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Comparison of Photothermal Activity of Iron- and Manganese-Chelated Natural Melanin Nanoparticles

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***Abstract*— Melanin is a pigment abundance in many living organisms and has been studied for its nanoparticle form in applications such as biosensors, drug delivery, imaging, and photothermal therapy. Previous research indicated that natural melanin nanoparticles (MNPs) conjugated with metal ions could serve as contrast agents for T1-weighted magnetic resonance imaging due to their high T1 relaxivity rates. MNPs have excellent ultraviolet (UV) and near-infrared irradiation absorption and photothermal activity in a wide range of wavelengths. This study investigated whether the photothermal properties of melanin nanoparticles persist after metal-ion chelation, comparing the efficacy of natural and metal-conjugated forms at different concentrations. The results demonstrate that metal ion chelation used for MR imaging does not affect the photothermal efficiency of MNPs, and the photothermal activity of the nanoparticles increase with nanoparticle concentration. The solution consisting of a 2 mg/ml concentration of manganese-MNP complexes (MNP-Mn^{2+}) showed the highest increase with 7°C temperature compared to distilled water under 633 nm wavelength laser irradiation for 30 minutes.**

Keywords—photothermal therapy; natural melanin nanoparticles; magnetic resonance imaging.

I. Introduction

Melanin is a pigment abundance in many living organisms like bacteria, fungi, squid, insects, octopus, squid, black tea, and humans [1,2]. The nanoparticle form of melanin pigment gained attention due to its high biocompatible and biodegradable properties, either extracted from natural origins or synthesized in the laboratory, particularly in research areas such as biosensor, biomaterial, drug delivery and wound healing [3-9]. MNPs-based agents present distinguishable photothermal activity when they are excited with light at a wide range of wavelengths, from ultraviolet (UV) to NIR. On the other hand, they do not suffer from long-term toxicity like metal-based photosensitizers. [10-13]. Previous studies by our research group have shown that metal ions chelated MNPs can be used as contrast agents in T1-weighted magnetic resonance imaging (MRI) due to their high relaxivity rates [14-16]. T1 shortening effect of Fe^{3+} ion chelated MNPs solution (MNP-Fe^{3+}) presented better contrast to noise ratio (CNR) compared to gadolinium containing contrast solution with same concentrations under 3T MR scanner [16]. In vitro cytotoxicity of MNP-Fe^{3+} nanoparticles, up to 1000 µg/ml concentration was found negligible when they were incubated with both human umbilical vein endothelial cells (HUVECs) and fibroblast (NIH 3T3) cells for 24 h using MTT assay [14]. There are also studies that focus on the theranostic applications of MNPs where they have been used as both contrast and photothermal agents for cancer [17-19]. Therefore, Fe^{3+}- or Mn^{2+}-MNPs complexes may serve as theranostic agents for cancer treatment under MRI, if the photothermal properties of MNPs persist after the metal-ion chelation.

This study compared the photothermal efficacy of MNPs, MNP-Fe^{3+}, and MNP-Mn^{2+} complexes at different concentrations under 633 nm laser irradiation for 30 minutes.

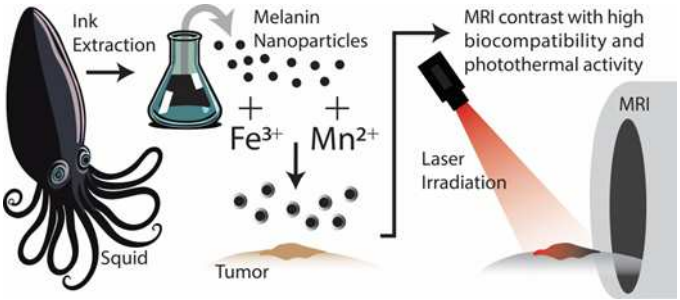


Fig. 1: Natural melanin nanoparticles are extracted from squid ink sac and are chelated with Fe^{3+} and Mn^{2+}. The final form of the nanoparticle may provide photothermal activity and visibility under MRI, which will allow theranostic applications in cancer research.

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II. METHODS

A. Extraction of the natural melanin nanoparticles

The extraction and purification of MNPs from squid (*Sepia officinalis*) ink sacs were performed following the procedures described by Eom et al. [20]. The ink was diluted five-fold with distilled water, and the resulting solution was centrifuged at 10,000 rpm for 20 minutes. After centrifugation, the supernatant was discarded, and the remaining pellets were washed three times with distilled water to remove salts and impurities. The washed pellets were then dried in an oven at 50°C for two days.

B. Determining the morphology of the nanoparticles

The morphology of MNPs was characterized using a scanning electron microscope (SEM) (FEI Quanta 400F, Hillsboro, OR, USA). Before the examination, a gold-palladium (Au-Pd) mixture was sputtered onto the samples. SEM images were captured at a voltage of 7.5 kV. ImageJ® software (NIH, MD, USA) was used to measure the average nanoparticle diameters for each formulation, with the data presented as the mean ± SD (n = 100).

C. Metal ion chelation of the natural melanin nanoparticles

To enhance the MRI visibility of MNPs, they were chelated with metal ions, Fe^{3+} and Mn^{2+}. Chelation of MNPs with Fe^{+3} was achieved by mixing 100 µL of Fe^{3+} (1 mg/mL) solution with the MNP solution and incubating for 3 hours at room temperature. The unbound Fe^{3+} ions were then removed from the solution by centrifugation at 10,000 rpm for 20 minutes. For Mn^{2+} chelation, MNPs (2 mg in 2 mL water) were mixed with 1 mL of $MnCl_2$ aqueous solution (2 mg/mL) and incubated for 2 hours at room temperature. The resulting complexes were washed 3 times, and unbound Mn^{2+} ions were removed by centrifugation at 10,000 rpm for 20 minutes.

D. Photothermal therapy experiments

MNP, MNP-Fe^{3+} and MNP-Mn^{2+} nanoparticles were dissolved in distilled water at 0.5, 1.0 and 2.0 mg/ml concentrations. An ultrasonic homogenizer (Bandelin Sonopuls HD 4000) was used to dissolve MNPs before each experiment. The solutions were irradiated using a 633 nm diode laser adjusted to 50 mW/cm^2 power density for 30 minutes. The temperature of the solution was measured every 2.5 minutes using a multi-thermometer (Voltcraft DT-300).

III. RESULTS

A. Morphology of the nanoparticles

Based on the SEM images of the MNPs, the average diameter was found to be 179.3 ± 40 nm (n=100) (Fig 2). Spherical shapes with rough surfaces were observed, and agglomeration was noted due to Van der Waals forces dominating at the nanoscale.

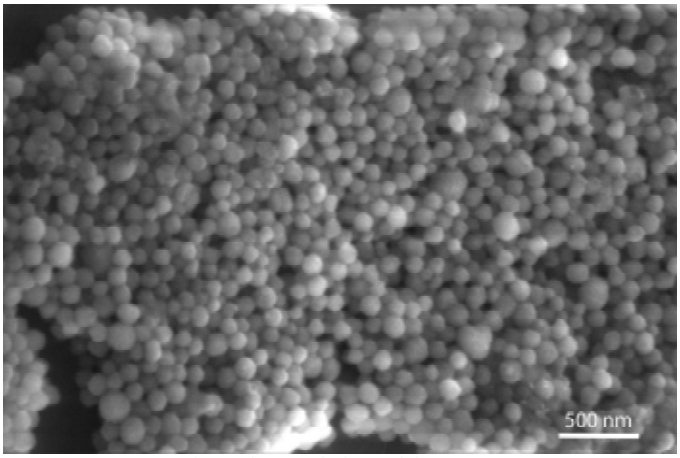


Fig 2. The morphology of the MNPs.

B. Results of the photothermal therapy experiments

Similar temperature increase curves were obtained when MNPs and metal ion-chelated MNPs at different concentrations were illuminated with 633 nm laser light. For simplicity, only the curves for a single concentration value (1 mg/mL) were shared in Figure 3. As shown in the graph, the temperature of the illuminated solutions initially increased by approximately 0.7°C per minute, then this value decreased and fell to approximately 0.2ºC per minute towards the end of the experiment. After 30 minutes of illumination, MNP, MNP-Fe^{3+}, and MNP-Mn^{2+} reached final temperatures that differed by 0.2ºC from each other. Among these samples, MNP-Mn^{2+} showed the highest increase in temperature with a final temperature of 30.6ºC.

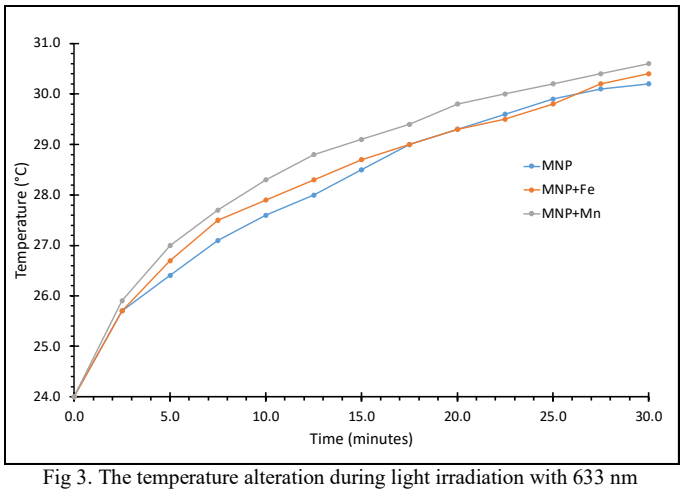


Fig 3. The temperature alteration during light irradiation with 633 nm wavelength.

As shown in Table 1, the increase in temperature of the distilled water was 0.6ºC and the temperatures of all the solutions were between 30.0ºC and 31.0ºC.

TABLE I. HIGHEST TEMPERATURES RECORDED

	Concentration (mg/ml)		
	0.5	1.0	2.0
MNP	30.0°C	30.2°C	30.6°C
MNP-Fe	30.2°C	30.4°C	30.9°C
MNP-Mn	30.5°C	30.6°C	31.0°C
Distilled Water	24.6°C		

The temperature of the solutions at the end of 30 minutes of irradiation with 633 nm wavelength. The initial temperature of all solutions and distilled water was around 24.0°C.

IV. Discussion

MNPs chelated with Fe^{3+} and Mn^{2+} can be used in theranostic applications for cancer. This study investigated whether the photothermal activity of MNPs at different concentrations is persistent after Fe^{3+} and Mn^{2+} chelation. The maximum temperature increases of distilled water (control), MNP, MNP-Fe^{3+} and MNP-Mn^{2+} were recorded as 24.6º C, 30.2ºC, 30.4ºC, and 30.6ºC, respectively under 633 nm laser irradiation with 50 mW/cm^2 power density. Results show that metal ion chelation does not affect the photothermal activity of MNPs. Consistent with the study of Sun et al. [19] the temperature increase initiated with the irradiation of MNPs was enhanced with higher MNP concentrations. On the other hand, the MNP concentration in the solutions and the thermal increase of the solutions was not directly proportional.

Mantso et al. presented that [21] when human malignant melanoma (A375) cells are exposed to temperatures higher than 43.0 °C, there is a significant reduction in viability. In this study, the peak temperature was 31.0°C, however, it should be kept in mind that our samples were at room temperature at the beginning of the experiments. Therefore, the temperature increase that we achieved (up to 7.0°C) with MNPs may provide cancer cell killing effect on in vivo applications.

Future studies are going to be conducted to further characterize the nanoparticles, such as measuring the zeta potential to evaluate aggregation. Furthermore, the photothermal activity of MNP-Fe^{3+} and MNP-Mn^{2+} under different optical settings, as well as their performance as MRI contrast agents on both in vitro and in vivo cancer models will be investigated.

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