2024 46th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC) | 979-8-3503-7149-9/24/$31.00 ©2024 IEEE | DOI: 10.1109/EMBC53108.2024.10781697

Nomination for-Electrosprayed Thin Films of Multifunctional Melanin Nanoparticles for Photoprotective and Antioxidant Properties

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***Abstract*— Melanin is an organic dark pigment that is naturally produced in mammalian epithelial tissues, as well as in certain bacterial and fungal species. This study investigates the creation of multifunctional thin films using melanin nanoparticles (MNPs), focusing on their photoprotective and antioxidant capabilities. The MNPs were synthesized via spontaneous oxidation of dopamine hydrochloride, followed by freeze-drying to produce a dry powder. This powder was then combined with polyvinyl alcohol and electrosprayed onto glass substrates to form thin films for four intervals (2, 4, 12, and 16 minutes). These films were characterized using spectroscopic and microscopic methods, confirming their uniformity and melanin-like composition. The films exhibited strong photoprotective properties against UV radiation and significant antioxidant activity, indicating their potential for various applications including protective coatings and biomedical devices. The study highlights the utility of MNPs as a versatile, bioinspired material, offering insights into their potential for advancing technology in fields requiring radiation shielding and oxidative stress mitigation.**

Keywords—melanin nanoparticles, film, nanocomposite, radiation shielding, antioxidant, electrospray.

I. Introduction

Melanin is a natural high molecular weight dark-pigmented heterogeneous biopolymer that is well known for its photoprotective properties, shielding cellular components from UV radiation damage in various organisms, including bacteria, fungi, plants, and animals [1]. However, this function is only one among its other optical, electronic, chemical, photophysical, and photochemical properties [2]. Natural melanin and synthetic melanin nanoparticles (MNPs) or analogs of naturally occurring eumelanin display biomedical-relevant functions, including metal ion chelation, free radical scavenging, antimicrobial and anti-fungal action. The polydopamine (PDA) is an active area of investigation in these analogs, playing a key role. Thanks to its electronic and optical properties, natural melanin and MNPs can be utilized for molecular imaging, including magnetic resonance, positron emission tomography, and photoacoustic imaging [3]. This plethora of properties renders melanin a promising multi-functional tool within the field of biomedical engineering[4]. Within the last ten years, utilization of the PDA MNPs not only provides a model to investigate the chemistry and structure of its natural counterpart [5] but also reproduces many functions of natural melanin-related nanotechnological advancements [5, 6]. Benefits include cost-efficiency, low toxicity, high dispersion, stability, and biodegradability [7]. Further, their size and easy functionalization render them promising multi-functional platforms for therapeutic applications. At the interface of nanoscience and biomedical engineering, understanding the structure of MNP films and coatings can offer novel nanotechnological insights and solutions to challenging biomedical issues. Recent advancements in thin film coatings and electronic functionalities have propelled melanin research [8] for applications including dye-sensitized solar cells (DSSCs), point-of-care applications more notably on skin sensors [9], high electrical conductivity devices [10], natural photo-protective films [11, 12], nanocomposite films with cellulose nanofiber [13], thanks the photoconductivity [10] and natural photo-protective qualities [14] of MNPs. The thin film coatings of MNPs are accomplished by self-assembly [15], laser deposition [16], spin coating, and electrochemical deposition [17, 18]. The films have been developed with agar [19], carrageenan [20], poly(lactic acid) [21], polyvinyl alcohol [22], ethylene-vinyl acetate [23] copolymer, chitosan [24] and polyurethane films.

In this paper, we report the electrospray technique as a novel coating method to obtain multi-functional thin films of melanin-like nanoparticles (MNPs). We synthesized MNPs by oxidation reaction of dopamine hydrochloride in sodium

NSF- Grant number 2050887 and Office of Undergraduate Research, ERAU

hydroxide and optimized their purification to obtain a dry powder. By conjugating the MNPs with polyvinyl alcohol, we obtained an electroconductive solution that was electrosprayed to form thin films on the glass substrate. Characterization of the MNPs confirmed their purity and melanin-like chemical profile. Using UV spectrophotometric testing and antioxidant assays, the photoprotective and antioxidant properties of MNPs were demonstrated.

II. MATERIALS AND METHODS

Materials: Dopamine hydrochloride (3, 4-Dihydroxy-phenethyl-amine hydrochloride, MW 189.64), 1.0 N Sodium hydroxide (NaOH, MW 40.0g/mol) solution, Polyvinyl alcohol (MW 15,000), Synthetic melanin powder (M8631), and an Antioxidant Assay Kit (MAK334) were purchased from Sigma-Aldrich (Germany).

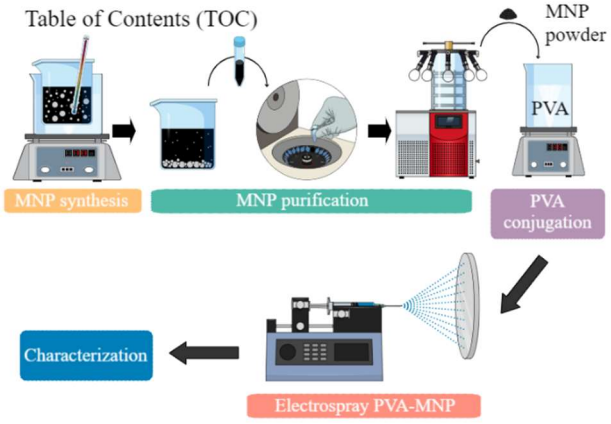

Fig. 1. Table of contents flow chart describing experimental design.

A. Synthesis of Melanin Nanoparticles

The synthesis of the MNPs is similar to the reference [25] with a slight modification. To synthesize MNPs, 5.3 mM sodium hydroxide and 10.5 mM dopamine hydrochloride solution were mixed to obtain a 1:2 ratio. The dopamine hydrochloride/sodium hydroxide solution was heated at 50°C while stirring at 800 rpm for 3.0 hours. After the 3-hour hot bath, the MNP solution was removed from the heat, allowed to cool to room temperature, and then stored at 9°C. The MNP solution was transferred into centrifugal test tubes and frozen in an upright position overnight. The test tubes were carefully removed from freezing and thawed without disturbing the sedimented MNPs. The supernatant was removed from the tubes and deionized water was added to the tubes. The test tubes were then centrifuged at 12500 rpm for 10.0 min before freezing overnight. The tubes were thawed, and the supernatant was removed, leaving the MNPs. The sedimented MNPs were then freeze-dried to obtain a dry powder form. The freeze-drying of the solution was conducted using the VirTis Lyo-Centre 3.5L DBT ES-55 Benchtop Lyophilizer instrument. The solution took approximately 40 minutes to freeze dry.

B. Characterization of Melanin Nanoparticles

Fourier Transform Infrared (FTIR) Spectrophotometer, ATR mode, Agilent was used to characterize the presence of specific chemical groups in purified MNP powder. Three independent dried polymeric samples of MNPs, PVA, and MNP-PVA complex mesh were obtained as 3-4 mm thick and analyzed by FTIR using attenuated total reflection mode. FTIR spectra were obtained in the range of wavenumber from 2000 to 700 cm-1 during 32 scans, with 2 cm^{-1} resolution at room temperature. The FTIR spectra were collected, and major vibration bands were individually interpreted. An elemental microanalysis of each coating was also examined by performing Energy Dispersive Spectroscopy (EDS). For control in EDS, commercial melanin powder purchased from Sigma Aldrich was used for the comparison.

To measure the antioxidant properties of MNPs, an antioxidant assay kit for Total Antioxidant Capacity (TAC) obtained from Sigma Aldrich was used. Reaction mixes and samples were prepared following the manufacturer's protocol and absorbance was measured at 570 nm. Shaking during incubation was used to prevent MNP sedimentation

C. Electrospray for Fabrication of MNP-PVA Composites

To prepare an MNP solution for electrospraying, a polyvinyl alcohol solution was prepared by dissolving polyvinyl alcohol (2.5% w/w) into deionized water at 80°C for 2 hours under constant stirring. The dried MNPs were added to the PVA solutions to obtain the desired concentrations. PVA was selected as a solvent for its water solubility, biocompatibility, and ease of electrospray. In this experiment, the SKE EF050 apparatus was used, which consists of a hypodermic syringe with a metal needle (20G, internal diameter), a syringe pump, with internal grounding available in the instrument, and a high-voltage power supply. The 2.0 mL syringe was filled with sample solutions, which were then electrosprayed at a target flat surface on the glass surface kept at a distance of 6.0 cm, with a flow rate of 0.010 mL/hr and an applied voltage of 13.93 kV. All electrospray studies were carried out at ambient temperature and relative humidity of 35-40%. The glass surfaces were made hydrophilic using plasma reactors. For control, only PVA solution was electrosprayed. The glass surface was coated for 4 min and was allowed to air-dry.

D. Characterization of Thin Films

The thin films of MNPs were then characterized by Scanning Electron Microscopy (SEM). SEM images of the MNP-PVA complex on the glass slides were obtained using an FEI emission scanning electron microscope where the glass films were sputter-coated in a 20 Å gold with the Denton vacuum cold sputtering system (Moorestown NJ). Samples were analyzed with an acceleration voltage of 3 kV and different magnifications up to 5000× were used. Optical measurements of the films were carried out using UV Spectroscopy (Genesys UV-Vis 150 Spectrophotometer). UV exposure setup was designed in-house using the Thorlabs PM400 optical power meter to measure UV transmittance through the MNP films.

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III. RESULTS AND DISCUSSION

A. Characterization of Melanin Nanoparticles (MNPs)

The optical microscopy image was obtained using the Axioscope 5 (Carl Zeiss) of the MNPs and is shown in Fig. 2A. The uniform coating of the glass is shown.

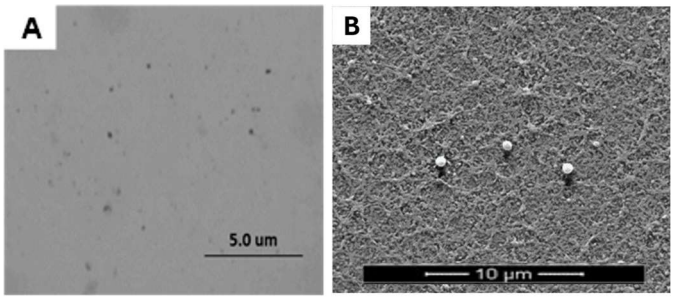

Fig 2: (A) Optical microscopy image of electro-sprayed MNP-PVA thin film. (B) Scanning Electron Microscopy (SEM) image of MNP.

Fig. 2B shows the scanning electron microscopy (SEM) image of an MNP with an approximate diameter of 500 nm ±0.2 µm.

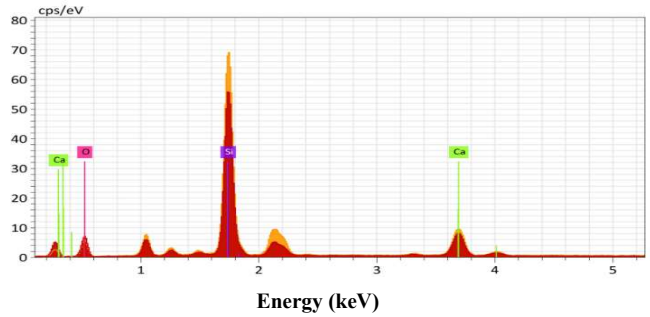

Fig 3: Energy Dispersive X-ray Spectroscopy (EDS) elemental comparison analysis of MNPs (in red) and commercial melanin powder (in orange).

Fig. 3 shows the Energy Dispersive X-ray Spectroscopy (EDS) data, which compares commercial melanin powder (shown in orange) and melanin nanoparticles (MNPs) (shown in red) and confirms a similar chemical composition and purity of the synthesized MNPs.

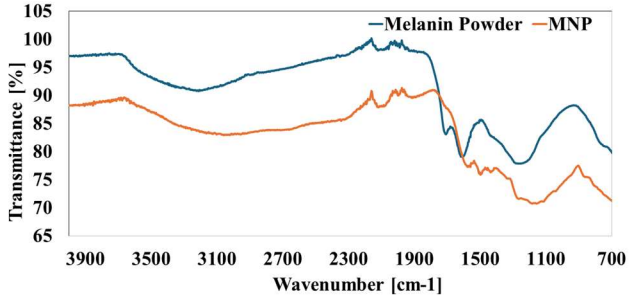

Fig 4. Fourier Transform Infrared (FTIR) plot of purified MNPs.

The plot helps to distinguish the peaks of C, Ca, O, Na, Mg, Cl, K as well as N even if the latter is significantly low in intensity. These can be due to chemical composition which contains $CaCO_3$, $MgCO_3$, NaCl, and Na_2SO_4, as well as other additional substances such as phenolic hydroxyl groups (-OH), carboxylic (-COOH) with amino (-NH_2) groups as potential binding sites of these elements. As there are no additional elemental additions as compared to the commercial one, it can be deduced that the purification of the investigated current melanin samples was effective.

Fig. 4 is the Fourier Transform Infrared (FTIR) plot, which shows broad bands in the range of 3000–3400 cm^{-1} assigned to the stretching vibration of phenolic O–H and N–H bonds

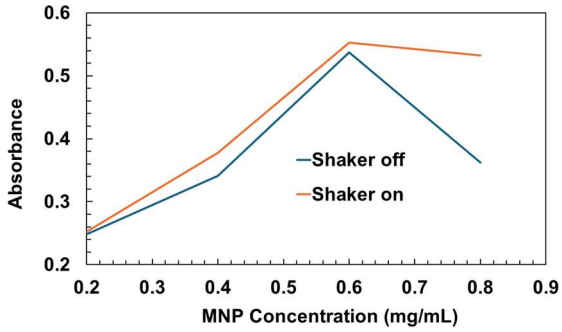

Fig. 5. Exploring Antioxidant Activity: analyzing absorbance in relation to MNP solution concentration.

vibrations of the carboxylic acid, and phenolic as well as aromatic amino functions present in the indolic and pyrrolic systems in L-DOPA. The bending vibration modes of the C=O double bond (COOH) and the C=C, C-N bonds of the aromatic system in addition to the C=O double bond of those carboxylic functions are observed in the spectral range between 1750 and 1550 cm^{-1}. The modes in the 1400-1300 cm^{-1} are attributed to OH bending of the phenolic and carboxylic groups while the out-of-plane bending of the aromatic C-H bonds are located in the 700-600 cm^{-1} region. The formation of nanoparticles is indicated through the bands 650 to 950 cm^{-1} that describe the out-of-plane deformation of aromatic C–H bonds.

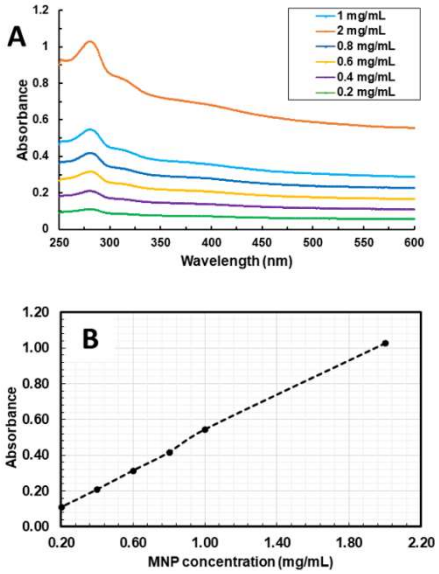

Fig. 6. (A) Ultra-violet absorbances of different concentrations of melanin-nanoparticles (MNPs). The 280nm wavelength shows the maximum

absorbance. (B) The linear relationship of the concentration vs absorbance at 280 nm.

Fig. 5 explores the antioxidant capacity of the MNPs using the antioxidant kit protocol and UV absorbance instrument. The absorbance saturation was reached at 0.6 mg/mL of MNP solution under shaking conditions. This suggests that concentrations above 0.6 mg/mL will have high antioxidant activity. The blue line experiment was conducted without shaking showing the sedimentation of the particles, which may have resulted in obstruction of the active sites of the MNPs for antioxidant activity. This experiment aided in including a shaker in the electrospray system to prevent the sedimentation of the nanoparticles. The addition of the shaking gave rise to more uniform film production on the glass surface.

In Fig. 6A, the UV-Vis plot shows the absorbance of 0.2, 0.4, 0.6, 0.8, 1, and 2 mg/mL melanin nanoparticles across 250-600 nm, highlighting a maximum absorbance at 280 nm. The linear relationship between the concentration of MNPs and absorbance at 280 nm was established in Fig. 6B.

B. Characterization of Melanin Nanoparticles Thin Films

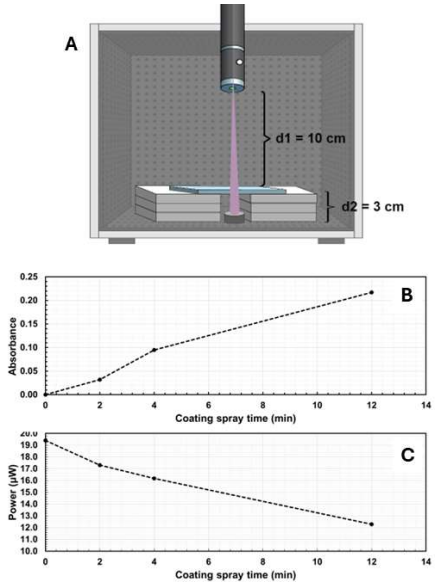

Fig 7. (A) The experimental setup for measurement of the UV light absorption. (B) The absorbance of thin films of MNPs with increasing coating spray times using UV Spectroscopy. (C) Coating time vs power plot of the thin films of MNPs.

Fig. 7A shows the setup for testing light absorption by the thin films of melanin nanoparticles (MNP). In Fig. 7B, the Ultraviolet (UV) absorbance at 280 nm of the coating using 2.5 mg/mL solution increases as the electro-spraying time is increased. As the time is increased, a high concentration of the particles is coated on the glass slide as the time of coating is increased. This means that the transmittance of the UV wavelength at 280 nm is decreased. Fig. 7C shows the results of the experimental setup for UV radiation blocking tests using a UV flashlight and optical power meter setup shown in Fig. 7A. The MNPs coated slides were located 10.0 cm below the UV light source and the optical power meter was kept 3 cm below the glass slide. Fig. 7B shows the plot of the coating spray time vs the power measured by the power meter. This shows that the power was reduced by 15.8% at the 4 min coating time and the power was reduced by 42% at the 16 min coating time. This means that the melanin particles at 16 min coating time measuring 2.56±0.4 μm were able to absorb 58 % of the UV light. For the control, a blank glass slide was used at 3 cm height.

IV. FUTURE DIRECTIONS

In the realm of future directions, the application of MNP-based thin films holds significant promise with potential implications for the field of wound healing, radiation shielding thin films, and sensors. These thin films, with properties including antioxidant activity and photoprotection (as well as potential antimicrobial and antifungal properties), present an intriguing avenue for the development of advanced wound dressings and bandages. By integrating MNPs into these dressings, enhanced healing properties could be achieved, such as accelerated tissue regeneration and protection against infection. Additionally, 3D printing technology offers exciting prospects for the fabrication of complex structures using MNPs. Incorporating melanin into 3D printing materials could pave the way for the fabrication of customizable scaffolds and implants for tissue engineering applications, providing tailored solutions for wound healing and regenerative medicine. Further research into the optimization of electrospraying, 3D printing parameters, and the development of biocompatible melanin-based materials will be essential to unlock the full potential of this innovative approach in biomedical engineering and healthcare.

ACKNOWLEDGMENT

This material is based upon work supported in part by the National Science Foundation under Grant number 2050887 as well as Dr. Mack Ronnie from the Office of Undergraduate Research for the Student grants from Embry Riddle Aeronautical University. The opinions, findings, conclusions, or recommendations expressed are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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