances NPC Differentiation. With immunohistochemical staining, the differentiation of NPCs was studied through the use of markers of differentiated cells, NSE (a neuron-specific enolase) [10]. In comparison with control group, more cells were stained

FIGURE 4: Low concentration of Gold/ Fe_3O_4 NPs decreases cell death rate of NPC. The percentage of dead cells of the low concentration of Gold/ Fe_3O_4 NPs (at the concentrations of $40 \mu\text{g}/10^4$ cells) was decreased compared with the control (Figures 4(a) and 4(b)). Cells cultured in $200 \mu\text{g}/10^4$ cells group showed higher cell death rate than other groups. * $P < 0.05$.

by NSE of the low concentration of Gold/ Fe_3O_4 NPs (at the concentrations of $40 \mu\text{g}/10^4$ cells) (Figure 5).

The expression of NSE has been detected in olfactory stem cells; the NSE levels implicate a crucial role as a regulator of neuronal differentiation in stem cell.

4. Discussion

In our study, Gold/ Fe_3O_4 NPs of low concentration group, at the concentrations of $40 \mu\text{g}/10^4$ cells, resulted in a remarkable enhanced cell viability, a decrease in the cell death rate,

and enhancement of neuronal differentiation. Surprisingly, when the concentration of Gold/ Fe_3O_4 NPs was raised to $200 \mu\text{g}/10^4$ cells, the death rates began to increase. Furthermore, the differentiation properties were enhanced at low concentration group. These findings suggest that Gold/ Fe_3O_4 NPs may thus be used as new nanotechnologies in stem-cell-based transplantation therapies for diagnosis and treatment of central nervous system diseases.

Stem-cell-based therapy provides the potential for repair and regeneration of damaged neural tissue in the therapy of central nervous system injury. OB NPCs derived from olfactory bulb tissue are accessible and abundant sources of

FIGURE 5: Low concentration of Gold/ Fe_3O_4 NPs enhances NPC differentiation. The differentiation of NPCs was studied through the use of markers of differentiated cells, NSE (a neuron specific enolase). Compare with control group, more cells were stained by NSE of the low concentration of Au/ Fe_3O_4 (at the concentrations of $40 \mu\text{g}/10^4$ cells) nanoparticles (Figure 5) ($\times 100$).

stem cells for translational clinical research and can be differentiated into multiple cell lineages, including chondrocytes, myocytes, and neuronal cells [23]. Compared with other stem cells, OB NPCs are superior seed cells for autologous cell transplantation in promoting nerve regeneration, as they can be obtained by less invasive procedures and cultured with higher proliferation rates [24]. Recently, OB NPCs have emerged as an alternative treatment option for degenerative spinal cord disease and CNS degeneration disease [5, 25, 26]. Therefore, the OB NPCs have important merits in regenerative clinical application and can be considered as a strategy for future tissue engineering.

Gold/ Fe_3O_4 NPs also represent an interesting tool for MRI measurement and cancer therapy [27]. However, the role of these Gold/ Fe_3O_4 NPs in the mammalian central nervous system is still unclear. This study has shown that concentration range of external Gold/ Fe_3O_4 NPs was found

to participate in regulation of stem cells survival and death. The findings may provide insight into future efforts to cell-based transplantation therapy. The application of this new technology will further depend on scientific and technological progress that does not depend on painful invasive procedures.

The differentiation properties induced by Gold/ Fe_3O_4 NPs were detected by NSE marker. NSE is widely recognized as a neuron differentiation marker. Neuron-astrocyte interactions play a leading role in the differentiation of NPCs, and a recent study showed that NSE-positive neurons can participate in the regulation of neurogenesis [28]. Region-specific cues are important in the neuronal differentiation of the original NPCs. Recently, tissue engineering has focused on the importance of developing *in vitro* stem cell microenvironment for transplanted cells proliferation and tissue-specific differentiation [29–32]. The present study revealed

that certain factors “guide” NPCs towards certain differentiation and that the process may be promoted by Gold/Fe₃O₄ NPs.

The present study demonstrates that Gold/Fe₃O₄ NPs may be used as an auxiliary strategy and might play an important role in the stem cell transplantation therapies. Although our work suggested that concentration range of external Gold/Fe₃O₄ NPs was found to participate in regulation of stem cells survival and death, future research efforts focus on the mechanism of Gold/Fe₃O₄ NPs and stem cells remained to be elucidated.

Conflict of Interests

The authors indicate no potential conflict of interests.

Authors' Contributions

Menghang Wang and Zhou Li contributed equally to this study.

Acknowledgments

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