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In Vitro Cytotoxicity and Intracellular Bioimaging of Dendritic Platinum Nanoparticles by Differential Interference Contrast (DIC)

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We demonstrated the excellent biocompatibility of dendritic platinum nanoparticles (DPNs) in vitro using a cancer cell line (BT-20) and MTT assay. In addition, a successful observation of DPNs within live cells using differential interference contrast (DIC) microscopy without labelling fluorescent dyes has been achieved.

The utilization of nanoparticles in the biomedical field has received much attention in recent decades.[1] Recent developments in the synthesis of new inorganic nanoparticle-based imaging/delivery devices with a controllable morphology have brought a lot of possibilities to this burgeoning bioresearch area. For example, gold nanoparticles (Au NPs) have been exploited for biotechnological applications because of unique properties including strong adsorption in the near-infrared (NIR) region and high affinity toward thiol groups of biomolecules.[2] Quantum dots (ODs) have attracted much attention in bioimaging because of their sharp and high-quantum-yield emission and size-dependent emission wavelengths.[3] Iron oxide nanoparticles (IONPs) have been used in magnetic resonance imaging (MRI) and hyperthermia because of their magnetic properties.[4]

Very recently, we have reported the synthesis of a new type of metal nanoparticles consisting of platinum nanocrystallites with a unique urchin-like morphology, namely, dendritic Pt NPs (DPNs).[5] Because Pt is a well-known catalyst for heterogeneous transformation (e.g., hydrogenation),[6] a high surface area in DPNs would enhance the catalytic performance of any Pt-based reaction. In addition to high surface area and the catalytic properties, the intrinsic inertia and high affinity toward the thiol groups of biomolecules could afford DPNs various biomedical applications such as drug delivery nanovehicles and intracellular biosensors.

The most important issue when using NPs in therapeutic or bioimaging applications is their safety and toxicological issues. Only full understanding of these issues can bring NPs to clinical trial. So far, the in vitro biocompatibility of NPs including Au NPs, IONPs, and QDs with cellular systems has been widely studied by different methods and in a variety of cell lines.[7] However, the cytotoxicity of DPNs has not been reported yet. Here we report for the first time the in vitro biocompatibility of DPNs with a cancer cell line (BT-20) using a colorimetric assay (i.e., MTT assay). For the study of the transmembrane permeability of DPNs, we used DIC microscopic technology to directly visualize the distribution of DPNs inside living cells. Unlike conventional fluorescence microscopic observation that needs the labeling of fluorescent dyes, nonlabeled DPNs can be clearly seen using DIC microscopy, as shown in Figure 1.

DPNs were synthesized following our previous paper.[5] Typically, an aqueous solution containing K_2PtCl_4 and a triblock copolymer Pluronic F127 was first prepared. To this solution, an ascorbic acid solution was quickly added as a reducing agent. The mixture was placed in an ultrasonic bath for Pt deposition. A typical TEM of the synthesized DPNs is shown in Supporting Information.[8]

BT-20 human breast cancer cells (ATCC® Number: HTB-19™) were purchased from the National Health Research Institute (NHRI), Taiwan. They were maintained in flasks using DMEM (Dulbecco's modified Eagle's medium) at 37 °C with 5% CO_2 in a 95% humidified atmosphere. For MTT assay, BT-

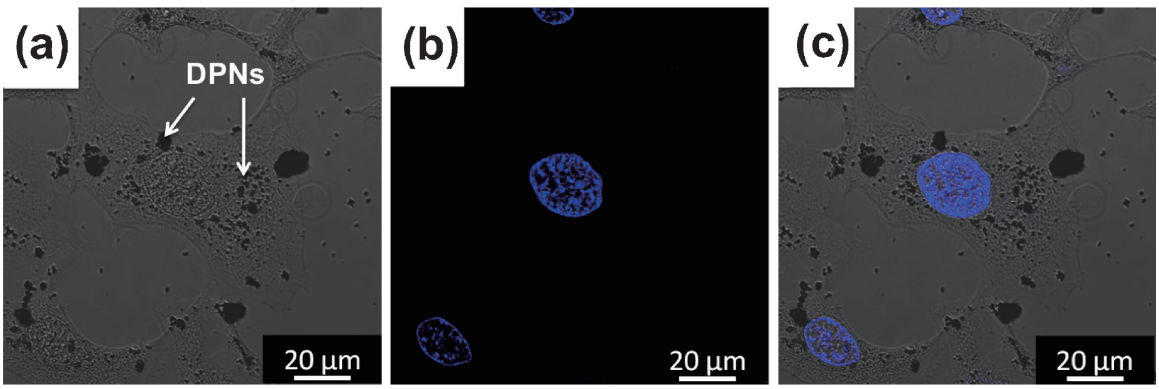

Figure 1. Confocal images of BT-20 cancer cells treated with DPNs with a dosage of 100 mg mL^{-1} for 8 h. (a) A DIC image indicates the distribution of DPNs inside the cells. (b) A fluorescent image indicates the DAPI-stained nuclei. (c) A merged image of (a) and (b).

20 cells were seeded at a density of 1×10^5 per well in 24-well plates in 0.5 mL DMEM for 24 h. After that, cells were incubated with different amounts of DPNs in serum-free medium for various durations, followed by the replacement of fresh serum-free medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.5 mg mL^{-1}) and allowed to grow for another 4 h. The absorbance at 570 nm was measured using a microplate reader (Bio-Rad, model 680). The LC_{50} (the lethal concentration of 50% cell death) value was directly determined from the cell viability data.

For confocal fluorescence microscopy measurements, BT-20 cells were seeded at a density of 1×10^5 cells per well in 4-well plates in 0.5 mL DMEM medium with Lab-Tek chambered coverglasses at the bottom of the wells. After incubation for 4 h, the DMEM medium was replaced by 1 mL of DPNs (100 μg mL^{-1}) in the serum-free DMEM medium for 4 h. The cell-plated coverglasses were then washed with medium and soaked in 4% formaldehyde in 1X PBS buffer (pH 7.4). The formaldehyde solution was then replaced by a solution containing DAPI (4′,6-diamidino-2-phenylindole) PBS buffer (1 μg mL^{-1}) for staining nuclei. The DAPI-stained coverglasses were examined with a confocal fluorescence microscopy system (Leica TCS SP5 II) using a 63 × oil immersion objective lens. The blue fluorescent, DAPI-stained nuclei could be clearly observed by exciting the cells with a UV laser. The DIC microscopy was then applied to directly observe DPNs.

The morphology and particle size were confirmed in the TEM image as shown in the Supporting Information.[8] The Pt NPs with a dendritic shape and particle sizes ranging from 13 to 23 nm with an average diameter of 17.4 nm were obtained. Each branch of the DPNs was seen to end with a rice-shaped 3 nm tip, indicating that the inner edge and corner area are accessible from outside.

In general, the transmembrane permeability of NPs was examined through observation of fluorescence microscopic images of cells. In such case, it is always necessary to label NPs with a fluorescent dye due to high transparency. However, we have found that the synthesized DPNs exhibited complete zero transparency, which appeared as black spots under microscopic observation. This unique property allows us to directly visualize DPNs using DIC microscopy. Several images with different focal depth along the *Z* axis were taken, and we chose the one where the nucleus can be clearly identified, as shown in Figure 1a. At this focal depth, many black aggregates representing the DPNs could be clearly seen, indicating the distribution of the DPNs inside the cells. The DAPI-stained nuclei could be observed as blue spots in Figure 1b. The merged image of Figures 1a and 1b is shown in Figure 1c, and it clearly shows that the black aggregates were at the same *Z* axis as nuclei, proving that the DPNs were indeed internalized by BT-20 cells. Although DIC microscopy has been used to observe mesoporous carbon nanoparticles,[9] we report here this technology is also applicable for Pt NPs, which provides a simple process for direct intracellular bioimaging.

The cytotoxicity of the DPNs was examined by MTT assay, and the result is depicted in Figure 2. The result showed the biocompatibility of the DPNs in vitro is concentration-dependent. More than 80% viability was obtained even under a high dosage of 100 μg mL^{-1}, indicating that the DPNs exhibited excellent in vitro biocompatibility. Although several studies

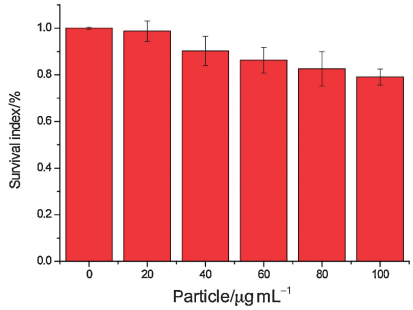

Figure 2. MTT assay of BT-20 cells treated with different amounts of DPNs, indicating good biocompatibility with a LC_{50} value larger than 100 μg mL^{-1}.

have reported that Pt NPs would reduce the cellular glutathione (GSH) level and impair DNA integrity,[10a] no obvious cytotoxicity or oxidative stress was found. In fact, the LC_{50} value of the DPNs was found to be more than 100 μg mL^{-1} in the present study. Such a high value is similar to other well-known biocompatible inorganic NPs such as Au NPs. In addition, a previous study has reported that flower-shaped Pt NPs exhibited higher cellular uptake than multipod-shaped Pt NPs,[10b] which implies that our DPN (similar to flower-shaped Pt NPs) would also have enhanced cellular uptake.

In conclusion, we prepared dendritic Pt nanoparticles and examined their in vitro cytotoxicity and transmembrane permeability using MTT assay and DIC microscopy with BT-20 human breast cancer cells. The results obtained here indicated that DPNs have a great potential as effective transmembrane delivery carriers for drugs or biomolecules, which will lead to a new generation of nanodevices for biomedical applications.

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