1. INTRODUCTION

Natural forms of melanin, a family of biopigments prevalent across many organisms, are distinguished from synthetic melanin analogues in a few aspects, among which is the involvement of additional molecular components. Pmel17, a melanosomal glycoprotein, is an indispensable component in natural melanogenesis. After sequential enzymatic cleavages, the intraluminal part of Pmel17 spontaneously self-associates into amyloid fibers, which provide a scaffold to which synthesized melanin species are deposited. Pmel17 fibers have long been suspected to physically keep potentially harmful intermediates of melanogenesis away from cytosol. However, recent findings have revealed that Pmel17 amyloid fibers have mysterious catalytic activities on oxidative association of catecholamines (an in vitro alternative of melanogenesis), along with abilities for tuning the water-dispersibility and morphology of the final melanin product. Being beyond only supportive in melanogenesis, amyloid fibers likely play multifaceted and critical roles in enhancing many beneficial properties of melanin, which are to be discovered yet. In particular, it remains completely unexplored whether amyloid scaffolds are capable of regulating/enhancing the intrinsic spin characters of melamins, which provide basis for their functional diversity.

Synthetic melanin analogues are often prepared as a form of melanin nanoparticles by simply oxidizing dopamine or its derivatives under alkaline conditions. Despite the absence of amyloid scaffold, they share some physicochemical properties with their natural counterpart, which facilitated their applications to photothermal conversion, photocatalysis, radical and ROS quenching, amylloid regulation, underwater adhesion, and structurally colored films. More recently, functional enhancement of synthetic melanin analogues was attempted by using additional molecular agents (metal ions or small organic molecules). For example, the stable free radical character of (2,2,6,6-tetramethylpiperidin-1-yl)oxyl was exploited to generate radical-enriched melanin with an advanced photothermal conversion efficiency. Interestingly, arginine alone was an effective additive for enhancing the efficiency of photothermal conversion of synthetic melanin analogues by narrowing their bandgaps. Amyloid-templated synthesis of melamins, however, has rarely been attempted previously, and thus capabilities of amyloid fibers in regulating materials properties of melamins remain largely elusive.

In this work, we showed that use of an amyloid scaffold greatly increases the quantity of the free radical species in synthetic melanins. We also demonstrated that the augmented spin characters could be exploited to prepare advanced synthetic melanin analogues better suited for spin-related applications. Instead of Pmel17, we used hen egg white lysozyme (HEWL) based on the following considerations: (i) the known functional properties of amyloid fibers in melanogenesis rely on the structure of amyloid rather than the identity of the protein. We previously have shown that fibers made of HEWL and Pmel17 are similarly active in promoting the...
formation of synthetic melanin and also in regulating its morphological features. (ii) Use of Pmel17 requires its massive recombinant production, whereas HEWL is commercially available and economically competitive. (iii) The synthesis of HEWL amyloid fibers is well established and reliably reproducible with plenty of information on their structural characteristics. The quantitative ratio of HEWL fibers and dopamine was systematically altered to synthesize various types of amyloid-melanin complexes (AMCs), whose optical properties and spin-related properties were subsequently investigated. According to the results, we show that the involvement of amyloid fibers largely narrows the bandgap of synthetic melanins and increases the density of intrinsic spins therein, which enables us to demonstrate their multimodal applications to solar water remediation and cytoprotection against ultraviolet (UV) irradiation.

2. RESULTS AND DISCUSSION

2.1. Synthesis of AMCs. Similar to the previously reported procedure for synthetic melanin analogues, synthesis of AMCs could be readily achieved by adding HEWL fibers to a dopamine solution and oxidizing by adding an NH$_4$OH solution. Different ratios between concentrations of HEWL fibers and dopamine were mixed to synthesize five types of AMCs (AMC-$i$; $i = 1$–$5$; Figure 1a). Increasing the proportion of HEWL fibers in AMCs led to the gradual improvement of water-dispersibility, consistent with our previous reports. Melanin particles are intrinsically hydrophobic and adhesive, which made handling them in water solutions unfavorable. AMCs, on the other hand, tended to form a compact complex among themselves, other than to stick to the external surfaces. They were still insoluble in water and slowly settled on the bottom of the container, but a brief vortexing or shaking caused their complete re-dispersion in the solution, forming a colloidal-like state for a relatively long time ($\sim$30 min; Figures 1b and S1). Improved water-dispersibility is useful in the context of melanogenesis, considering that melanin must be synthesized, handled, and recycled in water. In addition, AMCs exhibited larger surface area than melanin particles (Figure S2), which would be beneficial for implementing their chemical functions and recycling. In the absence of HEWL fibers, melanin particles exhibited a spherical morphology with a uniform size distribution (with a diameter of 230 nm, in our experimental condition, Figures 1b and S3). This is consistent with numerous previous works related to the synthesis of melanin nanoparticles. AMCs, on the other hand, had an elongated morphology and became mostly fibrillar when the content of HEWL fibers was maximized (Figures 1b, S3, and...
Reflecting this morphological trend, the shortest diameter of AMCs linearly decreased as the content of HEWL fibers increased (Figure 1c). In the case of AMC-5, the morphology was indistinguishable from HEWL fibers, implying that the fibers became the major component in this condition. The observed morphological trend suggested that HEWL fibers, as expected, templated the synthesis of AMCs. In a previous work, we suggested that initial melanin-coating of HEWL fibers results in their bundling and subsequent formation of micron-scale, physically rigid complexes.

This was further supported by a time-dependent morphological analysis during the formation of AMCs (Figure S5). It is also likely that melanin-like species would form preferentially at the surface of HEWL fibers (when they are present), considering that the solutions of sedimented AMCs were transparent, as opposed to that of melanin-like materials. The presence of intact amyloid fibers in AMCs was confirmed by Fourier transform infrared (FT-IR) spectroscopy analyses. The spectra collected from AMC-3, but not from melanin particles, showed a peak at 1630 cm$^{-1}$, which is attributable to the amide I vibrational mode of the amyloid structure (Figure 1d). In addition, X-ray photoelectron microscopy (XPS) analysis was conducted for melanin particles and AMCs (Figure S6). In both cases of melanin particles and AMC-3, the C 1s peak was composed of three peaks (C−H/C−C (284.4 eV), C−O/C−N (285.6 eV), and C=O/C−COOH (287.7 eV) (Figures 2a,b and S7).
spectra showed that AMCs generally have higher C==O/C−
COOH content than melanin particles. This was consistent in
N 1s peaks of both samples, where the increase of the amide
peak (NHC==O: 400.5 eV) and the concurrent decrease of
N−R (398.6 eV) and R−NH2 (401.9 eV) peaks were observed
from AMC-3 in comparison with melanin particles (Figures 2c,d and S7).

2.2. Light Absorption of AMCs Regulated by HEWL Fibers. Light absorption of melanin is intimately related to
their photothermal conversion properties. Li and Gianneschi
groups used a strategy of providing additional donor-acceptor pairs within melanin analogues, in turn narrowing their
bandgaps.15,16 Such donor-acceptor pairs were designed by
using a set of small organic molecules that can covalently
derivatize indolequinone species which strongly associate
together via π−π stacking to consist of major parts of the
melanin analogues. It is accepted that modulating intramolecular interactions among such indolequinone species is a key
to regulating optical properties of melanins.25

In line with this, we first set out to analyze light absorption
of AMCs. While the melanin without HEWL fibers exhibited
monotonically decreasing absorbance from the UV region to
the near infrared (NIR) region, those with HEWL fibers
(AMC-i, i = 1−5) showed absorbance relatively independent
of wavelength (Figure 3a). Remarkably, AMCs showed higher
absorption both at the visible and NIR regions in comparison
to melanin particles, with AMC-3 being the most efficient in
light absorbance. AMC-4 and AMC-5 absorbed less light
throughout the entire regions, which is consistent with the
scanning electron microscopy (SEM) data above in which
those two were mostly fibrillar in morphology. Using the
absorption spectra of AMCs, optical bandgaps of each sample
were calculated by the Tauc plot method (Figure S8). As
expected, bandgaps of AMCs were measured to be significantly
lower than that of melanin particles. Particularly, AMC-1,
AMC-2, and AMC-3 had lower bandgaps than the others,
which are lower than that of melanin particles by more than 1.5
eV (Figure 3b).

2.3. Electrochemical Bandgaps Decrease in the
Presence of HEWL Fibers. To confirm the measured trend
in bandgap, we also tried to measure bandgaps of each sample
electrochemically by using cyclic voltammetry (Figure 3c).
This method enables to give energy levels of the highest
occupied molecular orbital (HOMO) and the lowest
unoccupied molecular orbital (LUMO). Electrochemical
bandgaps of AMCs had a trend similar to that in their optical
bandgaps. Involvement of HEWL fibers led to an increase in
the energy level of HOMO as well as a decrease in that of
LUMO, resulting in lower electrochemical bandgaps. The
onset points were 0.56/−0.32, 0.27/−0.15, 0.33/−0.15, 0.31/
−0.15, 0.31/−0.12, and 0.28/−0.14 V, for melanin particles,
AMC-1, AMC-2, AMC-3, AMC-4, and AMC-5, respectively
(Figure S9). Using these values, HOMO and LUMO could be
calculated to be −4.94/−4.06, −4.69/−4.23, −4.65/−4.23,
−4.71/−4.23, −4.69/−4.26, and −4.66/−4.24 eV for melanin
particles, AMC-1, AMC-2, AMC-3, AMC-4, and AMC-5, respectively (Figure 3d). We note that the absolute values of
bandgaps measured optically and electrochemically may differ
as the definition of the two bandgaps is slightly different;
nonetheless, their general trends are consistent with each
other. Measurements of optical and electrochemical bandgaps
collectively suggest that using HEWL fibers as a scaffold would
enhance photothermal conversion behaviors of melanin by
regulating its light absorption.

2.4. Spin Augmentation of Melanins by HEWL Fibers. The permanent radical character of melanins is deeply related to
the energy levels and identities of their HOMO and LUMO. Previous attempts to add external radical characters in
synthetic melanin analogues brought about concomitant
alterations in their light absorption and bandgaps. To shed
light on the free radical features of melanin particles and AMC-
3 in solid state, X-band electron paramagnetic resonance (EPR)
spectroscopic analysis was performed.

The absorption signals in this region are known to probe the
chemical natures of various organic radicals. At first glance, it
was clear that the EPR signal of solid AMC-3 was significantly
stronger than that of solid melanin particles due to the
presence of radical species (Figure 4). The spin quantification

![Figure 4](https://doi.org/10.1021/acsami.3c07909)

Figure 4. Spin characters of melanin particles and AMC-3. X-band
EPR spectra of melanin particles (black line) and AMC-3 (red line)
measured at 77 K.
aligned hydrophobic planar spaces provided by HEWL fibers offer a suitable environment for the intercalation of spin-containing oligomeric structures, such as 5,6-dihydroxyindole derivatives. This intercalation may result in the directional alignment of the oligomeric components, which has been suggested to be crucial in determining the properties of intrinsic spin in melanin-like materials.

2.5. Enhanced Radical Scavenging of AMCs. Having observed that AMCs with HEWL fibers have more unpaired electrons and lower bandgaps with respect to melanin particles, we next asked if AMCs scavenge radicals more efficiently. We used 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a radical indicator and measured its gradual decrease in the presence of each melanin sample. As shown in Figure 5a, all AMCs except AMC-5 were more efficient in radical scavenging compared to melanin particles (Figures 5a and S10). In particular, AMC-3 scavenged more than 80% of DPPH radicals at the concentration of 50 μg/mL, while the same amount of melanin particles only captured less than 30% of them (Figure 5b). Interestingly, the trend of radical scavenging property among AMCs is the same with that of light absorption at 600−800 nm, suggesting that light absorption at this range and the relative content of intrinsic radical character may be correlated. Extending the reaction time for the synthesis of AMC-3 more than 6 h did not cause any significant difference in their radical scavenging efficiency (Figure S11). Similarly, AMC-3 was more efficient in capturing O₂ radical species than melanin particles, as measured by conducting the nitroblue tetrazolium assay (Figure S12). In addition, AMC-3 was stable at high temperatures and acidic/basic conditions (Figure S13).

2.6. Role of the Amyloid Supramolecular Structure of AMCs in Their Radical Scavenging. To elucidate the role of HEWL fibers in the improved radical scavenging activity of AMC-3, we designed variants of AMC-3. The soluble form of HEWL (i.e., their non-aggregated form) or a peracetylated version of HEWL fibers (via reacting them with an excess amount of acetic anhydride) was used instead of HEWL fibers, which led to the formation of HEWL-melanin complex (HMC) and acetylated AMC-3 (ac-AMC), respectively. As shown in Figure 5c, radical scavenging efficiencies of these variants against DPPH radicals were significantly lower than that of AMC-3 but were higher than that of melanin particles. Interestingly, ac-AMC exhibited a better radical scavenging efficiency than HMC, indicating that there are additional factor(s), other than lysines exposed on the surface of HEWL fibers, in the structure of amyloid fibers per se in improving radical-related properties of melanins. To this end, we subsequently examined if the individual molecules of amino acids that constitute HEWL fibers are sufficiently active for improving the radical scavenging property of melanin. We added molecules of lysine, arginine, or histidine, instead of HEWL fibers, in the synthetic procedure of melanin, which led to the formation of variants of AMC-3. We chose these amino acids among others since they can covalently (or non-covalently) bind to indolequinone derivatives. The equivalent concentrations of each amino acid to those in AMC-3 were used for the synthesis. As shown in Figure 5d, incorporation of each amino acid gave moderate improvement in radical scavenging activity of melanin, with lysines being the most efficient among others. They, however, were significantly less efficient than AMC-3 (92.3, 43.6, 39.2, 65.8% for AMC-3, and those with arginine, histidine, and lysine at 50 μg/mL of each sample, respectively), indicating the conditional presence of a synergistic relationship between the amino acids only when they are assembled into amyloid fibers. Interestingly, the
arginine-containing derivative of melanin, which have previously been reported to have a narrow bandgap energy and high photothermal conversion efficiency, was not remarkable in its radical scavenging activity. Interactions between a catechol group and many functional groups displayed on an amyloid fiber are likely complex and multifaceted. We previously have suggested that amine groups on amyloid fibers would primarily interact with oxidized derivatives of dopamine (or other catechol-containing molecules). Amine groups mostly exist as a protonated form (>99%) in a physiological condition, but they can be deprotonated when brought in proximal to each other or when put in a local environment with a decreased dielectric constant, which frequently occurs inside binding pockets of enzymes. Such a \( pK_a \) shift can also occur on the surface of amyloid fibers, which well explains the reason why the amino acids were mostly active when they were assembled into amyloid fibers. We believe a \( pK_a \) shift likely is responsible for many mysterious functions of amyloids, either beneficial or detrimental to organisms.

2.7. Application of AMCs to Solar Steam Generation. To ask if the augmented spins in AMCs are also beneficial for applications other than radical scavenging, we sought to examine their photothermal conversion properties. Powders of melanin particles and AMC-3 were coated on cylindrical wood pieces, and their optical and photothermal properties were characterized. Light absorption of melanin- and AMC-3-coated woods (wood@melanin and wood@AMC-3, respectively) in the UV region (200–400 nm) was comparable to each other (Figure 6a). At 400 nm, for example, wood@melanin and wood@AMC-3 absorbed 98.4 and 97.8% of light, respectively. However, wood@AMC-3 exhibited lower light absorption in the visible to near-IR region (400–2500 nm) than wood@melanin. At 1100 nm, wood@AMC-3 absorbed 74.0% of light.
which was 1.28-fold lower than that of wood@melanin (92.7%), and such a trend became stronger at longer wavelength regions, showing that light absorption of AMC-3 was inferior to that of melanin particles in their dried solid states. Such a trend was, however, reversed when the photothermal conversion behaviors of wood@melanin and wood@AMC-3 floated on water were compared under 1 sun irradiation (Figure 6b). After 5 s of irradiation, the surface temperatures of wood@AMC-3 and wood@melanin increased to 25.8 and 24.9 °C, from the initial temperature of 23.3 °C, respectively. With further irradiation, the surface temperatures of wood@melanin and wood@AMC-3 maintained such a trend and finally reached 47.6 and 48.7 °C after 30 min, respectively. It is notable that the AMC-3 layer had a slightly higher photothermal conversion rate and efficiency than the melanin layer, despite the lower light absorption. Interestingly, when melanin particles and AMC-3 were dispersed in water, this trend became more pronounced (Figure 6c), reflecting that the photothermal conversion properties of melanin-like materials differ between their wet and dry states. It is worth noting that the initial photothermal conversion rate of a solar steam generating device is important to achieve high performance because it quickly compensates the energy loss caused by water evaporation. Subsequently, the solar steam generation performances of wood@melanin and wood@AMC-3 were explored under 1 sun irradiation with feeding distilled water. When the mass change of water was traced for 1 h, the evaporation rates on wood@melanin and wood@AMC-3 were measured to be 1.34 and 1.22 kg m⁻² h⁻¹, respectively (Figure 6d). These values were much higher than those of bare wood and water, while wood@AMC-3 showed an evaporation rate 10% higher than that of wood@melanin, proving its superior solar steam generation performance. The energy efficiency (η) of solar-steam conversion of each sample was calculated to be 70 and 62% for wood@AMC-3 and wood@melanin, respectively (Figure 6e). This superiority of wood@AMC-3 might be due to the high rate of photothermal conversion which enables effective evaporation of water. The measured efficiency value was bit lower than previously reported materials specifically designed for enhancing photothermal conversion (82.8% for graphene oxide, 77% for laser engineered graphene, 88.6% for carbon nanotube/Fe₂O₃ nanocomposite, and 88.6% for Ag nanoparticles loaded polydopamine films) but was in a practically applicable range, despite the absence of inorganic materials. Meanwhile, the evaporation rate and efficiency of bare wood measured to be 0.77 kg m⁻² h⁻¹ and 36% respectively. With these characterizations, the desalination performance of wood@AMC-3 was examined for verifying stability and purification ability in an actual condition. To assess the evaporation stability of wood@AMC-3 for all day, the change of evaporation rate with a floating wood@AMC-3 was measured for 12 h under 1 sun irradiation. From 60 to 120 min, wood@AMC-3 maintained a high evaporation rate of 1.30 kg m⁻² h⁻¹ for seawater, similarly to the case of distilled water (Figure 6f). After 2 h, the evaporation rate of wood@AMC-3 gradually decreased presumably due to the formation of salt crystals on its surface. The crystalized salts impede the water evaporation process by lowering solar light absorption through reflecting light and plugging water transport channels. As such, the

Figure 7. Cytoprotective properties of AMCs against UV irradiation. (a) Phase-contrast images of HaCaT cells incubated with nothing (left), melanin particles (center), and AMC-3 (right). (b) A cross-sectional transmission electron microscopy (TEM) image of HaCaT cell incubated with AMC-3. (c) Viability of HaCaT cells incubated with 10 and 20 μg/mL of AMC-3. (d) Viability of HaCaT cell incubated with 20 μg/mL of melanin particles, AMC-3 with and without UV.
evaporation rate decreased to 0.89 kg m\(^{-2}\) h\(^{-1}\) after 12 h, but it was still 16% higher than that of bare wood floated on distilled water. The water evaporation rate can be also affected by the hydrophilicity of the device surface. A water droplet (10 µL) was dropped on wood@melanin and wood@AMC-3, and their adsorption rate was assessed. The whole water droplet was adsorbed completely into wood@AMC-3 within 2 s, whereas it took 20 s for wood@melanin (Figure 6g), proving that an AMC-3-coated layer is more hydrophilic, and this can better facilitate the water transport from the wood supports than a melanin-coated layer. With these characterization data in hand, the desalination effect of wood@AMC-3 was explored by analyzing concentrations of four major ions such as Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) in the water condensed through solar steam generation from seawater. The concentrations (ppm) of four ions decreased by 3 or 4 orders of magnitude (which fell in the standards of drinkable water) after distillation through solar steam generation using wood@AMC-3 (Figure 6h). Additionally, purification of a solution of an organic dye (3 ppm of methylene blue) was tried by solar steam generation with wood@AMC-3. In Figure 6i, UV−Vis spectra indicate disappearance of methylene blue peak at 605 and 669 nm, and the inset photos show a color change from blue to transparent during the purification. The salt resistance of wood@AMC-3 was also confirmed (Figure S14).

2.8. Application of AMCs as a Cytoprotecting Agent against UV Exposure. Finally, we set out to test if incorporation of HEWL fibers into melanin particles would also facilitate their known cytoprotective capabilities from UV irradiation.\(^{38}\) We incubated HaCaT cells, a human epidermal keratinocyte line, with AMC-3 or melanin particles (20 µg/mL). As previously described, melanin nanoparticles were actively engulfed by HaCaT cells and primarily located near the nuclei (Figure 7a). AMC-3 was uptaken and located in cells similarly to melanin particles, but the uptake amount was remarkably larger than that of melanin particles (Figure 7a,b). In addition to perinuclear areas, AMC-3 was also located at cell membrane. The data indicate that cellular uptake of melanins relies on their morphology, which has been a critical factor in cellular uptake of metal nanostructures. Melanin particles and AMC-3 did not show significant cytotoxicity up to 20 µg/mL, as measured for 72 h (Figure 7c). To compare the photoprotection capability of melanin particles and AMC-3, HaCaT cells incubated with each melanin sample (20 µg/mL for 72 h) was irradiated with UV (40 mJ/cm\(^2\)) for 10 min. UV irradiation led to significant cell death in normal HaCaT cells (from 100 to 35.19%) but not in those treated with melanin particles and AMC-3 (from 96.14 to 63.91% and from 92.73 to 72.49%, respectively; Figures 7d and S15). Compared to melanin particles, the same amount of AMC-3 exhibited a stronger photoprotection property in line with the above photothermal conversion results.

3. CONCLUSIONS

In summary, we showed that including amyloid fibers in synthetic melanin as a scaffold (i.e., AMCs) greatly augments their intrinsic spins, concomitantly narrowing their bandgaps. This was advantageous for synthetic melanin simultaneously for its capabilities related to photothermal conversion and radical scavenging, greatly enhancing its functional versatility for seemingly unrelated applications. In the current work, we demonstrated that AMCs are superior to conventional melanin particles for being applied to solar steam generation and cytoprotection from UV irradiation. The observed spin augmentation was not expectable—as amyloid fibers do not contain any spin-related moieties—and strongly suggests that Pmel7 fibers would fulfill a function analogous to that of HEWL fibers in this work in melanogenesis. In addition, the results imply the presence of an unexplored realm regarding the potential roles of various natural supramolecular structures in tuning properties of biomaterials.

4. EXPERIMENTAL SECTION

4.1. Materials. Sodium chloride was purchased from Daejung Chemicals (Siheung, South Korea). Dopamine hydrochloride, DPPH, ammonium hydroxide, L-histidine, tetrabutylammonium hexafluorophosphate acetoneitrile were purchased from Sigma-Aldrich. Ethanol and L-methionine were purchased from Daejung Chemicals, and HEWL was purchased from Thermo Fisher Scientific. L-arginine was purchased from Samchun Chemicals. Cylindrical pieces of balsa wood (FM7310, USA) were used as a support having water transport channels for solar steam generation. The diameter and height of the supports were set as 25.4 and 10 mm, respectively. Seawater samples were collected from Songdo beach (Busan, South Korea). Methylene blue was purchased from Alfa Aesar (USA).

4.2. Synthesis of AMCs. To synthesize melanin particles and AMC-1−3, 0.055, 0.0125, 0.025, 0.05, 0.10, or 0.2 g of HEWL were added to a 50 mL flask containing 90 mL of distilled water, 40 mL of ethanol, and 2 mL of ammonia hydroxide (28–30%), respectively. Then, a dopamine solution (0.5 g of dopamine hydrochloride in 10 mL of distilled water) was added to the flask, and the resultant solution was incubated with vigorous stirring (1500 rpm) for 24 h at room temperature. After incubation, AMCs were centrifuged five times and lyophilized overnight.

4.3. Preparation and Quantification of HEWL Amyloids. HEWL was dissolved in a solution of potassium phosphate buffer (20 mM; pH 6.3) containing 2 M of GdnHCl to a concentration of 2 mg/mL. The concentration of HEWL was measured based on the absorbance at 280 nm using an Eppendorf BioPhotometer D30 (extinction coefficient of HEWL at 280 nm: 38,904 M\(^{-1}\) cm\(^{-1}\)). The solution of HEWL was then incubated at 60 °C with a vigorous and continuous stirring for 4 h. The HEWL solution became turbid as the reaction progressed. The solution was centrifuged three to five times at 13,500 rpm to remove remaining salts and monomeric HEWL from the solution. In between each centrifugation, the supernatant was discarded, and the same volume of distilled water was added. Here, the concentration of monomeric HEWL in each discarded supernatant was measured, which was then subtracted from the initial concentration of HEWL to give the final concentration of HEWL fibers synthesized. The presence of HEWL fibers was confirmed by a Thioflavin T assay (Figure S15). The EPR measurement was performed using Jeol X-band EPR at the National Research Facilities and Equipment Center (NanoBio-Energy Materials Center) at Ewha Womans University.

4.4. DPPH Assay. For preparing a DPPH stock solution, a fresh 1 mM ethanol solution of DPPH was incubated in a refrigerator for 30 min and diluted 10 times. Each AMC solution (8 mg/mL) was sonicated for 1 h. After sonication, each AMC solution was repeatedly diluted to concentrations of 4, 2, 1, and 0.5 mg/mL. In a semi-micro cuvette, 0.1 mM DPPH and 100, 50, 25, or 12.5 µL/mL of AMCs were mixed in total volume of 1 mL, respectively, with which the absorbance of the cuvettes was measured.

4.5. Nitroblue Tetrazolium Assay. In a cuvette, 20 µM of riboflavin, 12.5 mM of methionine, and 75 µM of 4-nitro blue tetrazolium chloride were mixed with 12.5, 25, 50, or 100 µg/mL of samples (i.e., melanin particles or AMC-3). Their light absorbance was analyzed after 30 min of visible light irradiation.

4.6. Cyclic Voltammetry. On an indium tin oxide glass (surface resistivity of 8−12 Ω/sq), 1 mL of methanolic solutions of AMCs (60 mg/mL) was spin coated for 35 s (5 s at 500 rpm and 30 s at 3000 rpm). Cyclic voltammograms of each substrate were then obtained using 0.1 M acetonitrile solution of tetrabutylammonium hexafluor-
oxygen electrode, and a platinum electrode as a counter electrode. The voltage range was set as −0.6−0.8 V, the scan rate was 0.1 V/s, and the number of scans was 5.

4.7. Optical Bandgap. 3 mg/mL of each AMC water solution was sonicated for 30 min. Then each of the solution was diluted to 0.125 mg/mL in cuvettes, and then absorbance was analyzed. Using the absorbance data, the bandgap of each particle was calculated. The Tauc plot ([hv]2 = B(hν − Eg)] was plotted, and indirect bandgap could be calculated using onset point of the plot.

4.8. Solar Steam Generation Setup. AMC-3 was coated on the surface of balsa wood support, which was thermally insulated by polystyrene foam except the top surface, and then floated on a Petri-dish containing distilled water or seawater. Mass change of seawater during solar steam generation was measured with an electronic balance (HT224RCEN, Shinko Denshi ViBRA) and recorded in real-time. Solar steam generation test was conducted under 1 sun condition by using a xenon lamp as the solar simulator (DTEX, Siheung, South Korea). All experiments were conducted at room temperature of −25 °C and relative humidity of ~60%.

4.9. Characterization of Photothermal Conversion. Temperature change was measured by an IR camera (FLIR E6, Teledyne FLIR). UV−Vis−NIR spectra were obtained with a UV−visible/NIR-spectrophotometer with integrating spheres (V780, Jasco). The absorption was calculated by A = 1 − R − T where A is absorption, R is reflection, and T is transmittance. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis was conducted using ICP-730ES (Varian, Australia) for measuring ion concentration of seawater before and after purification. The energy efficiency (η) of solar-steam conversion was calculated by eq 1.

\[ \eta (%) = \frac{\text{H}_\text{eva} \times \text{P}_\text{eva}}{\text{H}_\text{dark} \times \text{P}_\text{dark}} \]

where \( \text{H}_\text{eva} \) and \( \text{H}_\text{dark} \) are evaporation rate under 1 sun and dark condition, respectively (kg m\(^{-2}\) h\(^{-1}\)), \( \text{P}_\text{eva} \) denotes evaporation enthalpy of the water (kJ kg\(^{-1}\)), and \( \text{P}_\text{dark} \) represents the irradiation power of 1 sun (1000 kW m\(^{-2}\)). Under different temperatures, \( \text{H}_\text{eva} \) could be changed following eq 2

\[ \text{H}_\text{eva} = \frac{2500.304 - 2.2521025 T - 0.02146584 T^{1.5} + 3.1750136 \times 10^{-4} T^{1.5} - 2.8607959 \times 10^{-5} T^{3}}{7} \]

4.10. Cell Culture and Assessment of Cryoprotective Properties. HaCaT cells (AddexBio, San Diego, CA) were incubated in a Dulbecco’s Modified Eagle Medium (Welgene, Seoul, Korea) containing 10% fetal bovine serum (FBS), 2 mM glutamate, and 100 unit/mL antibiotics (Gibco BRL, Rockville, MD) at 37 °C in a humidified atmosphere of 5% (v/v) air/CO\(_2\). HaCaT cells, pre-treated with or without AMC-3, were cultured in the PBS-free (serum-free) medium for 24 h. Next, cells were gently washed with PBS (phosphate buffer saline, Biosolutions, Suwon, Korea) and then irradiated with UV (40 mJ/cm\(^2\)) using a UV lamp (Bio-sun Lamps, Vilber Lourmat, Marine, France). HaCaT cells, pre-treated with or without melanin samples, were incubated for 48 and 72 h in the same way as the cell culture conditions.

4.11. SEM Analysis. 200 µL of each aqueous solution of AMCs (1 mg/mL) was dropped onto a cover glass and dried in an oven at 60 °C overnight. LEO SUPRA S5, GENESIS 2000 (Carl Zeiss EDAX) was used for analyses.

4.12. TEM Analysis. HaCaT cells incubated with melanin particles or AMC-3 were washed with PBS three times and fixed using 2% glutaraldehyde-2% paraformaldehyde in PBS for 4 h at 4 °C. Cells were washed again to remove the fixatives and collected by centrifugation at 1800 rpm for 2 min. The resultant cell pellets were washed again to remove the fixatives and collected by centrifugation at 1800 rpm for 2 min. The resultant cell pellets were

4.13. XPS Analysis. Synthesized AMCs were analyzed as a powder form using K-Alpha (Thermo Electron).

4.14. FT-IR. Synthesized AMCs were analyzed as a powder form using K-Alpha Spectrum One System (Perkin-Elmer).

4.15. BET Surface Area. Synthesized AMCs were analyzed as a powder form using BELSORP-max (MP).

## ASSOCIATED CONTENT

## Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.3c07909.

Water dispersity of AMC-3, surface areas of AMC-1−5, TEM images of HEWL amyloid fibers, melanin particles, AMC-1−5, EDAX elemental mapping images of melanin particles and AMC-1−5, C 1s and N 1s XPS analysis results of AMC-1, 2, 4, and 5, Tauc plots and cyclic voltammograms of melanin particles and AMC-1−5, photo of differently concentrated melanin particles and AMC-3 with DPPH in cuvettes and their radical and O₂ scavenging activity, cell viability of HaCaT cell treated with melanin particles and AMC-3 with and without UV irradiation (PDF)

## AUTHOR INFORMATION

### Corresponding Authors

Tae Hoon Lee — Department of Applied Chemistry, Kyung Hee University, Yongin, Gyeonggi 17104, Republic of Korea; Email: thlee@khu.ac.kr

Seungwoo Hong — Department of Chemistry & Nanoscience, Ewha Womans University, Seoul 03760, Republic of Korea; Email: hswh@sm.ac.kr

Young-Kwan Kim — Department of Chemistry, Dongguk University, Seoul 04620, Republic of Korea; orcid.org/0000-0002-9929-9510; Email: kimyk@dongguk.edu

Kyunghae Kang — Department of Applied Chemistry, Kyung Hee University, Yongin, Gyeonggi 17104, Republic of Korea; orcid.org/0000-0003-4236-8922; Email: kkang@khu.ac.kr

### Authors

Daehong Ha — Department of Applied Chemistry, Kyung Hee University, Yongin, Gyeonggi 17104, Republic of Korea

Joo Hyung Lee — Department of Applied Chemistry, Kyung Hee University, Yongin, Gyeonggi 17104, Republic of Korea

Hyeri Jeon — Department of Chemistry & Nanoscience, Ewha Womans University, Seoul 03760, Republic of Korea

Yoo Jin Kang — Department of Applied Chemistry, Kyung Hee University, Yongin, Gyeonggi 17104, Republic of Korea

Junnho Jeon — Department of Chemistry, Dongguk University, Seoul 04620, Republic of Korea

Complete contact information is available at: https://pubs.acs.org/10.1021/acsami.3c07909

### Author Contributions

K.K., Y.K.K., S.H., and T.H.L. conceptualized the research. K.K. and Y.K.K. decided the methodology. D.H., H.J., Y.J.K., J.J., and J.H.L. implemented investigation under supervision of K.K., Y.K.K., S.H., and T.H.L. D.H. and K.K. wrote the original draft. All authors have given approval to the final version of the manuscript. D.H. and K.K. wrote the original draft. All authors have given approval to the final version of the manuscript. D.H. and K.K. wrote the original draft. All authors have given approval to the final version of the manuscript.

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### ABBREVIATIONS

HEWL, hen egg white lysozyme

AMC, amyloid-melanin complex

CCR, carbon-centered radical

SQR, semiquinone free radical

DPHH, 2,2-diphenyl-1-picrylhydrazyl

HMC, HEWL-melanin complex.

### REFERENCES


