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Exploiting Mycosporines as Natural Molecular Sunscreens for the Fabrication of UV-Absorbing Green Materials

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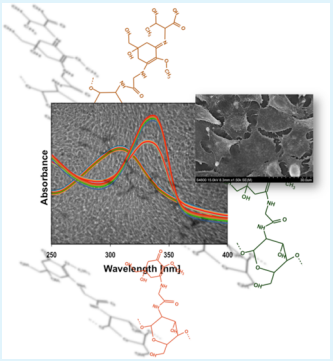
Supporting Information

ABSTRACT: Ultraviolet radiations have many detrimental effects in living organisms that challenge the stability and function of cellular structures. UV exposure also alters the properties and durability of materials and affects their lifetime. It is becoming increasingly important to develop new biocompatible and environmentally friendly materials to address these issues. Inspired by the strategy developed by fish, algae, and microorganisms exposed to UV radiations in confined ecosystems, we have constructed novel UV-protective materials that exclusively consist of natural compounds. Chitosan was chosen as the matrix for grafting mycosporines and mycosporine-like amino acids as the functional components of the active materials. Here, we show that these materials are biocompatible, photoresistant, and thermoresistant, and exhibit a highly efficient absorption of both UV-A and UV-B radiations. Thus, they have the potential to provide an efficient protection against both types of UV radiations and overcome several shortfalls of the current UV-protective products. In practice, the same concept can be applied to other biopolymers than chitosan and used to produce multifunctional materials. Therefore, it has a great potential to be exploited in a broad range of applications in living organisms and nonliving systems.



KEYWORDS: *mycosporine, natural sunscreen, chitosan, ultraviolet-absorbing material, biocompatibility*

1. INTRODUCTION

Populations in western countries tend to expose themselves to sun or artificial UV light to improve physical appearance,[1] while people in other geographic areas have limited awareness of the importance of photoprotection. These behavior and lack of consciousness complicate the fight against skin diseases such as cancer. Furthermore, UV exposure modifies the properties and durability of nonliving materials, for instance, outdoor and textile materials.[2] As a result, such materials need to be replaced more frequently or surface treated, which has an economic impact on private life and budget of public organizations. Many synthetic organic and physical UV-proof products are available. Typical examples are dioxybenzone, avobenzone, TiO_2, ZnO_2, Mexoryl, and Tinosorb. However, these agents are not always friendly to the environment, fully efficient for protection against both UV-A and UV-B, or sufficiently stable.[3−6] In addition, their regular use may have an impact on human health.[3−6] Thus, there is an urgent need to develop new materials that outperform the currently used UV-protective products.

We demonstrate here that this issue can be addressed by exploiting the UV-absorbing properties of small metabolites to develop novel highly efficient UV-protective green materials. Mucus from reef fishes and fish lenses are known to contain natural molecular sunscreens designated as mycosporines and mycosporine-like amino acids (altogether abbreviated here as MAAs).[7−9] Different types of MAAs also occur in microorganisms and algae exposed to UV in their natural habitats.[10−14] These organisms synthesize MAAs as a natural way to protect themselves from the damaging effects of UV radiations. Among all natural UV-absorbing compounds, MAAs exhibit the highest absorbing capacity at 310−360 nm, that is, in both the UV-A and the UV-B regions, with typical molar extinction coefficients in the 28 000−50 000 M^{-1} cm^{-1} range.[15] Examples of such compounds are mycosporine-glycine (MGly), porphyra-334 (P334), and shinorine (SH).[13,14,16−18] These

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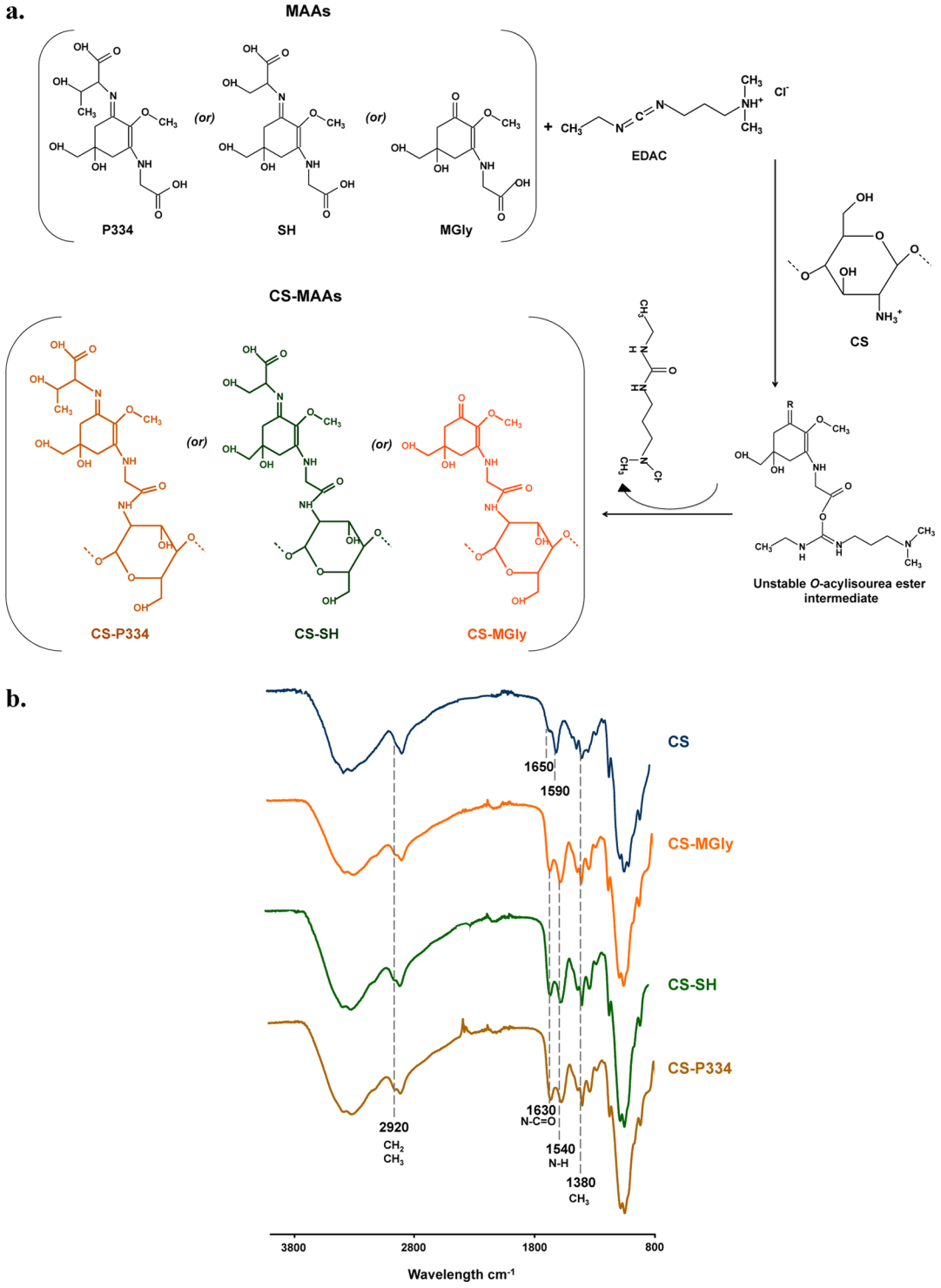

Figure 1. Chemical structures and synthesis of CS-MAA conjugates. (a) Schematic diagram illustrating the carbodiimide-based procedure used for grafting P334, SH, or MGly on chitosan. EDAC first reacts with the MAA carboxyl group to form an unstable amino-reactive *O*-acylisourea intermediate ester (R= $C_3H_4O_3N$ for P334, $C_4H_7O_3N$ for SH, and O for MGly). The latter subsequently reacts with the amino group of CS, thereby forming an amide bond between CS and MAA and yielding a stable CS-MAA conjugate. (b) ATR-FTIR spectra of CS and CS-MAAs. The characteristic absorption bands of CS[30] are visible at 1650 (C═O stretching vibrations coupled to the N−H bending vibration), 1590 ($-NH_2$ bending), 1380 ($-CH_2$ bending), 3365 (−OH groups), 1152 (antisymmetric stretching of the C−O−C bridge), 1084, and 1040 (skeletal vibrations involving the C−O stretching) cm^{-1}.

molecules contain amino acid residues linked to a central ring and form resonance tautomers whose extensive conjugation facilitates the absorption of UV radiations (Figure 1a). Because of these excellent properties, MAAs have recently been exploited as additives in products for skin protection[19,20] and as photostabilizers in nonbiological materials for increasing the durability of plastics, paints, and varnishes.[21] In addition, the UV-protective effect and positive action of MAAs on the

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viability of human skin fibroblasts were demonstrated, with no adverse effect.[22] Another advantage of using MAAs is the possibility to produce some of them in large scale by genetic engineering in bacteria.[10,11] However, to date MAAs have been exploited in formulations as free molecules only and in very few instances.[19] Yet a much broader range of UV-protective materials with a large variety of applications can be envisioned after immobilization of MAAs on polymers. Indeed, conjugation of these small molecules has multiple advantages over their use as free bioactive compounds. For instance, depending on the type of polymers used for grafting, multifunctional (bio)materials with limited solubility and diffusion capacity can be generated, thereby favoring local application and efficiency. The data presented here illustrate this potential through the generation of novel chitosan-MAA conjugates with unique physicochemical and biological properties. Chitosan (CS) is produced by deacetylation of chitin, which is a naturally abundant polysaccharide encountered in many extracellular matrices such as the exoskeleton of crustaceans and insects, and the cell walls of fungi.[23,24] As opposed to chitin, CS has the advantage of being soluble in mildly acidic aqueous media (pH < 6.3), which allows the easy preparation of many physical forms of products, for example, solutions, emulsions, gels, films, and fibers.[23,24] The polymer is also biocompatible, biodegradable, and exhibits antimicrobial properties.[23] Because of this range of physical, chemical, and biological properties, CS is being used in a vast array of applications, including, but not limited to, biomedical, pharmaceutical, cosmetic, food, textile, and packaging products.[23,24] Thus, CS represents a biopolymer of choice for the further development of functional materials relevant to many sectors. For this reason, it was selected here as a matrix for functionalization by MAAs.

2. METHODS

Chemicals and Materials. Medical-grade CS was purchased from Mahtani Chitosan PVT Ltd. (India). CS was purified before use by precipitation.[25] Briefly, CS was dissolved in 1% (v/v) aqueous acetic acid, and the solution was filtered on 1.2-μm filters (Millipore). The polymer was precipitated by increasing the pH of the solution to 8.5 using a 1 M NaOH solution. The precipitate was washed with distilled water until neutrality, and air-dried. The deacetylation degree of CS (98%) was determined by ^{1}H NMR spectroscopy in D_2O (Acros Organics) containing 1% of CD_3COOD (Sigma-Aldrich) using a DRX-300 Brüker spectrometer.[26] The viscosity-average molar mass of the polymer (500 000 g mol^{-1}) was determined by viscosimetry using the published Mark–Houwink constants.[27] All MAAs were from lyophilized extracts of marine organisms collected in 2011 and purchased from the Laboratory of Photobiology of the Central Research Services of the University of Málaga (Spain). MGly was extracted from *Lichina pygmaea*, P334 was isolated from *Porphyra rosengurttii*, and SH was from *Gelidium corneum*. The extraction, characterization, and quantification of the MAAs were performed as described previously,[14] and the purified molecules were stored at −20 °C until use. The UV absorption spectra, chemical structure, and molar extinction coefficient of MGly, P334, and SH are presented in Supporting Information Figure S2. EDAC (commercial grade) was purchased from Sigma-Aldrich (Saint Louis, MO).

Synthesis and Characterization of the CS-MAA Conjugates. All conjugates were synthesized by forming amide bonds between the primary amino groups of CS and the carboxylic acid groups of the MAAs using EDAC as the carbodiimide coupling agent (Figure 1). Briefly, 1% (w/v) CS solutions were prepared by dissolving the purified CS in 100 mL of aqueous acetic acid (1%; v/v). EDAC was then added at the same molar concentration of CS and kept under vigorous stirring until complete dissolution. Each MAA was subsequently added in separate reaction vessels to independent CS solutions (same molar concentration as CS). Conjugation was performed in the dark for 3 h at pH 5 and 25 °C under permanent stirring. At the end of the reactions, the free acetic acid, NaOH, isourea, and unreacted MAAs were eliminated by dialysis using membranes with a molecular weight cutoff of 12 000–14 000 (Spectra/Por, Spectrum Laboratories, Inc., Canada). The samples were then lyophilized and stored at 4 °C until further use.

Conjugation of the MAAs was verified by ATR-FTIR spectroscopy using a Nicolet Nexus 670 FTIR spectrophotometer equipped with a KRS-5 crystal (refractive index 2.4; incidence angle 45°). The spectra were recorded in the transmittance mode between 800 and 4000 cm^{-1}, with a resolution of 4 cm^{-1} and after 128 scan accumulations. The degree of substitution of CS was determined by element analysis. CHN elemental composition of CS and the CS-MAA conjugates was determined using a Eurovector EA 3000 element analyzer. The degree of substitution (DS) of CS in the CS-MAA conjugates was calculated using the following equation of Inukai at al.,[28] which was adapted to each of the samples to take into account their varying nitrogen content: $DS = ((C/N) - (C/N)_{CS})/n$, where C/N is the ratio (w/w) of carbon/nitrogen in the CS-MAA considered, $(C/N)_{CS}$ is the ratio (w/w) of carbon/nitrogen in pure CS, and n is the ratio between the number of carbon and nitrogen atoms in the considered MAA molecules. The molar extinction coefficient (ε) of each conjugate was determined spectrophotometrically using the Beer–Lambert law.

Preparation of CS-MAA Films. CS-MAA films were prepared by a casting/solvent evaporation approach. Briefly, the CS-MAA conjugates (1.0%; w/v) were dissolved under vigorous stirring in aqueous acetic acid (1.0%; v/v) for 24 h at 25 °C. After complete dissolution, liquids were poured into acrylic plates (5 × 5 cm^2), and the solvent was evaporated in a ventilated oven at 30 °C. After 20 h, the films were removed from their molds and kept in a conditioning cabinet at 50% relative humidity and 25 °C to ensure the stabilization of their moisture content before characterization. CS films used as controls were prepared using the same approach.

Absorption Capacity of the CS-MAA Films. Absorption and transmittance spectra were recorded using a Shimadzu UV–vis–NIR spectrophotometer UV-3600 (Japan) in the UV–visible region (250–800 nm).

Photostability of the CS-MAA Films. The CS and CS-MAA films were irradiated at room temperature using a 254 nm UV bench lamp (XX-15S UVP, Inc.) positioned at a distance of 10 cm from the sample. The light power was 0.6 W cm^{-2}, and the films were irradiated for up to 12 h. The absorption capacity of the samples was recorded at 307 and 334 nm every 2 h during the irradiation period as above.

Thermoresistance of the CS-MAA Films. The thermoresistance of all films was evaluated in the Shimadzu UV-3600 spectrophotometer fitted with a S-1700 thermoelectric cell holder. The temperature was maintained at 80 °C for up to 12 h, and absorbance spectra were recorded every 2 h in the range 250–400 nm.

Biocompatibility of the CS-MAA Films. Murine L-929 fibroblasts from an immortalized cell line were cultured in MEM medium (Gibco, Paisley, UK) supplemented with 1 mM sodium pyruvate (Gibco, Paisley, UK), 1% nonessential amino acids (Gibco, Paisley, UK), 1% penicillin-streptomycin (Lonza, Verviers, Belgium), and 10% fetal bovine serum (Biochrom AG, Berlin, Germany) (complete medium). CS-MAA films (6 cm^2, thickness ≤0.5 mm) were sterilized in 70% ethanol for 2 h and washed three times in sterile phosphate buffered saline (PBS) for 10 min. Extracted media were prepared for short-term in vitro cytotoxicity assays according to the ISO 10993-12 standard. For this purpose, each sterilized CS-MAA film was incubated for 24 h at 37 °C in 1 mL of complete culture medium. The collected extracted media were then added to cultures of murine L929-fibroblasts that had preliminary been seeded at a density of 4 × 10^3 cells/well in 96-well plates (Sarsted, Newton, U.S.) and allowed to attach for 24 h. Positive and negative controls were obtained in the same conditions as above by replacing the CS-MAA films with polyvinyl chloride (Portex Ltd., UK) and high-density polyethylene (USP Rockville, U.S.) foils, respectively. A colorimetric test based on methyl tetrazolium tetrabromide (MTT; Sigma-Aldrich, U.S.) was used to assess cell viability. Viable cells only are able to reduce MTT to

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formazan, which is then dissolved in dimethyl sulfoxide. The absorbance of formazan measured at 550 nm is proportional to the number of viable cells (Synergy HT spectrophotometer, Biotek, U.S.). Cell adhesion, morphology, and viability were evaluated by SEM and the Live/Dead assay[29] based on bright-field and fluorescence confocal microscopy. In all cases, CS, CS-SH, CS-MGly, and CS-P334 films of 0.5 cm^2 were placed in 24-well ultralow attachment culture plates (Corning, NY). After sterilization as described above, the films were prewetted in complete culture medium for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. Aliquots containing 5×10^4 L-929 fibroblasts were then seeded onto the films. The Live/Dead assay was performed after 14 and 21 days culture by incubating the adhered cells with 4 μM calcein-AM (Sigma-Aldrich, Saint Louis, MO) and 5 μM ethidium homodimer-1 (Molecular Probes, Eugene, OR). Each sample was examined under a confocal microscope (Olympus LV500, Japan) to visualize adhered viable cells (green fluorescence; λ_{ex}–λ_{em} = 490–515 nm) and dead cells (red fluorescence; λ_{ex}–λ_{em} = 490–630 nm). Bright-field and fluorescence micrographs were recorded on each sample. SEM observations of the L-929 fibroblasts attached to the films were performed 72 h after incubation. The samples were first rinsed three times in Sorensen buffer (Panreac AppliChem, Spain), fixed using 2% glutaraldehyde (Panreac) in a 0.1 M cacodylate buffer, and successively postfixed in 1% OsO_4 for 1 h, washed with PBS, dehydrated using a series of graded ethanol solutions, and dried in hexamethyldisilazane for 10 min. The samples were then sputtered with a thin layer of gold under an argon atmosphere and observed with a Hitachi S-4800 scanning electron microscope using an accelerating voltage of 15 kV and a magnification of 1500×.

3. RESULTS AND DISCUSSION

The UV-protective materials described here were prepared by first activating the carboxylic acid groups of selected MAAs, that is, MGly, P334, and SH, with *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC). This activation step leads to the formation of unstable *O*-acyl-isourea ester derivatives of MAAs that readily react with the primary amino groups of CS to form CS-MAA stable conjugates (Figure 1a). The successful covalent modification of CS was demonstrated by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy (Figure 1b).

As compared to the spectrum obtained from unmodified CS, the spectra corresponding to CS-P334, CS-SH, and CS-MGly exhibited well-defined absorption signals at 1540 and 1630 cm^{-1} that are characteristic of the MAA molecules. These bands are assigned to the N–H bending and N–C═O stretching in amide II, respectively. In addition, the absorption band at 2920 cm^{-1} that corresponds to the C–H stretching of the CH_2 and CH_3 groups from the MAA molecules was detectable in the CS-MAAs spectra only. Supporting evidence for the successful conjugation of MAAs is the occurrence of an absorption band at 1380 cm^{-1} of a higher intensity in all three CS-MAAs spectra (Figure 1b). This signal is attributable to the CH_3 group symmetrical angular deformation mode.

The CS polymer used for chemical grafting had an average molecular mass of 500 000, and its deacetylation degree was of 98% (see Methods). Thus, it can be estimated that each molecule of CS contains an average of ca. 3000 free amino groups. The degrees of substitution of the modified CS determined by element analysis were of 0.04 for CS-P334 and CS-MGly, and 0.08 for CS-SH.

These low values indicate that the CS-P334 and CS-MGly macromolecules were modified by an average of 120 P334 and MGly molecules, respectively, whereas the CS-SH conjugates carried twice as many SH molecules. As opposed to unmodified CS and despite their low degrees of substitution, the CS-MAA conjugates exhibited remarkably high UV-absorbing properties in both the UV-A (320–350 nm) and the UV-B (290–320 nm) regions (Supporting Information Figure S1). The absorption spectra and molar extinction coefficients (ε) of all three conjugates were comparable to those of the corresponding free MAA molecules (ε_{334} = 43 200 M^{-1} cm^{-1} for P-334;[14] ε_{334} = 44 700 M^{-1} cm^{-1} for SH;[16] ε_{307} = 28 100 M^{-1} cm^{-1} for M-Gly[8]) (Supporting Information Figures S1 and S2). Thus, the coupling reaction with EDAC did not alter the spectral characteristics of MAAs. Indeed, the experimentally measured ε values at the maximal absorption wavelengths were of 46 470, 40 270, and 30 150 M^{-1} cm^{-1} for CS-P334 (λ_{max} 334 nm), CS-SH (λ_{max} 334 nm), and CS-MGly (λ_{max} 307 nm), respectively. These are well above the 20 000 M^{-1} cm^{-1} value generally considered effective for UV protection in sunscreen products, and also higher than the $\varepsilon_{\lambda max}$ of current commercial UV-protective compounds, such as octyldimethyl *p*-aminobenzoic acid (28 400 M^{-1} cm^{-1}) and ethylhexyl *p*-methoxycinnamate (24 200 M^{-1} cm^{-1}).[15]

On the basis of these excellent properties, we can conclude that the CS-MAA conjugates have a potential protection capacity superior to that of current products. They are therefore expected to be efficient against both short-wavelength UV-B radiations responsible for erythema and sunburn, and long-wavelength UV-A radiations, which play a role in multiple disorders, for example, acute and chronic photodermatoses, photoaging, and immunosuppression.[31] Another interesting feature of the materials is their low degrees of substitution, leaving many free amino groups on CS, thereby allowing further modification of the polymer with other active molecules to generate multifunctional materials.

Films consisting of CS (control) and the different CS-MAA conjugates were prepared by a casting/solvent evaporation approach from 1% aqueous acetic acid solutions. This allowed the assessment of their transparency, stability under UV light, thermoresistance, and biological properties.

Transmittance spectra in the visible region (400–800 nm) revealed that all three CS-MAA films were equally as transparent as the films made from unmodified CS, with ca. 85% transmission capacity (Figure 2). The films exhibited absorption properties in the UV (280–380 nm) region (Figure 2) similar to those of the free MAA molecules in solution

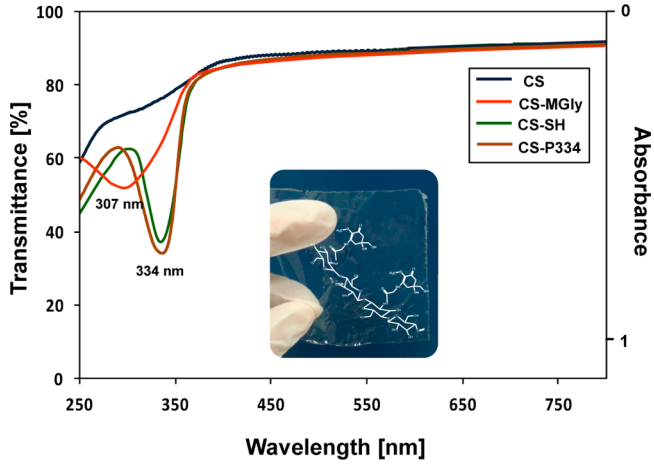

Figure 2. UV–visible spectra of CS-MAA films. Transmittance and absorbance spectra measured between 250 and 800 nm (film thickness ~27 μm). Picture of the CS-MGly film.

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(Supporting Information Figure S1). As opposed to CS-MGly, the absorption capacity of the CS-P334 and CS-SH films was higher in the UV-A than in the UV-B region. This indicates that films with a maximum UV absorbance over the whole UV region could be obtained by making hybrid conjugates that would integrate on the same CS macromolecule a mixture of CS-MGly together with either (or both) CS-P334 or CS-SH.

In the next step, we checked whether the remarkable UV absorption capacity of the films was preserved under stringent physical conditions. In the first instance, the photostability of 27-μm CS and CS-MAA films was evaluated. The films were exposed to intense UV irradiation for up to 12 h, and their absorbance capacity was assessed at both 307 and 334 nm (Figure 3).

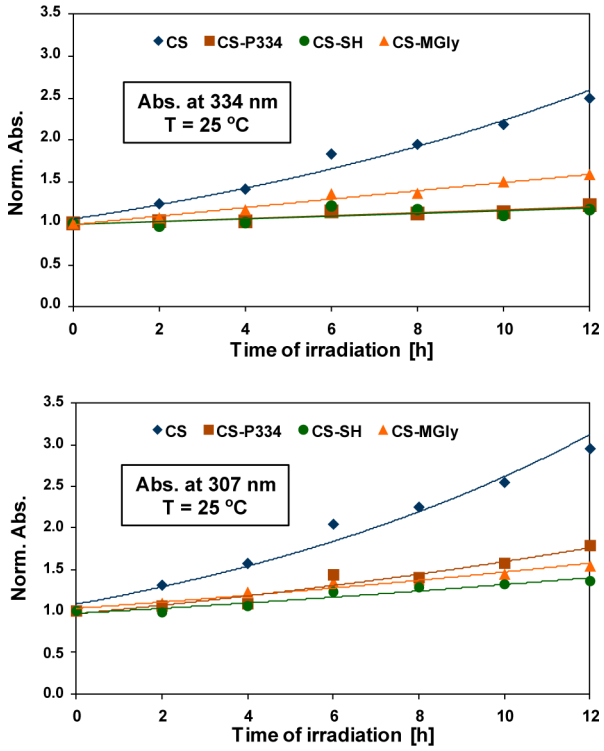

Figure 3. Photostability of CS-MAA films. Changes in the absorption capacity of the CS, CS-P334, CS-SH, and CS-MGly films were monitored at 307 and 334 nm after UV irradiation for up to 12 h.

At 334 nm, a slight, but detectable, increase in absorbance was observed over time for CS-MGly as compared to CS-P334 and CS-SH. The rate of absorbance increase was somewhat higher at 307 nm for CS-P334 and CS-SH, reflecting a slightly lower stability at this wavelength. The increased absorbance observed for all materials under UV irradiation is attributable to the CS polymer. Indeed, an increase of absorbance at around 300 nm is known to be a consequence of an increased formation of carbonyl groups in CS upon UV irradiation.[32] Most importantly, the increased absorbance at each time point was systematically higher for the CS film at both wavelengths than for the CS-MAA films (Figure 3), indicating that all three CS-MAA films are more stable under UV irradiation than the CS control. Thus, another salient advantage of the covalent modification of CS with any of the three MAA molecules is an increased photostability. An additional remarkable property of all films was their resistance to temperatures as high as 80 °C (Figure 4). None of the UV absorption spectra were altered at

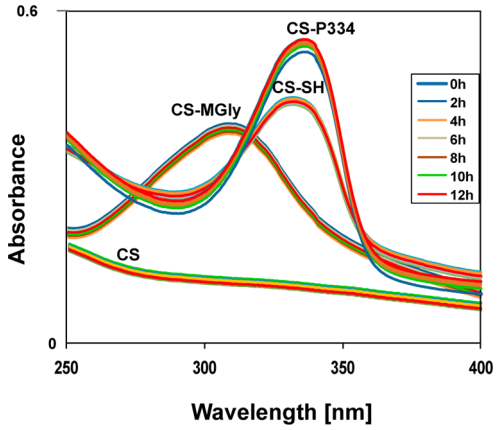

Figure 4. Thermoresistance of CS-MAA films. Absorption spectra of the CS and CS-MAA films recorded after heating the samples at 80 °C for up to 12 h.

this temperature after up to 12 h treatment, indicating that the conjugated MAAs molecules were stable under these conditions. In addition, it can be concluded that thermoresistance is independent from the chemical modification of CS by MAAs molecules as the same behavior was observed for the CS and CS-MAA films. A comparable stability has been reported for the free P334 and SH mycosporines extracted from the algae *Gracilaria cornea*.[33]

Altogether, our data demonstrate the potential to use the CS-MAA materials in environments subject to both UV exposure and moderately high temperatures. Typical examples would be the use of CS-MAAs for the protection of outdoor materials, textiles, or skin directly exposed to sun for prolonged periods of time. In addition, the CS-MAA conjugates may be usable for biomedical applications, in particular for the development of analogues of extracellular matrixes for cell growth and tissue regeneration. Thus, cytotoxicity/viability and cell adhesion tests were performed to assess the biocompatibility of CS-MAA films using L-929 murine fibroblasts as a model system.

First, short-term cytotoxicity assays were performed according to the ISO 10993-12 recommendations. In these assays, cell proliferation is measured in so-called "extracted media", that is, culture media that have been preincubated independently with each type of CS-MAA film at 37 °C for 24 h. Control extracted media were performed in the same conditions by replacing the CS-MAA films with either high-density polyethylene (negative control with no cytotoxic effect) or polyvinyl chloride (positive control with cytotoxic effect) foils. Cell responses to negative and positive controls were as expected, confirming the validity of the test. The viability of the L929 cells cultured in the CS-MAA extracted media for 24 and 48 h was higher than the established acceptance limit of 70% of the value of the negative control (Figure 5a). This indicates that all three CS-MAA materials are not cytotoxic. After 48 h culture, cell proliferation in the CS-MAA extracted media was nearly identical (CS-SH and CS-P334) or 1.2-fold higher (CS-MGly) than in the polyethylene negative control (Figure 5a). These data demonstrate the total absence of cytotoxicity of all CS-MAA extracted media.

In a second type of assay, L-929 cells were cultured directly in contact of the different CS-MAA films (Figure 5b and c). The viability of adhered cells to the materials was assessed at 14 and 21 days of culture, using the Live/Dead green/red fluorescence-based double staining procedure.[29] At day 14,

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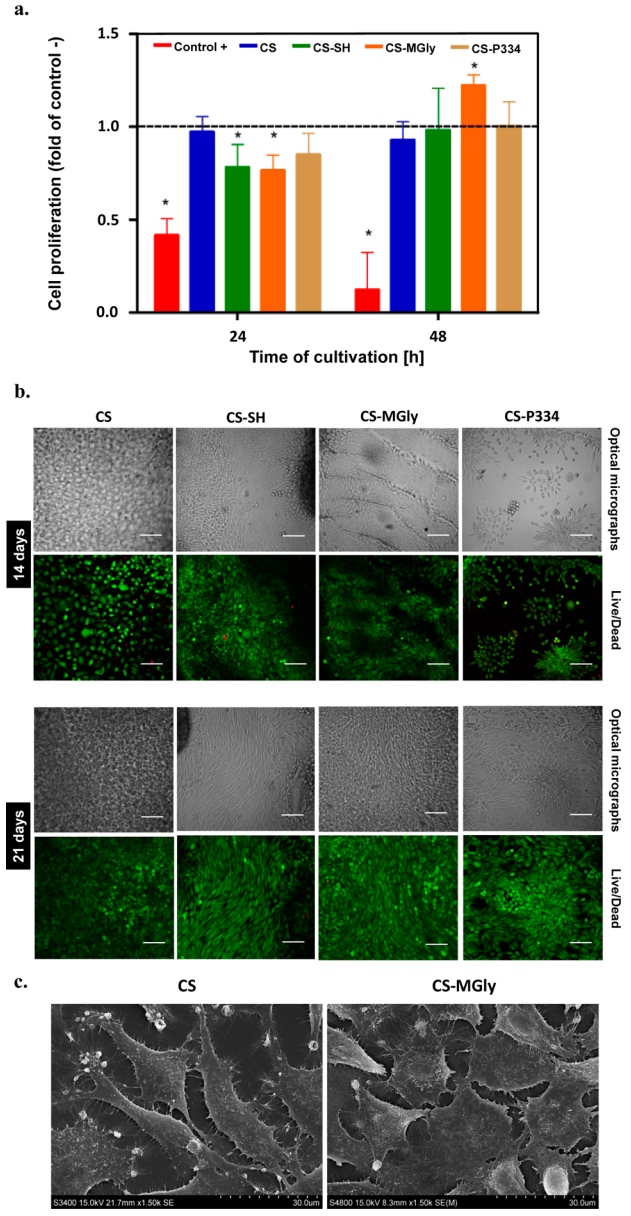

Figure 5. Evaluation of the biocompatibility of CS-MAA films. (a) In vitro cytotoxicity tests performed on L-929 murine fibroblasts using extracted media corresponding to CS and CS-MAA films. The results are presented relative to the polyethylene negative control (broken line) performed in the same conditions (see Methods for details) (*p < 0.05). (b) Confocal microscope images (bright-field and fluorescence) recorded from Live/Dead assays of cells grown in direct contact with the CS (control) and each CS-MAA film for 14 and 21 days (scale bar = 100 μm). (c) SEM micrographs of L-929 murine fibroblast cells cultured on the surface of CS and CS-MGly films after 72 h (1500×).

cells grown on all three materials were metabolically active (green), and only a few dead cells (red) were observed on each film (Figure 5b, fluorescence micrographs). The cells spread and closely attached to the surface of the different materials, although the CS-P334 film was colonized to a lesser extent than the two others (Figure 5b, bright-field micrographs). Cell density became significantly higher at day 21, and the cells remained attached to the films and preserved their elongated morphology. However, the CS-SH and CS-P334 films were still not homogeneously and completely colonized by the cells, whereas full confluence was observed on the CS control and CS-MGly materials (Figure 5b). Cells adhered to these two materials were further observed by high-resolution scanning electron microscopy (SEM). Representative SEM images revealed that cells adhered equally well to the CS control and CS-MGly films. However, in the case of CS-MGly, cells showed a more flattened morphology, which corresponds to an increase in cell surface area attached to the material (Figure 5c).

Altogether the biocompatibility assays show that (i) no toxic compounds are released from the films, (ii) L-929 murine fibroblasts are able to grow in contact with the different CS-MAA materials, and (iii) the CS-MGly material exhibited the highest biological performance as judged by the proliferation rate, extent of cell confluence, and completeness of adhesion during in vitro cultivation. On the basis of these observations, it can be concluded that all three CS-MAA materials have the potential to be exploited in a whole range of biomedical applications involving living cells.

4. CONCLUSIONS

Aquatic organisms such as fish, algae, and cyanobacteria have evolved biological strategies to counteract the negative effect of UV radiations. Inspired by these natural systems, we have developed UV-protective materials that combine individual components normally encountered in separate natural biological systems. As such, the materials are unique. They are based on CS as a matrix on which MAAs were grafted through amide bond formation based on carbodiimide coupling. The CS-MAA conjugates hold an unprecedented combination of properties as compared to existing UV-protective products. Indeed, the materials generated here are concomitantly (i) inspired by nature; (ii) biodegradable because they are made entirely from natural biological molecules; (iii) able to potentially provide protection with a high efficiency against both UV-A and UV-B radiations; (iv) able to maintain high UV absorption after prolonged UV irradiation; (v) thermoresistant; (vi) biocompatible, that is, noncytotoxic and compatible with both cell proliferation, adhesion, and tissue formation; (vii) amenable to further engineering to generate multifunctional materials through the modification of the numerous remaining free amino groups on CS; and (viii) comply with current requirements for use in cosmetic products and potentially also for biomedical and biopharmaceutical applications because the carbodiimide-based grafting procedure and all components are already used individually in some or all of these areas. Thus, it can be concluded that the CS-MAA materials have a great potential to be exploited in a whole range of applications in living organisms (e.g., cosmetics, wound healing, artificial skin, contact lenses, artificial cornea, etc.) and nonliving systems (e.g., outdoor materials and textiles, food and drug packaging, coatings, etc.). A further extension of the proof of concept presented here is the applicability of the same approach to modify other biopolymers, such as cellulose and proteins, thereby increasing the range of UV-protective materials and potential areas of application.

■ ASSOCIATED CONTENT

Supporting Information

Chemical structure, absorption spectra, and molar extinction coefficients of the CS-MAA conjugates and absorption properties of free P334, SH, and MGly molecules. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b04064.

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Notes

The authors declare no competing financial interest.

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