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Exploring marine algae-derived phycocyanin nanoparticles as a safe and effective adjuvant for sunscreen systems

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Abstract

Background UV radiation (UV) exposure risks skin damage and cancer due to DNA damage and oxidative stress. Synthetic chemical sunscreens that protect against UV radiation can have health and environmental concerns. This study explores phycocyanin (PC), a marine algae-derived natural photoprotective compound, and its crosslinked nanoparticles (PCNP) as safe and effective adjuvants for sunscreen systems.

Methods PCNP was synthesized via genipin-crosslinking. PC and PCNP biocompatibility were assessed on mouse embryonic fibroblast cells. ABTS evaluated antioxidant activity, and the UV absorption capacity of PC and PCNP were analyzed. PCNP skin permeability was tested in vitro and in vivo. Gel formulations with PCNP were examined for UV absorption effects.

Results PCNP showed good biocompatibility, maintaining cell viability above 90% across concentrations. Both PC and PCNP demonstrated concentration-dependent antioxidant activity, efficiently scavenging free radicals. PCNP exhibited enhanced UV absorption in the UVB range compared to PC alone. Skin permeation studies displayed limited PCNP penetration through skin layers. In vivo, absorption assessments indicated PCNP localized mainly in the stratum corneum. PCNP-containing gels displayed improved UV absorption compared to gels without PCNP.

Conclusion This study showcases PCNP's potential as a natural and safe adjuvant for sunscreen with enhanced UV protection capabilities. PCNP preserved antioxidant activity, displayed limited skin penetration, and enhanced UV absorption. The findings suggest PCNP's promise as a viable alternative to synthetic sunscreen agents, delivering effective photoprotection while minimizing health and environmental concerns.

Article Highlights

- Phycocyanin nanoparticles (PCNP), derived from marine algae, show promise as natural and safe adjuvants for sunscreens.
- PCNP exhibit good biocompatibility, maintaining cell viability and demonstrating concentration-dependent antioxidantactivity.
- PCNP exhibit limited penetration through skin layers, primarily localizing in the stratum corneum.

Keywords Phycocyanin nanoparticle · UV radiation · Photoprotective · Marine algae

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1 Introduction

Ultraviolet radiation (UV) is an electromagnetic wave with 200–400 nm wavelengths. It can be classified into three groups based on its physiological effects on the human body: short-wavelength ultraviolet (UVC), medium-wavelength ultraviolet (UVB), and long-wavelength ultraviolet (UVA), with the wavelengths of 200–280 nm, 280–320 nm, and 320–400 nm, respectively. Exposure to both UVA and UVB can lead to erythema, DNA damage, photoaging, and the production of reactive oxygen species (ROS), which can cause acute or chronic harm to the skin [1–3]. Additionally, prolonged exposure to UV radiation can damage DNA and increase the risk of skin cancers, such as basal cell carcinoma, squamous cell carcinoma, and melanoma [4, 5]. So, it is critical to develop sunscreen agents and formulations to protect against the harmful effects of UV radiation [6–8].

The active ingredients in sunscreen products can be classified into inorganic and organic, also known as physical filters and chemical absorbers, respectively [9]. Chemical absorbers protect against ultraviolet radiation by absorbing it, while physical filters reduce its effects through reflection, scattering, and absorption. Each type of ingredient has its own set of advantages and disadvantages. Chemical absorbers are widely used and have noticeable effects. Still, they can contaminate water environments through various means, such as wastewater treatment, swimming, and runoff, leading to pollution and negatively affecting aquatic ecosystems [10]. Moreover, small molecules in these compounds can potentially penetrate the skin and be absorbed by the body [11]. On the other hand, physical filters, like ZnO and TiO2, are safer and have a broader spectrum of efficiency, but they are thick in texture and can leave a white film on the skin, which can be an unpleasant experience for users [7].

Marine algae have been a rich source of photoprotective compounds in recent years. These include mycosporine-like amino acids, sulfated polysaccharides, carotenoids, and polyphenols. Such compounds have been found to have various bioactive properties that enable marine algae to thrive in extreme environments. These properties include UV absorption, antioxidant, inhibition of matrix metalloproteinases, immunomodulatory, and others [12–16]. As a result, these compounds have potential applications in skincare products, cosmetics, and pharmaceuticals. The presence of UVA- and UVB-absorbing compounds in a large number of marine microalgae has been examined [15, 17].

Phycocyanin (PC) is a photoprotective compound of interest. This pigment-protein complex is found in cyanobacteria and is characterized by its blue color. PC has been found to have potent antioxidant and anti-inflammatory properties and protection against UVB-induced apoptosis. Its ability to absorb light makes it a potential candidate for sunscreens, while its low toxicity profile makes it safe for cosmetics [14, 15, 18].

The current study investigates the application of genipin, a biocompatible crosslinker, in covalently crosslinking PC molecules to generate PC nanoparticles (PCNP). The research focuses on examining the size and morphology of the resultant PCNP particles, as well as comparing the cytotoxicity, free radical scavenging ability, and UV radiation absorption capacity of PC and PCNP. Additionally, a carbomer gel incorporating PCNP was formulated, and its ability to absorb UV radiation at different wavelengths within the UVB range was assessed. To ensure the safe utilization of sunscreen components, it is crucial that they remain on the skin surface without being absorbed or penetrating the underlying tissues. Hence, the skin permeability of PCNP was evaluated using an ex vivo permeation chamber method, and the absorption level of a gel containing PCNP was assessed in nude mice.

2 Materials and methods

2.1 Materials

Phycocyanin was obtained from Binmei Biotechnology (Zhejiang, China). Genipin and Carbomer (974P NF) were acquired from Yuanye Bio-Technology Co., Ltd (Shanghai, China). The 3T6 cell line was purchased from Mingzhou Biological Technology Co., Ltd. (Zhejiang, China). Dulbecco's Modified Eagle Medium (DMEM) and penicillin–streptomycin were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Methyl thiazolyl tetrazolium (MTT), 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS), Trypsin, and EDTA were obtained from Biosharp Life Sciences (Anhui, China). Fetal bovine serum was purchased from Tianhang Biological Technology Co., Ltd. (Zhejiang, China). Ethanol, 7-(Diethylamino) coumarin-3-carboxylic acid (DEACCA), and other chemical reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. of analytical grade.

2.2 PCNP preparation

The preparation of phycocyanin nanoparticles (PCNP) was achieved through the genipin-crosslinking method. Specifically, 20 mg of phycocyanin was dissolved in 2 mL of distilled water, and the pH level was adjusted to 8.3 with a 0.1 N NaOH solution. Methanol was then gradually added at a 1 mL/min rate, followed by 200 µL genipin solution under continuous stirring. After being stirred for four hours under dark conditions, the mixture underwent centrifugation at 11,000 rpm for ten minutes. The supernatant was discharged to obtain a blue precipitate, resuspended in 1 mL distilled water, and sonicated for 1 min to disperse the PCNP.

2.3 PCNP characterization

Dynamic light scattering (DLS) was utilized to determine the size distribution and polydispersity index (PDI) of PCNP nanoparticles (DLS, NanoBrook 90Plus, PALS, Brookhaven Instruments, USA.). At the same time, transmission electron microscopy (TEM) was employed to examine their morphology (TEM, Tecnai 12, Philips, Netherlands). The fluorescence spectra of both PC and PCNP were measured using a Shimadzu RF-6000 spectrophotometer and subsequently compared.

2.4 Gel formulation

Carbomer (974P NF) was utilized to prepare a gel containing PCNP. Initially, 0.3 g of Carbomer was added to 7.5 mL of distilled water while stirring to facilitate swelling. Subsequently, 3 g of propylene glycol and 0.54 g of triethanolamine were added to the mixture under continuous stirring. Simultaneously, three replicates were prepared, two of which were combined with 1.5 mL of PC (10 mg/mL) and PCNP (10 mg/mL PC portion) solutions, respectively. The remaining replicate served as a blank gel without PC or PCNP. Consequently, the final concentration of the PC portion in the gel was estimated to be approximately 1.3 mg/g.

2.5 Cell culture

Mouse embryo fibroblasts 3T6 cells were cultured in a DMEM medium containing 10% fetal bovine serum, streptomycin (0.1 mg/mL), and penicillin (100 U/mL). Cells were cultured in a humidified atmosphere of 5% CO_2/air at 37 °C until subsequent treatments. Furthermore, the cells were treated with trypsin EDTA/PBS to detach them for subsequent analyses.

2.6 Animal

The nude mice used in the in vivo experiments (female, 8 weeks old, No. 202216659) were sourced from the Experimental Animal Center at Jiangsu University (Supplier Production License Number: SCXK(Su) 2018-0012, Operation Site/Use License Number: SYXK(Su) 2018-0053). This study adhered to the ethical principles of laboratory animal utilization and implementation, and the experimental protocol was approved by the Ethics Committee of Laboratory Animals at Jiangsu University (Application Number: 11733).

2.7 Cell viability assay

The MTT method was employed to evaluate the biocompatibility of PCNP and PC by determining cell viability. Mouse embryo fibroblasts 3T6 cells in the logarithmic growth phase were diluted with DMEM to a final concentration of 5×10^3 cells/mL. The cell suspension (100 µL) was then inoculated into each well of a 96-well plate and kept in an incubator for 24 h. Afterward, the medium was replaced with a solution containing PCNP and PC at concentrations of 10, 50, 100, and 500 µg/mL, followed by incubation for 48 h. The subsequent steps were performed according to the MTT standard process. The optical density (OD) was measured at 490 nm, and cell viability was calculated using Eq. 1.

$$\text{Cell viability}\,(\%) = \left(\text{OD}_{\text{sample}}/\text{OD}_{\text{Control}}\right) \times 100\%. \tag{1}$$

2.8 Antioxidant activity of PC and PCNP

The ABTS method was employed to evaluate the antioxidant activity of PC and PCNP. A vitamin C solution was used as a control, and the absorbance was measured at 734 nm to calculate the ABTS free radical scavenging rate and the half-maximal inhibitory concentration (IC_{50}). The scavenging rate was calculated using the equation: IR (100%) = $[1-(A_S-A_0)/A_0]\times 100\%$, where A_S and A_0 represent the absorption values of the sample and buffer, respectively. A regression equation was established between the sample concentration and the free radical scavenging rate, and the sample concentration at a rate of 50%, which is the IC_{50}, was calculated.

2.9 In vitro skin permeability of PCNP

The permeability of PCNP on nude mouse skin was investigated using a Franz diffusion cell (PermeGear, Inc, Pennsylvania, USA). The receptor compartment contained a sample volume of 6.5 mL; the membrane area through which the molecule could permeate was 1 cm^2. The skin from 8-week-old nude mice was placed between the donor compartment and the receptor compartment, with the stratum corneum facing the donor compartment and the dermis in contact with the receptor compartment. 0.4 mL of a 10 mg/mL PCNP suspension was present in the donor compartment, while the receptor compartment contained blank PBS. The system's temperature was maintained at 37 °C, and the rotation speed was set at 300 rpm. The experiment was initiated, and at 2, 4, 6, 8, 10, and 12 h, 0.5 mL of the sample was withdrawn from the receptor compartment, and the same volume of PBS was added to maintain the volume. To quantify PC and PCNP, the fluorescence intensity was measured using a fluorescence spectrophotometer (RF-6000, Shimadzu, Japan). The cumulative permeation amount, Qn (μg/cm^2) per unit area, was calculated using Eq. 2. The obtained values of Qn were plotted against time to create the cumulative permeation curve.

$$Qn = \left(\sum_{i=1}^{n} Cn \times V\right)/S \tag{2}$$

Cn is the test concentration of the sample taken each time, V is the volume of the solution in the receptor compartment, and S is the membrane area. Cumulative permeability per unit area K = (Qn/m) × 100%, while m is the total PCNP in the donor compartment.

2.10 UV absorptions of PC solution and PCNP suspension

The UV absorption of PC and PCNP were determined by a UV spectrophotometer (UV-8000, Shanghai Metash Instruments, Co., Ltd, Shanghai, China), mainly described by Mansur et al. A series of PC and PCNP in ethanol with concentrations ranging from 10 to 2000 μg/mL were prepared. Each preparation's absorbance (290–320 nm with 5 nm interval) was determined using a spectrophotometer.

2.11 UV absorption effects of gels

This study utilized ultraviolet photometry to evaluate the UV absorption effect of PC and PCNP gels in the UVB region (280–320 nm). The transparent surfaces of the quartz cuvettes were covered with 3 M medical tapes (1 cm × 4 cm) to simulate skin texture. Five parallel samples were prepared for each, with 8 ± 0.2 mg of gel applied to one cuvette and dried at 35 °C for 30 min. A gel without PC or PCNP was used as a blank control. The absorption of optical densities at 285, 290, 295, 300, 305, 310, 315, and 320 nm was measured to estimate the photoprotective properties of the gels containing PC or PCNP.

2.12 In vivo absorption of PCNP gel

Gels containing either 0.7 g PCNP or an equal amount of 7-(Diethylamino) coumarin-3-carboxylic acid (DEACCA) were evenly applied to the skin on the back of the nude mice (Laboratory Animal Resources Center of Jiangsu University, No. 202216659), and sterile gauze was used to cover it to prevent the animal from licking the gel. 24 h later, the mice were euthanized to collect their back skin. Skin samples were rinsed in PBS three times to remove excess gel and dried with paper tissue. Following that, skin samples were embedded and frozen with a microtome for cross-sectional skin slices.

These slices were placed on glass slides and observed under a fluorescence microscope (DeltaVision Elite, General Electric Company, USA) to examine the localization of the molecules in the skin over different time intervals. PC exhibits inherent red fluorescence, whereas DEACCA exhibits green fluorescence.

2.13 Statistical analysis

The measurements were done in triplicate. The data are presented as the mean ± standard deviation (SD). One-way analysis of variance test was performed using SPSS version 28. ANOVA was conducted in OriginPro 9.1, and the post hoc analysis method used was Tukey's HSD test.

3 Results

3.1 Phycocyanin nanoparticles preparation and characterization

The fabrication and characterization of phycocyanin nanoparticles (PCNP) were conducted, with results shown in Fig. 1. Figure 1a demonstrates the blue color of the PCNP suspension. Dynamic light scattering (DLS) in Fig. 1b indicated a normal particle size distribution with an average diameter of 175.2 nm. Figure 1c's transmission electron microscopy (TEM) image displayed consistent spherical PCNP (indicated with arrows) with sizes ranging from 70 to 100 nm. Importantly, DLS tends to overestimate nanoparticle size, while TEM provides the projected area diameter. Furthermore, fluorescence spectra analysis (Fig. 1d) displayed PCNP retaining PC's fluorescence characteristics, with a slightly blue-shifted maximum emission wavelength compared to PC.

3.2 Cell viability

The impact of different concentrations of PC and PCNP on the viability of mouse embryonic fibroblast cells was assessed using the MTT assay. As shown in Fig. 2, in the concentration range of 0–600 µg/mL, both PC and PCNP made the cell viability slightly decrease with the increase of concentration, but the survival rate of each group still remained above 90%, showing a good biocompatibility in vitro. These findings suggest that PC and PCNP can potentially be used as photoprotective agents for further research on sunscreen systems.

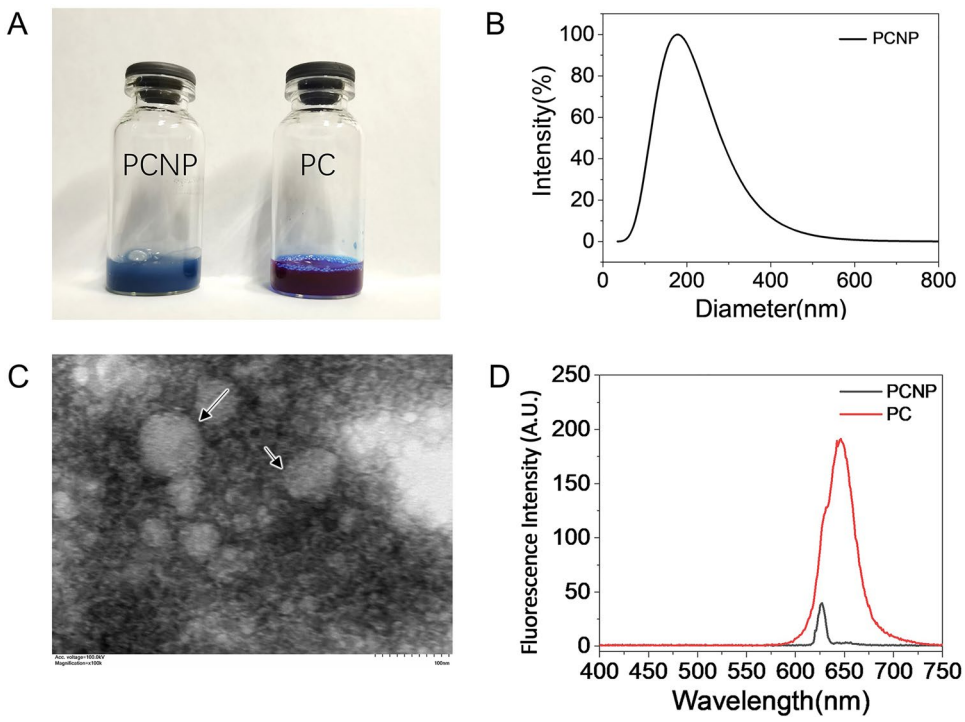

Fig. 1 Fabrication and characterization of PCNP. **A** PC solution (right) and PCNP suspension (left). **B** Particle size distribution of PCNP. **C** Transmission electronic microscope (TEM) image of PCNP (indicated with arrows). **D** Fluorescence emission spectrum of PC (red), and PCNP (gray)

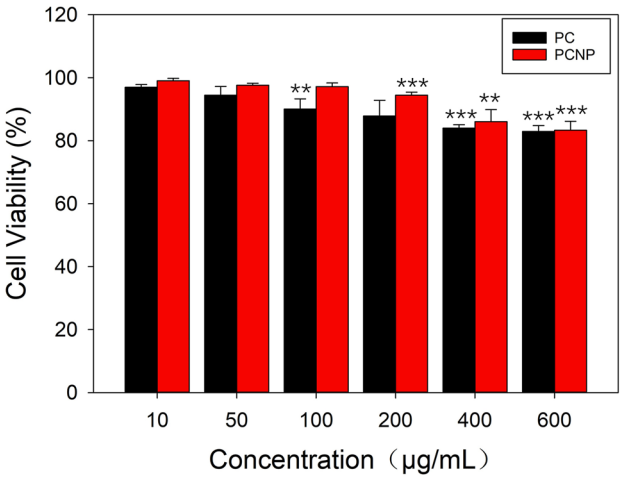

Fig. 2 Cell viability to evaluate PC and PCNP biocompatibility to 3T6 cell (n = 6, all data are presented as the mean ± SD. Significant difference analysis was performed when compared with 10 μg/mL group, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$).)

3.3 Antioxidant activity of PC and PCNP

The antioxidant activity of PC and PCNP was evaluated using the ABTS method, with vitamin C (VC) serving as a control. The ABTS free radical scavenging rate and the half-maximal inhibitory concentration (IC50) were calculated and plotted in Fig. 3. The IC50 values for PC, PCNP, and VC were 19.75 μg/mL, 65.22 μg/mL, and 0.42 μg/mL, respectively. Given the larger molar mass of PC molecules, this result can demonstrate that both PC and PCNP possess good antioxidant capabilities.

3.4 UV absorptions of PC solution and PCNP suspension

The UV radiation absorptions of PC solution and PCNP suspension were analyzed, as shown in Fig. 4. Within the 290 to 320 nm range, as the concentrations of PC or PCNP increased, their ultraviolet absorption values at the same wavelength significantly increased. Notably, PCNP exhibited greater absorption capacity in the shorter wavelength UVB region.

3.5 UV absorption effects of PC and PCNP gels

The UV absorption effects of PC and PCNP gels (Fig. 5a) in the UVB region (280–320 nm) were evaluated using a UV spectrophotometer. The absorbance values were compared and shown in Fig. 5b. It was observed that both gels exhibited significant absorption in the UVB range. The PCNP gel showed slightly higher absorbance values than the PC gel, particularly at wavelengths of 320 nm and 280 nm.

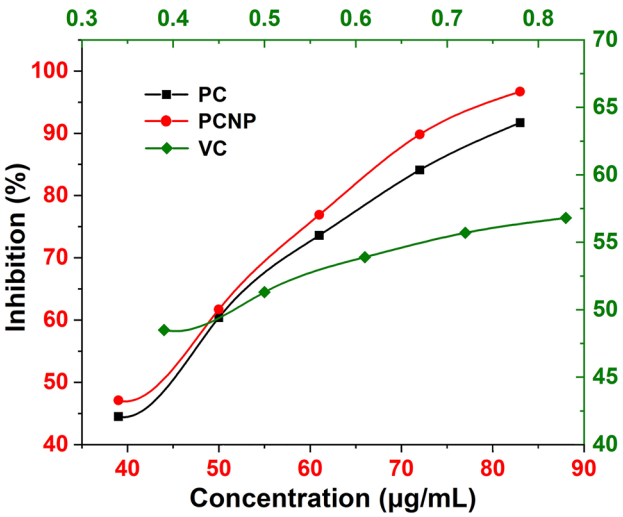

Fig. 3 Fitting curves of ABTS free radical scavenging rate for PC (black) and PCNP (red), while vitamin C (green) as a control

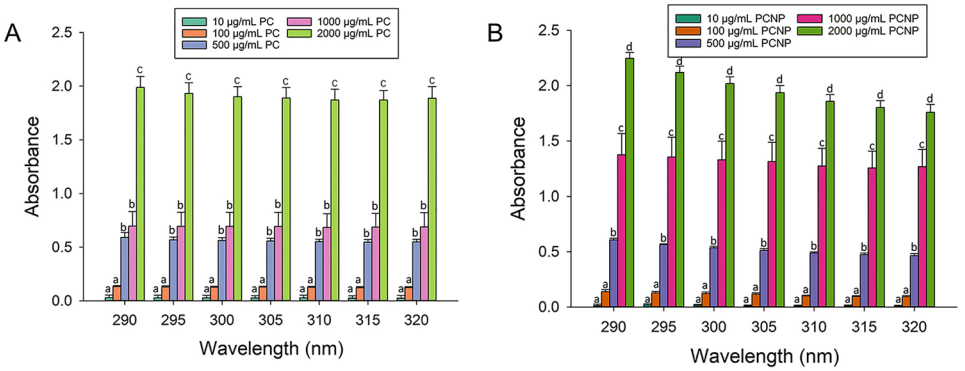

Fig. 4 UV radiation absorptions by PC (**A**) and PCNP (**B**) determined by UV spectrophotometer (n = 3, all data are presented as the mean ± SD (n = 5, all data are presented as the mean ± SD. different lowercase letters a, b, c and d represent the significance levels in the post Tukey HSD test))

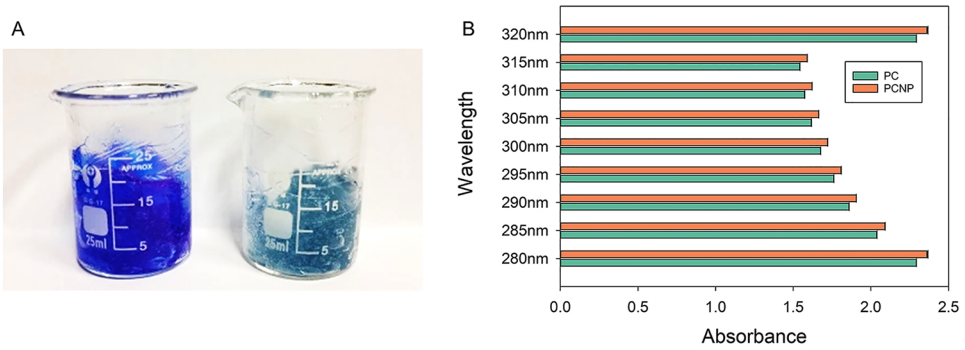

Fig. 5 UV absorption effects of PC and PCNP gels. **A** Carbomer gels containing an equal concentration of PC (left) and PCNP (right). **B** UV absorption of PC and PCNP gels in UVB region (280–320 nm)

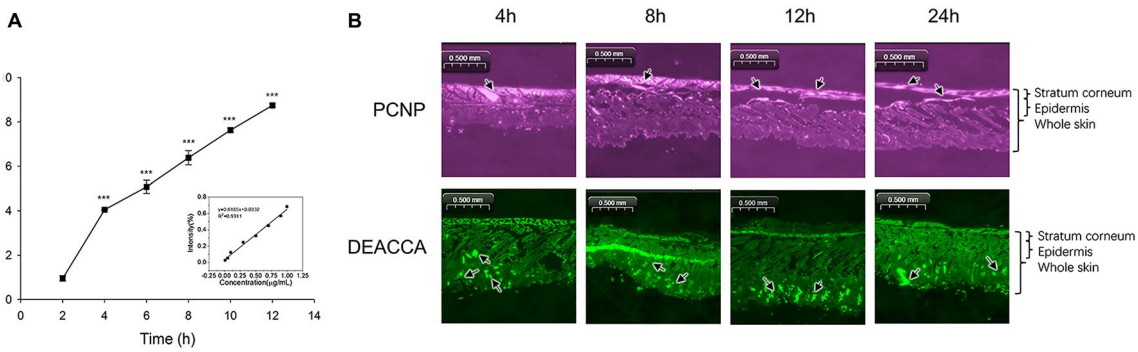

Fig. 6 Results of skin permeation study. **A** In vitro skin permeability of PCNP (n = 3, all data are presented as the mean ± SD. Significant difference analysis was performed when compared with 2 h group, ***$p < 0.001$). **B** In vivo absorption of PCNP gel. DEACCA as a small molecule control, emits green fluorescence, while PCNP inherently exhibits a purplish-red fluorescence, both of which serve to self-trace their locations (indicated with arrows)

3.6 In vitro skin permeability of PCNP

The safety of using PCNP and PCNP gel on the skin was evaluated by studying the skin permeability of PCNP in vitro using the Franz diffusion cell. The cumulative permeation of PCNP in 12 h was 8.746 μg/cm^2, with a cumulative permeation rate of 0.219% (Fig. 6a). Comparing these results with the permeation rate of benzophenone in hairless mice [19], it was observed that the permeation rate of PCNP was significantly lower.

3.7 In vivo absorption of PCNP gel

The in vivo skin absorption study of PCNP in gel form was conducted on nude mouse skin, with 7-(Diethylamino) coumarin-3-carboxylic acid (DEACCA) as a small molecule control. The results (Fig. 6b) indicated that DEACCA penetrated the skin and reached the bottom layer after 4 h, while PCNP remained mainly on or within the stratum corneum layer of the skin. This suggests that PCNP, due to its larger molecular weight and enlarged particle size through crosslinking, has a higher tendency to remain in the outermost layer of the skin, indicating low absorption into the deeper tissues or bloodstream.

4 Discussion

Some research has explored the potential use of fluorescent proteins from marine organisms as photoprotective agents and antioxidant resister [20–22]. The advantages of using fluorescent proteins as photoprotectors in sunscreen preparations could include their natural origin (since they are derived from living organisms) and their ability to absorb light in a specific wavelength range [23, 24]. However, one disadvantage is that fluorescent proteins may not be as effective at blocking UV radiation as other synthetic UV filters commonly used in sunscreens. There may also be concerns about the potential for allergic reactions or other adverse effects.

The present study aimed to investigate the fabrication and characterization of phycocyanin nanoparticles (PCNP) and evaluate their potential as adjuvants for sunscreen system.

After PLGA preparation, the DLS analysis demonstrated a normal distribution of particle sizes, while the TEM image confirmed a consistent spherical shape of the PCNP. These findings align with previous studies that reported successful synthesis of phycocyanin nanoparticles with similar characteristics [18, 25]. The fluorescence spectra analysis revealed a slightly blue-shifted maximum emission wavelength for PCNP compared to PC, which may be attributed to the crosslinking agent concentration and the phycocyanin molecule's size after crosslinking [26, 27].

The assessment of cell viability indicates that PCNP, as well as PC, can be considered as potential adjuvants for further research on sunscreen systems. However, it is essential to note that in vitro cell viability assays provide a preliminary evaluation of biocompatibility, and further studies, such as in vivo experiments, are necessary to assess the safety and efficacy of PCNP.

The antioxidant activity of PC and PCNP showed concentration-dependent free radical scavenging ability. This finding is consistent with previous studies that have reported the antioxidant properties of phycocyanin and its potential to protect against oxidative stress and inflammation [19, 28–30]. The ability of PCNP to retain good free radical scavenging ability even after crosslinking further supports its potential as a molecule and nanoparticle additive with skin protection functions. These characteristics endow PCNP with the potential to exert photoprotective effects and protect the skin from light-induced oxidative stress, inflammation, and aging, thereby demonstrating promising applications.

The UV absorption capacity of PC and PCNP showed increased absorption of ultraviolet radiation with increasing concentration. PCNP exhibited a greater absorption capacity in the shorter wavelength UVB region, which suggests that PC and PCNP could serve as natural sources of adjuvant for sunscreen systems, offering UV protection. The synergistic effect between PC and nanoparticles in PCNP contributes to the enhanced UV absorption ability of the gel, providing a novel approach for developing safe and effective sunscreens.

The PCNP's skin permeability results indicate limited absorption through the skin, primarily confined to the stratum corneum layer. This suggests a reduced potential for penetration into deeper tissues or systemic circulation. Using protein nanoparticles in sunscreen formulations addresses concerns regarding the toxicity associated with skin penetration of small molecule sunscreens. Their larger size and surface characteristics contribute to their diminished skin penetration, thereby mitigating potential toxicity risks [7]. Furthermore, being derived from biopolymers, PCNP demonstrates biocompatibility, supporting its safety profile. These findings underscore the potential of PCNP as a safe addition to skin cosmetics, offering, adequate sun protection without significant systemic absorption or associated toxicity. Further toxicological investigations are warranted to fully ascertain the long-term safety and suitability of PCNP in skin care applications.

As shown in the reults, PCNP retains PC's antioxidant and UV absorption abilities. Moreover, gels containing PCNP exhibit a moderate UV absorption. This approach addresses two aspects: first, it reduces the likelihood of instability

of PC protein molecules in sunscreen formulations, and second, the polymerized nanoparticles can scatter light while retaining their inherent UV absorption characteristics. Thus, PCNP can provide both chemical and physical sun protection functionalities. Furthermore, PC and PCNP exhibit excellent biocompatibility with cells, and skin permeation experiments on nude mice demonstrate that PCNP can avoid penetrating the inner layers of the skin, unlike small-molecule substances. Additionally, PC's anti-aging and antioxidant properties could enhance its suitability as a sunscreen component. Overall, these findings highlight the potential of PCNP as a natural and safe adjuvant for a sunscreen system with enhanced UV protection capabilities.

While the results are encouraging, there are still inherent limitations that could impact the translation of findings to practical applications. Notably, the skin permeability model, although insightful, may not fully replicate the complexity of human skin permeation dynamics, emphasizing the need for further in vivo studies for a more accurate understanding of the skin absorption profile of PCNP. Additionally, while the results obtained demonstrate moderate sun protection performance, further investigations should explore methods to enhance the UV protection efficacy of PCNP, potentially through complementary formulations or synergistic combinations with other photoprotective agents. Moreover, considering the growing emphasis on safety in cosmetic products, there is a critical need for comprehensive long-term safety evaluations to assess the potential risks associated with prolonged and repetitive use of PCNP-based formulations. Future research efforts should also aim to explore novel delivery systems and formulation strategies to optimize the performance of PCNP-based sunscreens, thereby ensuring enhanced skin protection and minimal adverse effects.

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Author contributions CJ, ZX, LZ, ZM, QC, SS, and QX all contributed significantly to this manuscript. They conducted experiments and analyzed results related to the fabrication, characterization, biocompatibility, antioxidant activity, and skin permeation studies of phycocyanin nanoparticles (PCNP). QC specifically performed cell viability experiments, demonstrating the non-toxic nature of PC and PCNP to 3T6 cells. ZM conducted ABTS free radical scavenging experiments and analyzed the data. SS performed experiments, analyzed data, and provided insights into the limited skin permeability of PCNP. SS and QX supervised the research process, including study conceptualization, experimental design, and data interpretation, and provided guidance throughout the manuscript preparation. All authors reviewed and approved the final version for submission to SN applied sciences.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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