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Communication

Citrate-Coated Platinum Nanoparticles Exhibit a Primary Particle-Size Dependent Effect on Stimulating Melanogenesis in Human Melanocytes

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Supplementary Table:

Table S1. Physicochemical characterization of SPtNP and LPtNP as provided by the manufacturer.

Parameter	SPtNP	LPtNP
Diameter	4.6 ± 0.8 nm	46.2 ± 5.1 nm
Zeta Potential	ND	−49.8 mV
Hydrodynamic Diameter	ND	53.2 nm
Particles/ml	8800×10^{11}	9.5×10^{11}
Surface Area	50.8 m^2/g	5.9 m^2/g

ND; not determined, due to limitations in measurement of small diameters; Hydrodynamic Diameter and Zeta Potential was measured on Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Diameter and size statistics were measured on JEOL 1010 Transmission Electron Microscope

Supplementary Methods:

1. Cell Viability of HEMn-MP and HaCaT Cells

HEMn-MP cells (5×10^4 cells/well) were seeded in a 96-well clear bottom black microplate (Corning Inc., NY, USA) for 24 hours after which PtNPs were added, and cultures were continued for another 48 hours. Subsequently, the Alamar blue viability assay was conducted, and the fluorescence was measured at excitation/emission of 570/585 nm and viability reported as % of untreated control.

Human keratinocytes (HaCaT cells) were purchased from AddexBio (San Diego, CA, USA) and cultured in DMEM supplemented with 1% antibiotics. For the estimation of viability of PtNPs to HaCaT cells, 1×10^4 HaCaT cells/well were cultured in a 96-well black plate for 48 hours followed by treatment with PtNPs for a duration of 72 hours. After this, culture medium was replaced by medium containing 10 µL of Alamar Blue reagent and incubated at 37 ^{0}C for 3 hours after which the fluorescence was read at excitation/ emission wavelengths of 570/585 nm and viability was reported as % of untreated control.

2. Melanogenesis Assay in Cocultures.

HEMn-MP and HaCaT cells were cultured together using a contact coculture based on the method reported previously with some modifications [68]. Briefly, HEMn-MP cells were cultured in six-well plates for 24 hours after which HaCaT cells were added at twice the seeding density for another 24 h in serum-free keratinocyte growth medium (SF-KGM; Gibco). Subsequently, the

medium was replaced by fresh SF-KGM medium containing PtNPs and the cocultures were maintained for a period of 48 h. After the exposure, the relative extracellular and intracellular melanin levels were evaluated based on the spectrophotometric method of NaOH solubilization.

Supplementary Figures:

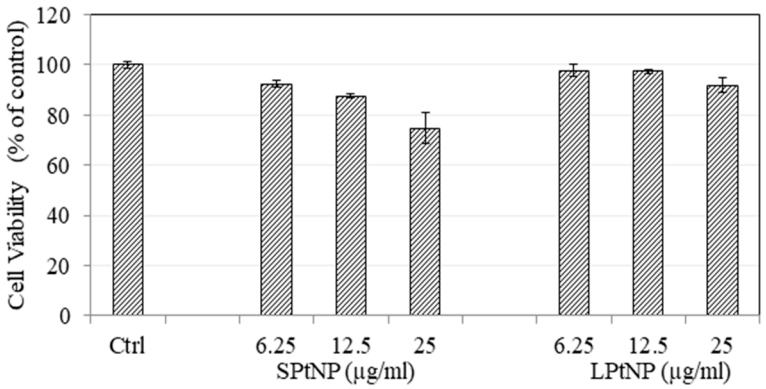

Figure S1. Viability of HEMn-MP cells treated with different concentrations (6.25–25 µg/mL) of SPtNP and LPtNP for 48 hours, evaluated by Alamar Blue assay; Data is mean ± SD of triplicate determinations.

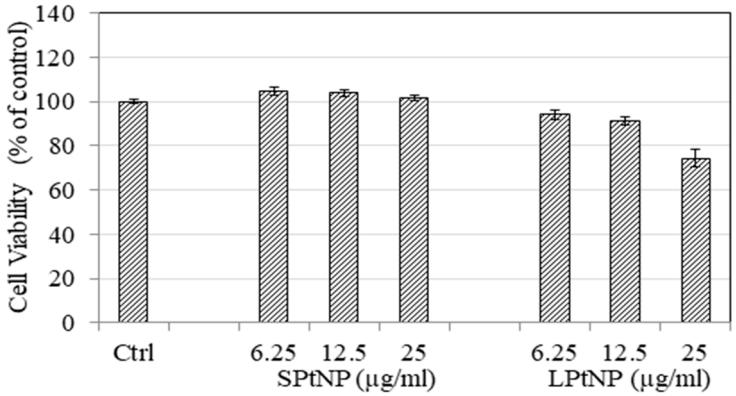

Figure S2. Viability of HaCaT cells treated with different concentrations (6.25–25 µg/mL) of SPtNP and LPtNP for 72 hours, evaluated by Alamar Blue assay; Data is mean ± SD of triplicate determinations.

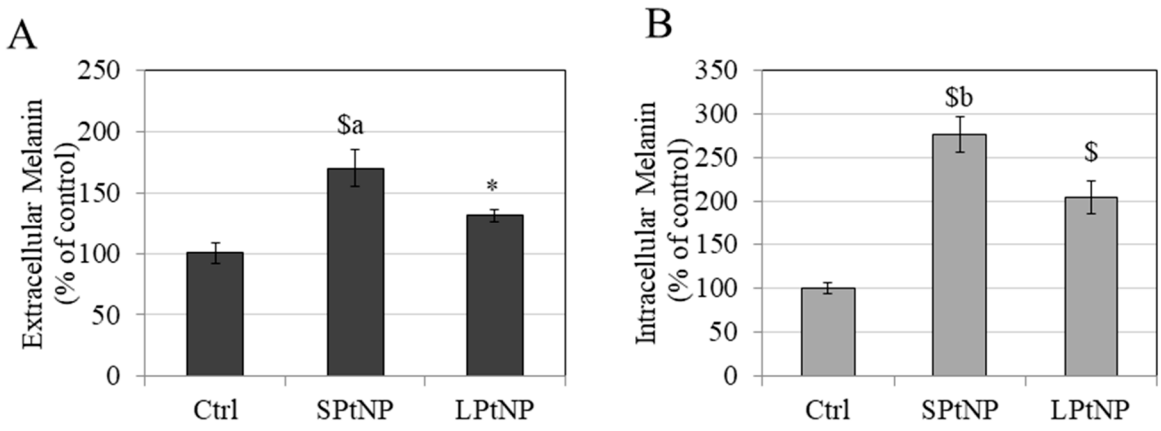

Figure S3: Melanin assay with (**A**) Extracellular and; (**B**) Intracellular levels in cocultures of HEMn-MP and HaCaT cells treated with SPtNP and LPtNP at 12.5 μg/mL; ($p < 0.001 and *p < 0.05 *vs.* Ctrl; letter a-p < 0.01 *vs.* LPtNP , letter b-p < 0.01 *vs.* LPtNP; Data is mean ± SD of triplicates.