The Protective Role of Natural Melanin Nanoparticles Under UVC Exposure

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Abstract

Ultraviolet C (UVC) irradiation may cause serious cellular and molecular tissue damage or ocular problems when it directly exposed to living organisms without filtering. Melanin is an organic UV absorbing biomaterial, that has no cytotoxicity or any harmful effects on human health. This study focused on UV blocking ability of natural melanin nanoparticles (MNP) against the damaging effects of UVC irradiation on the gram-negative bacteria Escherichia Coli (E. coli). Experiments showed that existence of MNPs enhanced cell viability of E. coli bacteria when exposed to different application time of UVC irradiation.

Keywords: Melanin nanoparticles, UVC irradiation, Nanomaterial, UV protecting agents

I. INTRODUCTION

Nowadays, the need in effective UV-shielding agent has increased in construction, paints, coating, packaging, cosmetic and biomedical industries [1]. Recent advances in the development of new nanoscale and multifunctional materials like nanoparticles, nanowires, nanotubes and/or thin films with the help of nanotechnology have brought a new dimension to UV-shielding technology. To date, significant progress has been made in the production of photo-stable nanoparticles like ZnO, TiO2 [2;3]. However, with the changing world standards, researchers have started to seek for new sustainable, biocompatible, and biodegradable nanoparticles produced with environmentally friendly techniques instead of nanoparticles produced by wet chemistry, which are harmful to the environment and results in high amount of solvent waste.

Melanin, produced in melanosomes, is a well-known pigment with free radical scavenging ability, antioxidant activity, electronic-ionic hybrid conductivity, metal-ion chelation, redox activities, and electrical stability, most importantly their photoprotective ability by absorbing the light in the visible and UV regions of the electromagnetic spectrum [4-6]. It is found in many living organisms such as bacteria, fungi, plants, catfish, and cuttlefish ink. Among these, melanin nanoparticle can easily be extracted from cuttlefish ink with a simple centrifugation and washing process without the need for performing extraction or purification steps using harsh chemicals [7]. Natural melanin nanoparticles (MNPs) have been started to take place of metallic and metal oxide nanoparticles as a “green and sustainable” alternative [8;9].

Herein, natural melanin nanoparticles with excellent biocompatibility and biodegradability were prepared via centrifugation from cuttlefish ink. We attempted to extract nanoparticles with sustainable and low-cost way, which has potential application as UV-shielding agent. Then, these nanoparticles were incubated with a commensal gram-negative bacterium, E. coli [10;11]. Protection efficacy of MNPs were evaluated and analyzed after different UV-C irradiation exposure durations.

II. MATERIALS AND METHOD

Preparation steps for experiments delineated in Fig. 1 starts with design of UV-C light source, extraction of adequate amount of melanin nanoparticles stock and bacterial cultivation of Escherichia Coli (E. coli) on TSA. A set of experiments were applied for varied groups incorporating control group, MNPs solution, the mixture of bacterial solution and MNPs solution. Following that cell viability was observed and analyzed for each experimental group under UV-C exposure time variations.

Fig. 1. Block diagram of the experimental steps
A. Personal Protective Equipment
The ISO 15858 Standard shares the necessary safety information for humans and other living organisms to use devices incorporating UV-C. According to the ISO 15858 – UV-C Devices – Safety Information Standard [12], UV-C irradiation may result in serious cutaneous or ocular damage on human body when it directly contacts with skin or eye without filtering. Unprotected and unfiltered exposure to UV-C increases the risk of developing skin cancer arisen from DNA and RNA damage [13]. Furthermore, long-term eye exposure to UV-C may lead to partial and permanent blindness [12]. Regarding the ISO 15858 standard, it is extremely important to use the necessary personal protective equipment which consist of EN 166 (personal eye protection) and EN 170 (UV filters) approved goggles, a non-porous glove with irradiation protection up to 400 nm of wavelength, clothing covering exposed skin known not to be transparent to UV-C penetration [12]. Moreover, warning signs should be placed for environmental protection. During this study, all personal and environmental protection were applied in accordance with ISO 15858 standard directives.

B. UV-C LED Light Source Design
Light emitting diode (LED) based ultraviolet (UV) lamps became a popular instrument in many industries, since they have mercury-free nature, low cost, small size and longer lifetime compared to traditional UV lamps [14]. Therefore, surface mounted device (SMD) 3535 UV-C LED sources with 270-280 nm wavelength and 10 mW power, supplied from Secol Electronic. Application-specific 3X3 UV-C LED array matrix was designed for a 24-well plate after soldering process and was fixed on the aluminum plate to prevent excessive heating during use.

C. Extraction of Natural Melanin Nanoparticles
Natural melanin nanoparticles extracted from commercially available cuttlefish (Sepia officinalis) ink according to a method described by Jakubiak et al., indicated in Fig. 2 [15]. Firstly, the cuttlefish ink (Ekol Food Products) was diluted five times with distilled water and adequate amount of melanin stock solution was obtained. The resulting melanin stock was re-diluted five times and centrifuged at 10000 rpm for 20 minutes.

D. Dynamic Light Scattering Analysis
The size distribution of melanin nanoparticles before and after 10 minutes UV-C exposure was measured by a Zetasizer (Nano ZS, Malvern, Worcestershire, UK).

E. Bacterial Strain
Escherichia coli ATCC (American Type Culture Collection) 25922 strain was used in this study. Bacteria from frozen stocks stored at -80°C was transferred on Tryptic Soy Agar (TSA) via streaking and incubated at 37°C overnight. After an overnight incubation period, a single colony was selected and removed from TSA to inoculate in 5 mL Tryptic Soy Broth (TSB) and cultured overnight at 37°C on an orbital shaker (180 rpm). The bacterial suspension was centrifuged, and the supernatant TSB was removed. The pellet (bacteria) was dissolved in phosphate-buffered saline (PBS) to obtain units / mL (CFU / mL) forming approximately 10^8 for the bacterial strain.

F. Installation of the UV-C Irradiation Set
In the UV-C irradiation system, the light sources are positioned perpendicularly to the plate. The distance between the plate surface and the light source was kept constant at 1 cm. Irradiation from each LED was exposed to single well of a 24 well plate and only 9 wells of the plates were irradiated by the UV-C LED light source which has 9 UV-C LEDs in total (Fig. 3). The application time for bacterial inactivation were selected in different time intervals including 10 seconds, 1 minute, 5 minutes, 15 minutes, and 30 minutes.

G. Experimental Design
UV-C shielding role of MNPs on bacteria (E. coli) were investigated into 4 different groups as follows;
- Control Group: Bacterial solution is neither treated with nanoparticles nor UV-C irradiation.
- Melanin Group: Bacterial solution is only incubated with melanin nanoparticles.
- UV Group: Bacterial solution is only exposed to UV-C irradiation.
- UV + Melanin Group: Bacteria solution is both incubated with melanin solution and exposed to UV-C irradiation.

Fig. 2. The extraction steps of natural melanin nanoparticles.
This washing and centrifugation process was repeated 5 times. At the end of process, the pellet was left in the oven to dry for 1-2 days at 80°C.
Melanin solution in 100µg/ml concentration was prepared and sonicated overnight using bath-type sonicator before the experiments. Each group was replicated with 3 samples on 9-well plates. For Melanin and UV + melanin group applications, 250 µl bacterial solution and 250 µl MNP solution were mixed in a well of 24-well plate and incubated for 4 hours. Then UV irradiation was applied for 10 seconds, 1 minute, 5 minutes, 15 minutes and 30 minutes for UV and UV + melanin group. A different set of experiments was prepared for each parameter change.

H. Colony Counting Method via Serial Dilution for Bacterial Cell Viability Analysis

To detect the number of bacteria that remained alive after each application, serial dilution method was used. Since there are too many bacteria to count in the original sample, the dilution process is carried out gradually. The dilution factor of this solution was 1/10 to calculate the number of bacteria in the wells. 20 µl of the treated sample were taken and combined with 180 µl of PBS in one well. Then 20 µl was taken from this well and combined with 180 µl PBS in the other well. This process was repeated gradually until the desired dilution was reached. 100 µl of diluted solutions were poured onto TSA containing petri dishes and incubated at 37°C overnight. At the end of this process, colonies became visible. After counting the number of bacteria on the agar, it was multiplied by the dilution factor and the number of viable bacteria was determined in each original sample at the end of the applications.

I. Data and Statistical Analysis

Student’s t-test was utilized to compare whether there was a statistically significant difference between the control and the experimental groups, and when this value was less than 0.05, the results were considered as significant.

III. RESULTS

A. Dynamic size distribution characterization

Dynamic light scattering analysis points out that the average size of melanin nanoparticles was found 248 nm with a standard deviation of 3 nm. After UV-C exposure, the size of melanin nanoparticles was slightly decreased to 213 ± 7 nm.

B. Cellular Protection at 100 µg/mL Melanin Concentration

The cellular protection effects of 100 µg/ml melanin concentration on E. coli under UV-C irradiation at different exposure times were investigated. In general, all bacteria in the UV-C groups were inactivated under 1, 5, 15, and 30 minutes of UV-C exposure. Only 10 s of UV-C irradiation did not induce complete eradication of the bacterial cells. Nevertheless, it also resulted in more than 98% decrease in cell viability. When 100 µg/ml melanin was applied on bacteria without any irradiation of UV-C, it only caused 18% decrease in bacterial cell viability, and it was not significantly different from the control group (Fig. 4).

On the other hand, when bacteria were incubated with 100 µg/ml melanin nanoparticles, the cell viability-enhancing effect of melanin was observed in all UV + Melanin Groups. The protective effect of MNPs in the UV + Melanin Group which was irradiated for 10 s was the greatest and the cell viability was enhanced more than 20% in this group. The increase in the duration of UV-C exposure resulted in a decrease in the protective effect of melanin. Nevertheless, the application of melanin nanoparticles prevented the complete eradication of bacterial cells after all UV-C exposure durations including 1, 5, 15, and 30 min as shown in Fig. 5.

Fig. 4. Effect of MNPs and different exposure times of UV-C applications on the cell viability of E. coli. * represents statistical significance between the control and the experimental groups (p≤0.05).

Fig. 5. Effect of MNPs on mean cell viability of E. coli bacteria under UV-C irradiation for 10 seconds, 1, 5, 15, and 30 minutes. * represents statistical significance between the experimental groups (p<0.05).
When the data of the UV and UV + Melanin Groups were compared, it was seen that the groups which were irradiated at 10 s, 1 and 5 min of UV-C were statistically different from each other.

IV. DISCUSSION

In the experiments using UV-C LED light source, complete eradication of bacterial cells was observed in the experimental groups that were exposed to UV-C irradiation on E. coli bacteria. However, when UV-C irradiation was applied to the experimental groups in which, bacteria were incubated with MNPs, an increase in cell viability was observed compared to the UV-C experimental group alone. In other words, MNPs exhibited the protective feature of microorganisms in the irradiation with UV-C LED. The set of the experiments for observing the bacterial photoinactivation of microorganisms using the combination of UV-C light source and melanin is insufficient. Nevertheless, we clearly observed the protective effect of melanin nanoparticles on bacteria after UV-C exposure and its efficacy increased by decreasing the irradiation time.

As a future perspective, we aim to continue this study by changing the parameters of not only the exposure time, but also the concentrations of the melanin nanoparticles to determine the exact ranges that exhibit protective effect. Besides, we will continue to study the protective effect of MNPs on healthy human cells such as fibroblasts or keratinocytes which are the primary cells exposed to UV-irradiation in daily life and have crucial roles in the skin against UV-C light.

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