Core/shell melanin/nanosilica as a novel material for improved sun protection

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Abstract.

Overexposure to ultraviolet radiation (UVR) and high-energy visible light (HEVL) can lead to health issues such as skin cancer, immunosuppression, and photoaging. Melanins, which give color to living tissues, play a crucial role in photoprotection and thermoregulation by absorbing and dissipating excess light energy as heat. Drawing inspiration from the natural photoprotective properties of melanin granules, we have synthesized a lightweight and biocompatible melanin-silica hybrid material for use in true broad-spectrum (UVA-UVB-Vis-NIR) sunscreen formulations. Our fabrication method overcomes the challenges associated with manipulating melanins, resulting in a thin layer of eumelanin-like pigment covalently bonded to non-toxic silica nanoparticles. We believe these findings will significantly impact the development of metal-free sun care products, enhancing the photoprotection of sunscreen formulations beyond the UVR region.

Keywords: sun care; melanin; singlet oxygen; visible light; NIR.

Introduction.

Excessive exposure to sunlight initiates several photochemical processes that can have detrimental effects on human skin, such as accelerated aging and cancer. While ultraviolet radiation (UVR; ISO-21348 standard: 100 ≤ wavelength (λ) < 400 nm) has long been recognized as harmful, recent studies indicate that high-energy visible light (HEVL; 400 ≤ λ < 500 nm) also plays a significant role in skin damage. Visible light, which constitutes approximately 45% of solar irradiance compared to only 5% for UV light, penetrates the deepest layers of the epidermis, inducing oxidative stress and cellular damage similar to that caused by UVA radiation. [1]

Current sunscreen formulations are limited as they do not protect against visible light. Additionally, many sunscreen ingredients can permeate human skin, leading to systemic side effects and environmental concerns, which have resulted in bans in several countries. This highlights the urgent need for new sun care technologies that utilize eco-friendly ingredients and provide comprehensive broad-spectrum protection. Most chemical sunscreens are designed to block UVA I (340–400 nm), UVA II (320–340 nm), and UVB (290–320 nm) light. However, broadening this protection to include visible light without altering skin appearance is challenging. Ingredients such as iron oxide, adapted from other cosmetic uses, have raised safety concerns when used for sun protection. There is a lack of suitable ingredients specifically developed for visible-light protection.

Melanin, a natural pigment composed of indole moieties such as indolequinone (IQ), dihydroxyindole (DHI), and their carboxylate derivatives, is synthesized, stored, and transported within melanosomes—organelles produced by melanocytes and pigment-epithelial cells. Melanosomes vary in size and shape, from sub-micrometer spherical grains to elongate particles up to 4-μm in length. Typically, eumelanosomes are ellipsoidal, whereas pheomelanosomes are spherical. Melanin particles, approximately 1-μm in size, possess numerous conjugated double

bonds that scatter and absorb light from the UV to visible regions, serving as a major natural photoprotector for many organisms. Despite its potential as a photoprotective additive in sunscreens, the use of melanin has been limited due to difficulties in stabilizing suspensions of whole melanin particles. Recent advancements have led to the development and patenting of new melanin-based materials. However, there are no reports on the development of biocompatible hybrid silica-melanin nanomaterials or their photoprotective properties.

Our research aims to develop an optimized platform for synthesizing silica nanoparticles coated with an ultra-thin film of melanin. We intend to demonstrate that this hybrid material can protect skin cells against visible-light-induced photodamage, thereby introducing a new paradigm in sun protection. This approach not only addresses the limitations of current sunscreen formulations but also paves the way for more sustainable and effective sun care solutions [2].

Materials and Methods.

Synthesis of Silica Nanoparticles Using the Stöber Sol-Gel Method

Ammonium hydroxide (28% m/v, 12.0 mL), ethanol (240.0 mL), and tetraethyl orthosilicate (TEOS, 8.00 mL, 7.50 g, 36.0 mmol) were added to a 500 mL round-bottom flask and kept under magnetic stirring for 24 h at room temperature. The resulting suspension was centrifuged at 38,000 ×g for 30 min at 25 °C. The white precipitate was collected, dried in a desiccator, and stored at room temperature until use.

Activation and Functionalization of the Nanoparticles

Silica nanoparticles (2.50 g) were dispersed in phosphate buffer (PB, 100 mmol L^{-1}, pH 6.5, 25.0 mL) in a 50 mL round-bottom flask. To this suspension, 3-aminopropyltriethoxysilane (ATPES, 1.20 mL) was added, and the mixture was stirred magnetically at 25 °C for 2 h. The suspension was then filtered under vacuum using a PTFE filter (pore size: 0.47 µm) and washed with PB. The resulting solid was re-dispersed in PB (25.0 mL) and functionalized by adding glutaraldehyde (1.20 mL). This mixture was stirred magnetically at 25 °C for 2 h, followed by

vacuum filtration and washing with PB. The functionalized nanoparticles were dried in a desiccator for 24 h and stored at –20 °C until use.

Immobilization of Tyrosinase and Oxidation of Tyrosine

Functionalized nanoparticles (2.60 ± 0.40 g) were dispersed in PB (25.0 mL). To this suspension, a tyrosinase solution in water (2.00 mg/mL, 1.00 mL) was added, and the mixture was stirred magnetically for 2 h. The suspension was filtered under vacuum and washed with PB. The solid was immediately re-dispersed in PB (25.0 mL), followed by the addition of a solution of L-tyrosine in PB (2.00 mg/mL, 1.00 mL, 11.0 µmol). The mixture was stirred in a shaker incubator at 180 rpm and 30 °C for 4 hours. After this time, the suspension was filtered under vacuum, washed with PB, and the resulting brown solid was dried in a desiccator for 24 h.

Adjustment of Eumelanin Nanoparticle Size

The dried melanin nanosilica was ground using a Retsch RM 200 mortar grinder (piston pressure: 6.5) for 10 min. The powder was then transferred to a 180-µm stainless steel mesh sieve and agitated in a Retsch AS 200 vibratory sieve shaker (amplitude: 2.08 mm/g) for 10 min. The monodispersed aggregated melanin nanosilica was collected and stored at room temperature.

Acquisition of reflectance spectra

The diffuse reflectance accessory (Barrelino, Harrick Scientific) was attached to a Varian Cary 50 spectrophotometer and the instrument was calibrated using a white standard (Spectralon) for 100% reflectance and a dark reference for 0% reflectance. After calibration, the sample holder with uniform size melanin particles was placed into the reflectance accessory, ensuring the sample surface was flat and evenly distributed. The spectrophotometer was set to scan the wavelength range (360–830 nm) and the reflectance spectrum of the microparticles was recorded. The reflectance spectrum was analyzed using the Color software, and the sample spectrum was compared to the baseline and white standard spectra for accuracy. The data were

saved for further analysis. After measurements, the sample holder was removed and cleaned if necessary, and the samples and standards were appropriately stored.

Cell Culture

3T3 murine fibroblast clone A31, obtained from the Rio de Janeiro Cell Bank (BCRJ, Paul Ehrlich Scientific-Technical Association) were grown in Dulbecco's Modified Eagle Medium (DMEM), 2 mM glutamine, and supplemented with 5% (v/v) of fetal bovine serum (FBS), 5% (v/v) fetal calf serum (SFV), 1% (v/v) streptomycin-penicillin. We used 80-90% confluence for the experiments, peeling the cells every 3 days and keeping them in 75 cm2 culture bottles.

Results.

Melanin nanosilica with 55 ± 6 nm diameter was prepared using the well-known Stöber synthesis of silica nanoparticles (Figure 1). The surface of silica nanoparticles prepared by hydrolysis of TEOS were functionalized with aldehyde groups through sequential activation with APTES and glutaraldehyde. The oxidation of L-tyrosine by oxygen in the presence of tyrosinase and functionalized nanoparticles results in melanin-coated silica nanoparticles.

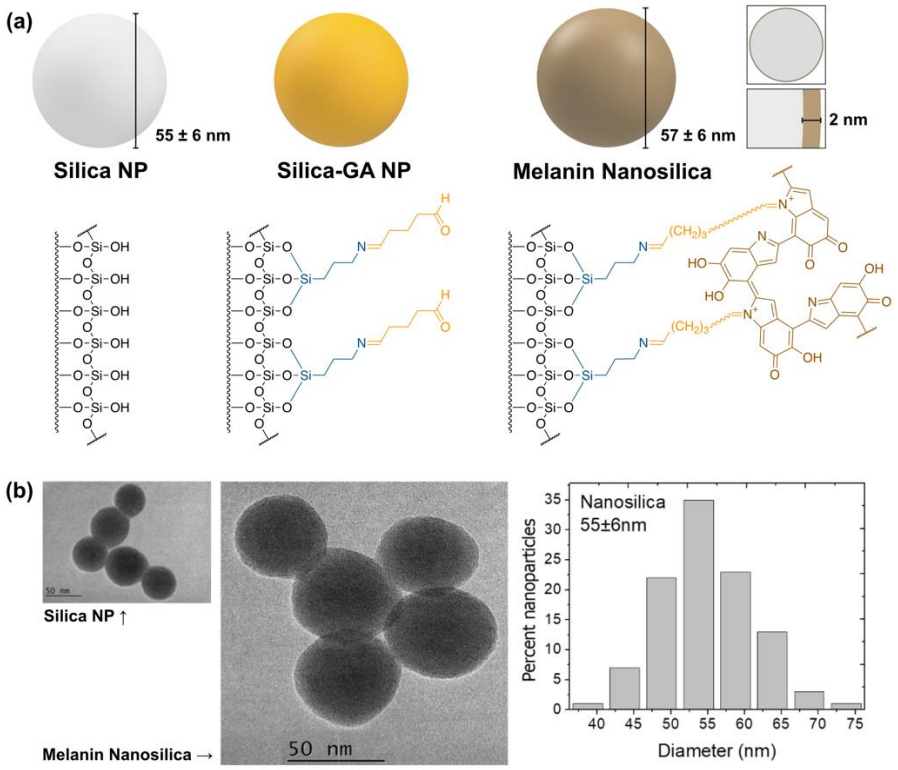

Figure 1. Fabrication of melanin nanosilica. (a) Details on the chemisorption of melanin precursors at the surface of aldehyde-functionalized silica nanoparticles. (b) Transmission electron microscopy (TEM) images of silica NPs with an average diameter of 55 nm.

Melanin/nanosilica exhibits broad-spectrum absorption across the UVR (280-400 nm), visible (400-750 nm), and near-infrared (NIR, 750-1400 nm) spectral regions, all crucial parts of the solar spectrum (Figure 2a and b). This is particularly importantly when considering that current skincare products do not yet provide protection against visible light and NIR. When incorporated into sunscreen formulations, it significantly increases wideband absorbance/reflectance in the visible range. The catalytic mechanism of tyrosinase catalysis is complex and was examined. The simplified sequence of reactions begins with the oxidation of tyrosine to form *o*-quinones, which are further oxidized to form the reactive monomers of melanin, specifically indolequinone (IQ) and dihydroxyindole (DHI). Due to this stepwise catalytic

mechanism, the absorption spectra of the tyrosine solution in the presence of tyrosinase change over time. Initially, 60 min after the start of the reaction, dopachrome accumulates, as indicated by absorption maxima at 305 nm and 475 nm (spectra labeled as “a” in Fig. 2c). As the reaction progresses to 3 h, other derivatives form, showing increased absorption in the red portion of the spectra (spectra “b”, Fig. 2c), due to the formation of IQ and DHI and subsequent melanin oligomers. These oligomers naturally tend to react with each other to form melanin granules. However, in the presence of silica nanoparticles, the result is primarily nanosilica covered with a thin film of melanin (Figs. 2d and 2e).

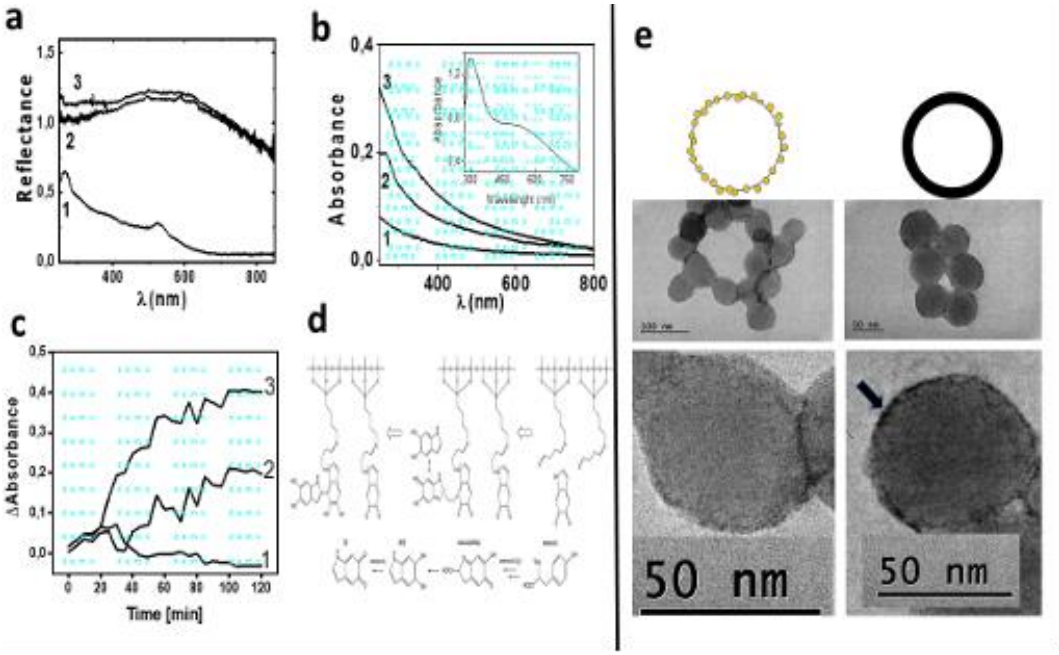

Figure 2. Spectroscopic and nanoscopic characteristics of Melanin/nanosilica. a: Reflectance spectra of surface-activated silica (1), melanin nanosilica (2) and synthetic melanin particles (3); b: Absorption spectra of bare nanosilica (1) of surface-activated nanosilica in the presence of Tyrosine and Tyrosinase 1 h after starting the reaction (2), and of Melanin/nanosilica (3); Insert shows the absorption spectra of the reaction solution of tyrosine and tyrosinase in the presence of bare nanosilica (not previously activated), 3 hours after starting the rection. T=25oC c: Absorbance Change at 800 (1 and 3) and at 475nm (as a function of time of tyrosinase/tyrosine

solutions containing bare silica (1 and 2) and functionalized silica (3), 35 ºC; d: Electron microscopy images of surface-activated silica (a), melanin nanosilica (b). e: Reaction scheme for the chemisorption of melanin precursors at the surface of aldehyde-functionalized silica nanoparticles.

Additionally, melanin/nanosilica protects 3T3 A31 fibroblasts from the harmful effects of blue light at physiologically relevant doses (Figure 3). Nanosilica is not only easy to surface-modify but also plays an active role by scattering light across the entire spectral region of interest (UV/visible/NIR) and possessing robust emissivity in the infrared region, which allows the release of heat generated both naturally and from light-to-heat converted solar energy.

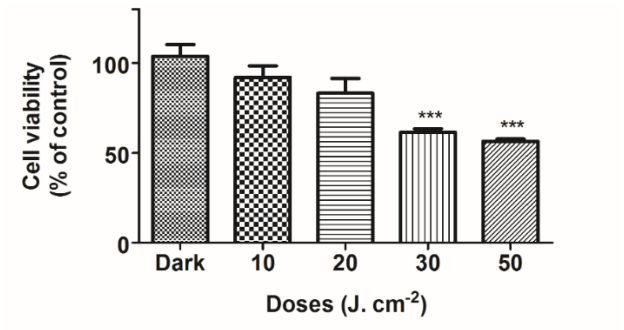

Figure 3. Viability test of 3T3 A3 fibroblasts as a function of blue light (408 nm, power: 2.4 mW/well) irradiation.

Discussion.

Although metal-derived nanomaterials have a strong capacity to absorb and scatter light, even greater than melanin, they have very low emissivity in the infrared, which can cause an uncomfortable warming sensation on the skin. Additionally, metal-based nanoparticles are not as easy to modify as silica. We believe that melanin/nanosilica will significantly advance sun care technology, enhancing sunscreen formulations to provide true broad-spectrum defense. It is

important to note that visible light protection typically affects appearance, but melanin nanoparticles offer the opportunity to develop products that match the user's skin tone.

Conclusion.

Melanin nanosilica has the potential to significantly advance sun care technology by providing true broad-spectrum photoprotection. It can offer visible light protection without changing the user's appearance, as melanin nanoparticles can be matched to different skin tones. Furthermore, melanin nanosilica has optical properties similar to whole melanin granules but with only a small fraction of their mass. This innovation paves the way for sun care products that deliver comprehensive protection while preserving natural aesthetics.

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Conflict of Interest Statement.

The authors disclose they hold a patent related to this research: "Process for obtaining coated nanosilica and their use" (2016) INPI: BR1020160242622, IPC: B82B 3/00 B82B 1/00 C01B 33/14.

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