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Cytotoxicity of gold nanoparticles in human neural precursor cells and rat cerebral cortex

Uhn Lee,[‡] Chan-Jong Yoo,[‡] Yong-Jung Kim, and Young-Mi Yoo[*]

Department of Neurosurgery, Gachon Medical College, Gil Medical Center, Incheon, 405-760, South Korea

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Nanoparticles are promising tools for the advancement of drug delivery, medical imaging, and as diagnostic sensor. Medical nanodevices should develop miniaturization, because it would be injected into a human body. Gold nanoparticles (GNPs) with different sizes and shapes have therapeutic potential as a result of their small size, robust nature, excellent biocompatibility and optical properties. However, the application of GNPs as medical nanodevices it is necessary to know the biodegradation, biocompatibility, and development of surface coating which avoid the accumulation of nanoparticles. In this study, we carry out an *in vitro* toxicity and *in vivo* gene expression study using two kinds of GNPs. We found that GNPs toxicity is dependent on the dose or size administrated after the injected GNPs into the brain, and small particle size GNPs appeared more nestin expression compared to large particle size at short term implantation. These findings of toxicity of GNPs may play an important role in development of *in vivo* tools for the safety of GNPs.

[**Key words:** Gold nanoparticle; Cytotoxicity; Cell viability; Nestin; Glial fibrillary acidic protein; Cerebral cortex]

Nanotechnology is the engineering and manufacturing of materials at the atomic and molecular scale to produce unique or enhanced materials, products and devices. Recent advances of nanotechnology in biomedical research have shown the capability of using nanomaterials to treat intractable disease (1). Using nanomaterials, it is possible to achieve improved delivery of poorly water-soluble drugs, targeted delivery of drugs into cells or tissue, delivery of large macromolecule drugs to intracellular sites of action, and the co-delivery of two or more drugs for combination therapy. Various nanomaterials with different sizes and shapes have been developed recently for delivery vectors and diagnostic markers (2,3). Nanomaterials are exquisitely sensitive chemical and biological sensor and are used in the construction of nanobiosensors for detection of chemical as well as biological materials. Gold nanoparticles (GNPs) are the most commonly used nanomaterial in diagnostics. GNPs with different size and shapes have therapeutic potential as a result of their small size, robust nature, excellent biocompatibility and optical properties (4–6). Thus, GNPs have recently emerged as an attractive candidate for delivery of various payloads, such as a drug molecules or bio-molecules into their targets and are used as a connecting point to build biosensor for detection of disease (7–9). This approach for a diagnostic test consists of an antibody attached to GNPs instead of a fluorescent molecule (10–12). When the antibody attaches to a protein associated with the disease, GNPs light up under ultraviolet light. GNPs are much more sensitive and accurate than the fluorescent molecule tests used. The antibody conjugated GNPs specially and homogeneously binds to the surface of the cancer type cells with 600% greater affinity than to the noncancerous cells (6). Furthermore, GNPs show great potential as photothermal therapy agents and as imaging agents in living system. However, for the application of GNPs in therapy and drug delivery it is necessary to know the cellular toxicity and histocompatibility associated to them. Furthermore, the interaction between cells and nanoparticles is influenced by plasma proteins, which have been shown to coat nanoparticles instantly once they get in contact with plasma, and then biomolecules may induce phase transformations, free energy release, restructuring and dissolution at the nanoparticles surface (13). In particular case of use in central nervous system (CNS), GNPs for brain probes and implants are commonly used for recording of electrical impulses and for brain stimulation like deep brain stimulation (DBS) (14). The brain regards these implants as a foreign material and encapsulates them with glial scar tissue formation and GNPs are one of the key elements of the biosensor might be degradable during glial scar formation. Therefore, it is important to assess whether GNPs are not biodegradable during glial scar formation and for short-term brain implantation. These studies were undertaken in order to determine the cellular toxicity of GNPs with an established human neural cell line. In this study, we injected GNPs into the cerebral cortex and examine whether reactive astrocytes in the brain responded by expression the undifferentiated protein, nestin.

MATERIALS AND METHODS

Preparation of neural precursor cells Human neural precursor cells (NPCs) were prepared from the spinal cord of a single five-week human fetus after an

* Corresponding author. Tel.: +82 32 4603090; fax: +82 32 4603899.
E-mail address: gilbrain@gmail.com (Y.-M. Yoo).
‡ The first two authors contributed equally to this work.

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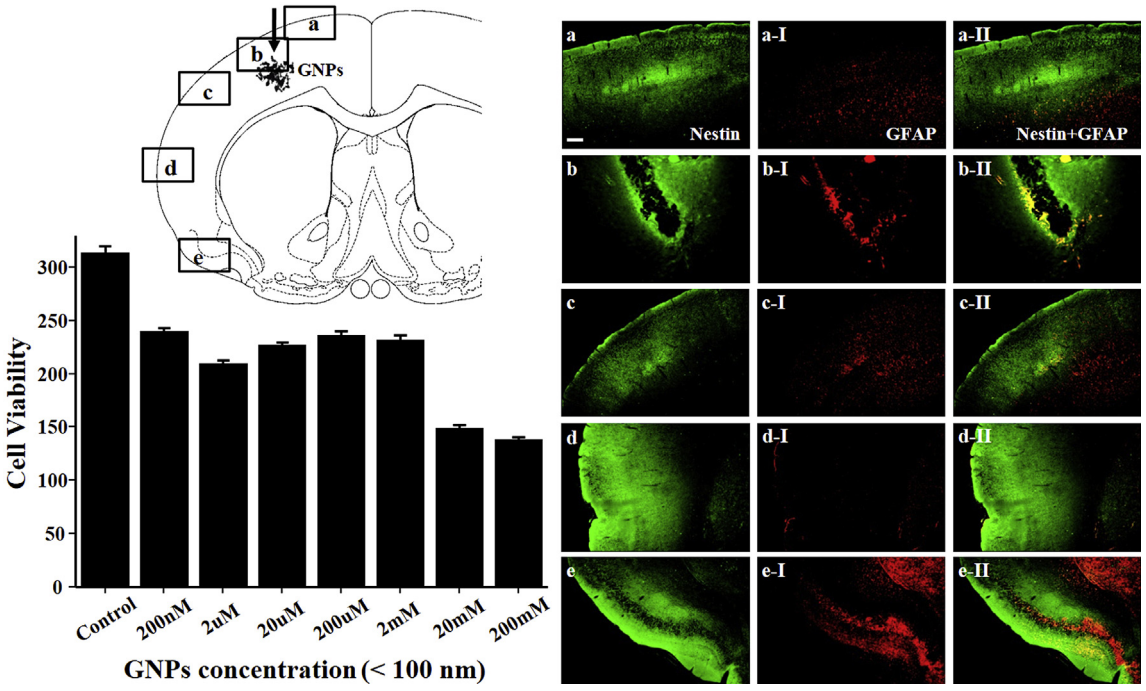

FIG. 1. Gold nanoparticles (GNPs) were injected into the rat right cerebral cortex (1.0 mm anterior to bregma, left up). The rectangles in brain map are the region that was taken a picture. The mitochondrial activity of neural precursor cells (NPCs) after 18 h exposure to the various concentrations of 100 nm GNPs injected group was determined by MTT assays (left down). To observe gliogenesis, immunohistochemistry (IHC) of nestin (green: a–e)/GFAP (red: a-I–e-I) was performed 1 week after GNPs implantation. Merged pictures showed in a-II–e-II. Scale bar is 100 μm.

elective abortion. The tissue was obtained following abortion after individual permission had been obtained by means of standard informed consent procedures and the approval of the ethics committee of the Gil Medical Center. Cells of the eighth passage were used for experiments. One cryopreserved vial of the eighth passage was thawed and plated onto one 25 cm^3 cell culture flask. After 1 day, these cells were washed, incubated, and dissociated from the culture flask with the trypsin-EDTA to make a single cell. Single cell suspension was centrifugated at 1500 rpm for 5 min and resuspended in the growth media. Cells were cultured in low glucose DMEM/F-12 with fetal bovine serum (5%). These cells were cultured at 37°C in water saturated air supplement with 5% CO_2. Culture media were changed every 3 days. For the MTT assays, dissociated cells were plate onto 96-well microtiter plates in DMEM/F-12 medium containing 5% FBS.

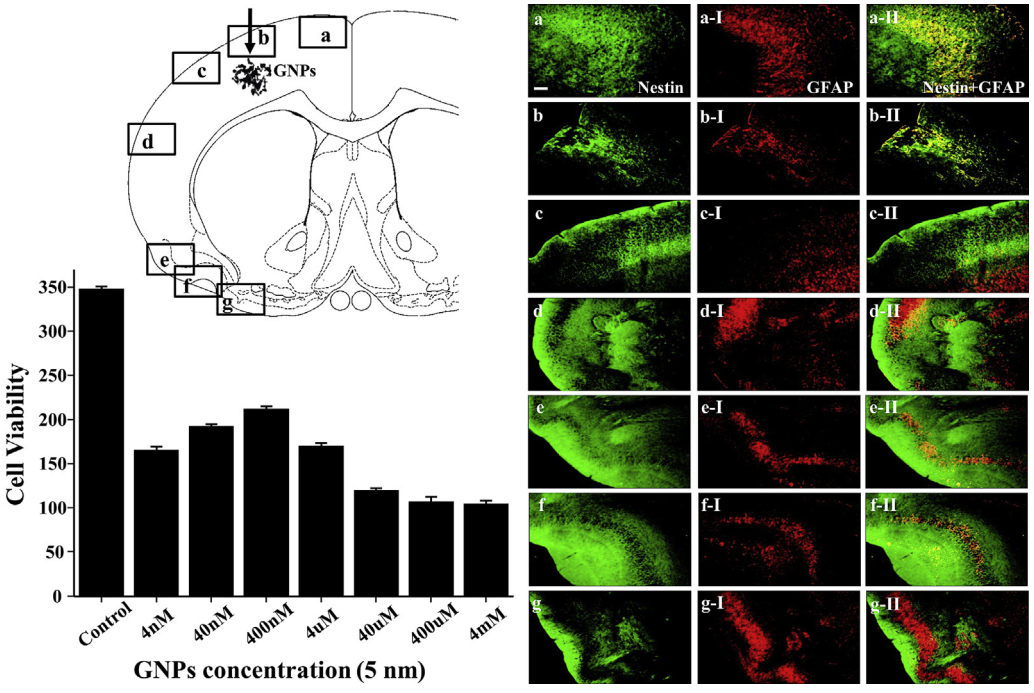

FIG. 2. Cell viability treated with different concentrations of 5 nm GNPs injected group was determined by MTT assay (left down). The rectangles in brain map are the region that was taken a picture (left up). Immunostaining images showing gliogenesis (nestin: green; a–e, GFAP: red; a-I–g-I) of 5 nm GNPs in various parts of the brain (a-II–g-II). Numerous nestin expressed cells distributed in not only injection site but also many different parts. Scale bar is 100 μm.