Biomacromolecules

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Triazinyl Porphyrin-Based Photoactive Cotton Fabrics: Preparation, Characterization, and Antibacterial Activity

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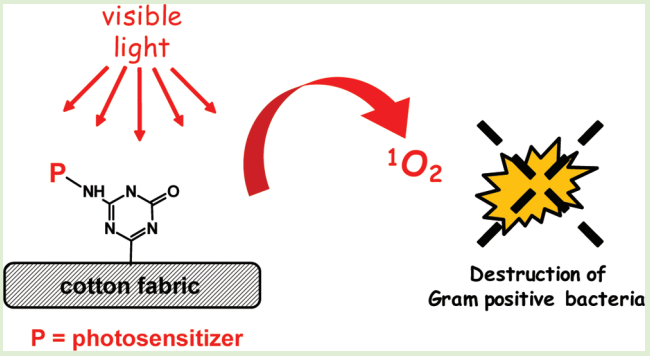
ABSTRACT: In the present work, we report on the synthesis of cellulose cotton fibers bearing different types of photosensitizers with the aim to prepare new efficient polymeric materials for antimicrobial applications. Anionic, neutral, and cationic *amino* porphyrins have been covalently grafted on cotton fabric, without previous chemical modification of the cellulosic support, using a 1,3,5-triazine derivative as the linker. The obtained porphyrin-grafted cotton fabrics were characterized by infrared (ATR-FTIR), diffuse reflectance UV—vis (DRUV) spectroscopies, and thermogravimetric analysis (TGA) to confirm the triazine linkage. Antimicrobial activity of porphyrin—cellulose materials was tested under visible light irradiation against *Staphylococcus aureus* and *Escherichia coli*. The results showed excellent activity on the Gram-positive bacterium, showing structure—activity relationship, although no photodamage of the Gram-negative microorganism was recorded. A mechanism of bacterial inactivation by photosensitive surfaces is proposed.



INTRODUCTION

Nosocomial infections have become a major concern, complicated by the emergence of multi-drug-resistant microbial strains such as the so-called superbugs belonging to *Staphylococcus aureus* and *Escherichia coli* bacterial species. Hospital equipment, including polymeric materials and textiles, are potential vectors for microbial dissemination. As a consequence, interest has grown in the preparation of materials with antibacterial properties, and numerous research works on antimicrobial surfaces have been published.[1−11] In addition to their antimicrobial properties, it would be desirable that these materials also possess a pronounced duration of properties and hence could resist repeated washing or exposure to cleaning products. Modifications by the means of coating, impregnating, or simple blending often lead to short-lived properties. Irreversible modifications resulting from covalent attachment of biocidal moieties are thus highly preferable, assuming that the chemical reactions do not affect the genuine properties of the starting material. Cotton fabrics, which essentially consist of cellulose fibers, can be easily modified by substitution of a relative small number of their numerous −OH nucleophilic groups. Among the solutions proposed, antibacterial cellulosic surfaces or fabrics have been transformed by grafting quaternary ammonium salts,[12−14] antibiotics,[15] *N*-halamines,[16] chitosan,[17−20] or guanidine polymer.[21]

During the last 20 years, photosensitizers (PS) such as porphyrins have been intensively studied for their use as photobactericidal agents in photodynamic antimicrobial chemotherapy (PACT).[22−27] Although the cellular mechanism of the photodynamic process is not yet fully understood, it is presently admitted that phototoxicity primarily relies on the formation of singlet oxygen ($^1O_2$) after illumination.[28,29] This highly reactive species is able to react with almost every cellular component, bringing irreversible damages that ultimately lead to cell death.[30] This strong reactivity, combined with a low specificity, gives rise to a promising approach for the photoinactivation of microorganisms, such as bacteria. It has been shown that porphyrins keep their antimicrobial properties when grafted to chitosan or cellulose and that these modified polymers can be cast into photobactericidal membranes or films.[31−33] The first photoantimicrobial porphyrinic textiles with covalent links have been obtained by grafting protoporphyrin IX and zinc protoporphyrin IX on nylon fiber.[34,35]

In connection with our research program on PACT,[32,33,36,37] we have recently presented the first elaboration of photobactericidal cotton fabrics with porphyrinic moieties using "click chemistry" reaction as a covalent binding protocol.[38] Nevertheless, this strategy needs prior modification of both cellulose substrate and photosensitizer. The present article describes a simpler and faster method which avoids the modification of cellulose. So, cyanuric chloride, a 1,3,5-triazine derivative, previously substituted by

Received: January 18, 2011
Revised: March 17, 2011
Published: March 25, 2011

photosensitizers, shows a very high reactivity with the hydroxyl groups of cellulose.[39] Three types of photosensitizers (neutral, anionic, and cationic) have been used, and chemical attachment of the porphyrinic macrocycle to the cellulosic surface was scrutinized by polymer analysis methods (ATR-FITR, DRUV, and TGA). The study of the structure−activity relationship of the photosensitizers attached to the polymer support is reported, and the photobiocidal activity of the modified cotton fabrics was tested against *E. coli* and *S. aureus*.

EXPERIMENTAL SECTION

Materials. Cyanuric chloride (99%, Acros), diisopropyl ethylamine (99%, Aldrich), and sodium carbonate (99%, SDS) were used as received at the highest purity. Photosensitizers were synthesized following a procedure available in the literature.[39−42] Cotton fabrics (dimension 3.5 × 3.5 cm, without optical bleach) were purchased from Avelana, France. Light source (LED model Luxéon Star white Lambertian LXHL-MW1D 5500K) for the photoinactivation system was obtained from Dioptik, France. Cultures of *Staphylococcus aureus* (S2375) and *Escherichia coli* (S2025) were obtained from Institut Pasteur, Paris. For the antibacterial tests, glassware was autoclaved at 120 °C for 15 min. Unmodified and porphyrinic cotton fabric samples were purified with acetone at 70 °C for 24 h, dried at 100 °C for 15 min, and autoclaved at 120 °C for 15 min before antibacterial assessment.

UV−Visible Spectroscopy (UV−vis). UV−vis spectra were recorded on a Perkin-Elmer Lambda 25 double-beam spectrophotometer using 10 mm quartz cells. Spectra were realized at adequate concentration (10^{-5}−10^{-6} M).

Determination of Singlet Oxygen Quantum Yield, Φ ($^1O_2$). Quantum yield of $^1O_2$ production was determined by direct analysis of the $^1O_2$ near-infrared luminescence at 1270 nm. Excitation occurred with a Xe arc, and light was separated in a SPEX 1680 spectrofluorometer, 0.22 μm double monochromator. Detection at 1270 nm was realized through a PTI S/N 1565 monochromator, and emission was monitored by a liquid-nitrogen-cooled Ge detector model (EO-817L, North Coast Scientific Co.). Absorbance of reference (ethanolic solution of Rose Bengal,[29] Φ $^1O_2$ = 0.68) and sample solutions (at 415 nm) was set equal (between 0.2 and 0.5) by dilution.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR). ATR-FTIR spectra of unmodified and functionalized samples were recorded on Varian 800 FT-IR Scinitar Series spectrometer using a single reflection, horizontal ATR accessory. For the measurement of ATR-FTIR spectra, each sample piece was placed under the ATR source and was then slowly pressed on the sample. Each spectrum was collected in the range of 4000−400 cm^{-1}, and the baseline correction was applied for all spectra using Varian Scinitar Series software.

Diffuse Reflectance UV−Vis Spectroscopy (DRUV). DRUV spectra of porphyrin-modified samples were obtained with a CARY 5000 Varian spectrometer using a 110 mm PTFE integrating sphere. Reflectance spectra were recorded against Teflon standard reflectance spectrum. Each spectrum was recorded in the range of 350−750 nm.

Thermogravimetric Analysis (TGA). Thermogravimetric analyses of unmodified and modified samples were carried out using a SETARAM series Setsys 2400 thermogravimetric analyzer under an air atmosphere. Samples (25−30 mg) were heated from room temperature to 500 °C at a rate of 5 °C min^{-1}. Calcined alumina was used as internal standard.

Scanning Electron Microscopy (SEM). Surface morphology of unmodified and functionalized cellulosic samples was observed by SEM using a XL30 Philips scanning microscope. Dried cotton samples were coated with a 17 nm gold−palladium layer using SCD 050, BAL-TEC coating unit accessory. Electron micrographs of the sample were recorded at 2000× magnification. All samples were conditioned on disk (diameter 1 cm) and purified with acetone at 70 °C for 24 h and dried at 100 °C for 15 min prior to SEM preparations.

Grafting Ratio. The molar grafting ratio (%) of each porphyrin-grafted cotton fabric was calculated from the difference between the initial porphyrin amount in the grafting reaction and the unreacted porphyrin present at the end of the grafting reaction, assuming that this difference represented the amount of porphyrin actually bound to each cotton sample, according the following formula

$$\text{grafting ratio}\ (\%) = \left[1 - \frac{A_{\text{Soret}}/\varepsilon_{\text{Soret}} \times V \times d}{n_{\text{initial}}}\right] \times 100 \quad (1)$$

where A_{Soret} is the Soret band absorbance of the porphyrin−triazine compound corresponding to the free photosensitizer in a solution consisting, for each cotton sample, of the final reacting solution mixed with the various washings operated after the grafting reaction; $\varepsilon_{\text{Soret}}$ is the Soret band molar absorption coefficient of the free photosensitizer; V is the volume of prepared solution for obtaining an absorbance value between 0 and 1; d is the dilution factor done for UV−vis measurement; and n_{initial} is the initial amount of photosensitizer (mol) present before initiating the grafting reaction.

Preparation of Neutral Photosensitive Cotton Fabric. Photosensitizer **1** (Figure 2), TPP-NH_2 (11 mg, 1 equiv), was solubilized in THF (15 mL), then cyanuric chloride (4 mg, 1.2 equiv) and DIPEA (4 μL, 1.2 equiv) were added.[39] After 15 min at 0 °C, the mixture was stirred at 25 °C for 15 min. Then cotton fabric (3.5 × 3.5 cm, 0.27 g), previously soaked in 100 mL of 0.5 M NaOH during 24 h, was introduced and grafting was carried out at 70 °C for 24 h under reflux and stirring. Neutral cotton was collected and thoroughly washed with THF (3 × 100 mL), $CHCl_3$ (3 × 100 mL), and finally with hot DMF (100 mL) at 120 °C for 24 h. Neutral cotton was obtained after drying at 100 °C for 1 h. The molar grafting ration was calculated using eq 1. Grafting ratio, 57%. FTIR (cm^{-1}): 1726, 1660 (−CONH− stretching, amido triazine), 880, 840 (C═C deformation, aromatic macrocycle). DRUV (nm): 422, 518, 555, 594, 649.

Preparation of Anionic Photosensitive Cotton Fabric. For photosensitizer **2** (Figure 2), TPPS-NH_2 (10 mg, 1 equiv), water was used as reaction solvent (15 mL). Cyanuric chloride (2.5 mg, 1.2 equiv), dissolved in a minimum of THF, and 1.2 equiv of saturated aqueous $NaHCO_3$ (1 mL) were added, and reaction mixture was stirred at 0 °C for 30 min then at 25 °C for 30 min. Cotton fabric (3.5 × 3.5 cm, 0.27 g), previously soaked in 100 mL of 0.5 M NaOH during 24 h, was introduced and grafting reaction was heated at 80 °C for 24 h. Then, anionic cotton was washed with water (3 × 100 mL) and finally with hot DMF (100 mL) at 120 °C for 24 h. Sample was obtained after drying at 100 °C for 1 h. The molar grafting ration was calculated using eq 1. Grafting ratio, 73%. FTIR (cm^{-1}): 1730, 1655 (−CONH− stretching, amido triazine), 880, 840 (C═C deformation, aromatic macrocycle). DRUV (nm): 425, 521, 559, 594, 650.

Preparation of Cationic Photosensitive Cotton Fabric. For elaboration of cationic porphyrinic cotton, the procedure was similar to the preparation of neutral cotton. Ten milligrams (1 equiv) of photosensitizer **3**, *trans*-$MePy^+$-NH_2, cyanuric chloride (3 mg, 1.2 equiv), and DIPEA (3 μL, 1.2 equiv) were used. Grafting ratio, 54%. FTIR (cm^{-1}): 1636, 1562 (−CONH− stretching, amido triazine), 1352 (−C═N^+- stretching), 805 (C═C deformation, aromatic macrocycle). DRUV (nm): 425, 520, 557, 595, 655.

Antibacterial Activity of Photosensitive Cotton. *Growth Conditions of Bacterial Cells.* Gram-positive bacteria, *S. aureus*, and Gram-negative bacteria, *E. coli*, were inoculated into liquid tryptic soy (pancreatic casein extract 17 g/L, soy flour papaic digest 3 g/L, dextrose 2.5 g/L, NaCl 5 g/L, and K_2HPO_4 2.5 g/L) and incubated at 37 °C overnight under aerobic conditions. The stock solution was further diluted to give a working suspension of approximately 10^6−10^7 CFU/mL.

Light Source and Exposure. We developed a derivative technique of a normalized protocol ("ATCC Test Method 100-1999, *Antibacterial Finishes on Textile Materials: Assessment of*"). Figure 1 shows a photograph of the photodynamic inactivation device inside an incubator. A LED (light-emitting diode) system for irradiation over the entire visible spectrum (400−800 nm) was obtained with Luxéon Star white Lambertian LXHL-MW1D 5500K LED model (45 lm, 3.42 V, 350 mA). The distance between the light source and the exposed sample surface was adjusted to 10 cm (light intensity = 690 lx). Samples of photosensitive textiles were exposed to this white light irradiation (0.16 mW/cm^2) under aerobic conditions at 37 °C in a humidified incubator for 24 h. Light fluence dose was equivalent to 9.5 J/cm^2.

Photodynamic Treatment with Photoactive Cotton Surfaces. Sterile photosensitive textiles (3.5 × 3.5 cm) were impregnated with 1 mL of bacterial inoculum at a cell density of approximately 10^6 CFU/mL, deposited on a sterile Petri dish, and then incubated at 37 °C for 24 h under white light irradiation in wet atmosphere. Then, each sample was removed and transferred into 20 mL of extraction solution: Triton X-100, 0.5% (v/v) for *S. aureus* and 0.05% (v/v) for *E. coli*. After 15 min of gentle stirring at room temperature, serial dilutions of these suspensions were prepared. Aliquots (100 μL) of diluted samples were then spread on tryptic soy agar plates. After incubation at 37 °C for 24−48 h, plates were examined and the number of colony forming units (CFU) was counted manually. The results, after adjustment by dilution factors, were expressed as mean CFU per square textile.

Each test was done in triplicate and was conducted along with necessary controls: a photosensitive sample was processed immediately after bacterial impregnation (t = 0), another one was incubated at 37 C for 24 h in the dark; unmodified cotton has also been processed in the same conditions (24 h at 37 °C in the dark and under white light irradiation). Finally, we checked Triton X-100 for its possible deleterious effect on tested organisms; this extracting agent did not affect bacterial viability when used at working concentrations (0.5% v/v for *S. aureus* and 0.05% v/v for *E. coli*).

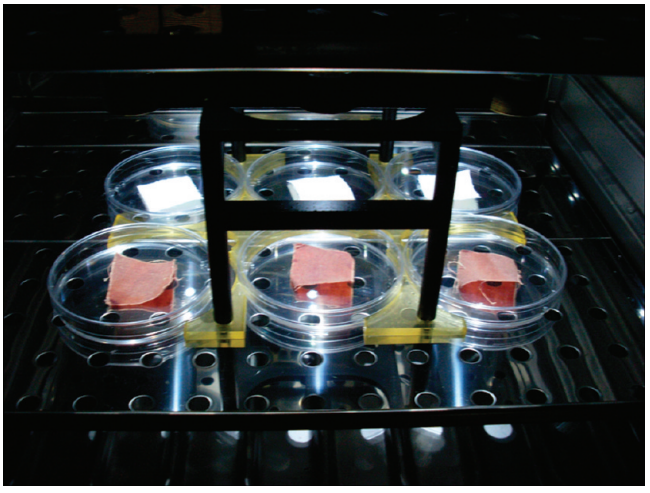


Figure 1. Photodynamic inactivation device. Triplicate samples of photosensitive surfaces (reddish) and native cotton (white) were placed in sterile Petri dishes and subjected to light irradiation in a humidified oven at 37 °C.

RESULTS AND DISCUSSION

Synthesis of Porphyrin-Bound Cotton Fibers via Triazine Linkage. According to our previous results, cyanuric chloride was used for the covalent grafting of porphyrins to cellulose fabrics. Functionalized *para*-aminophenylporphyrin derivatives **1**−**3** were used as nucleophilic agents to react with cyanuric chloride; these photosensitizers were synthesized according to classical methods as previously described.[39−42] Structures of different photosensitizers used, neutral aminoporphyrin **1** (TPP-NH_2), anionic sulfoned aminoporphyrin **2** (TPPS-NH_2), and cationic *trans*-pyridinium aminoporphyrin **3** (*trans*-$MePy^+$-NH_2), are illustrated in Figure 2.

In a first step, porphyrins were reacted with cyanuric chloride at 0 °C, which allows substitution of a first triazine chlorine atom with the amino function conducing to porphyrin−triazine derivatives **1-a**, **2-a**, and **3-a** (Scheme 1). From these intermediates, a highly reactive and not isolated porphyrin−triazine link has been characterized after the complete substitution of chlorine atoms with the use of piperidine (for neutral and cationic product) and sodium sulfanilate (for anionic compound).[39] Then, alkali-treated cotton fabrics (3.5 × 3.5 cm surface) were introduced in the porphyrin−triazine reaction mixture, as described above (see Experimental Section).

Grafting reactions were followed by washing cycles in order to remove unbound photosensitizer. Neutral and cationic cotton was intensively washed with chloroform and THF and anionic cotton with water; these washes were followed by hot DMF (24 h, 120 °C). These cycles were repeated until washing solutions did not show any trace of porphyrins (UV titration at 420 nm). After drying at 100 °C, the resulting cotton samples gained a reddish-brown tint (Figure 3).

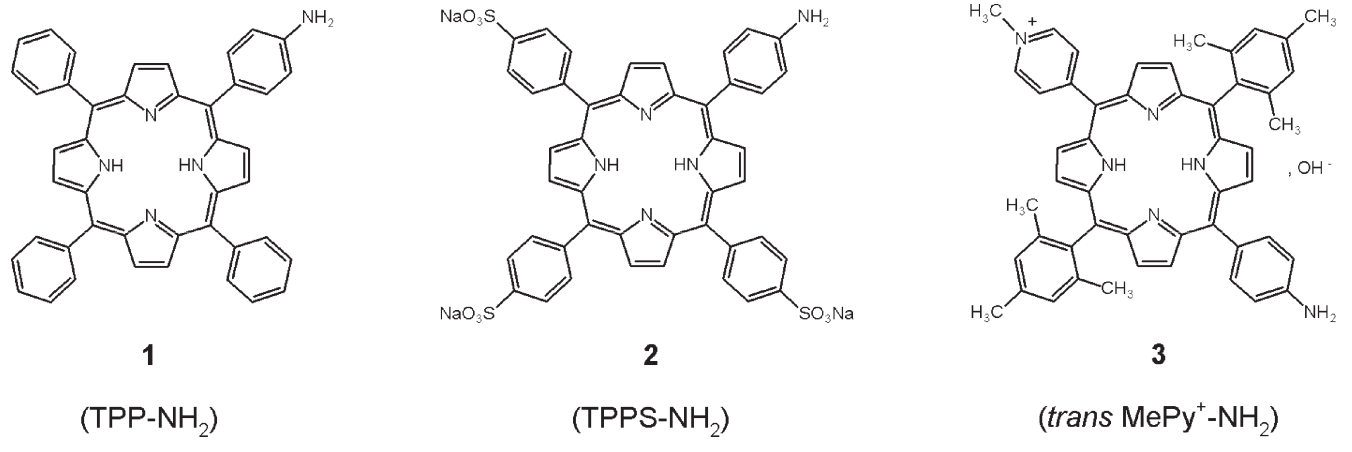
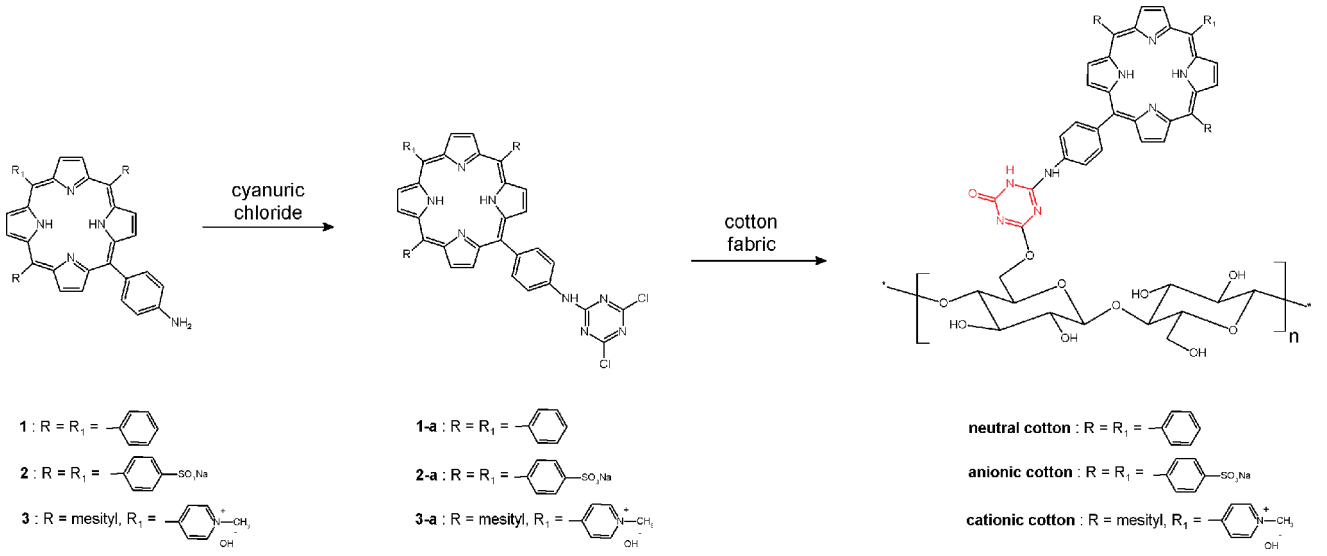


Figure 2. Chemical structure of anionic, neutral, and cationic photosensitizers used in this work.

Scheme 1. Synthetic Route to Photoantimicrobial Cotton



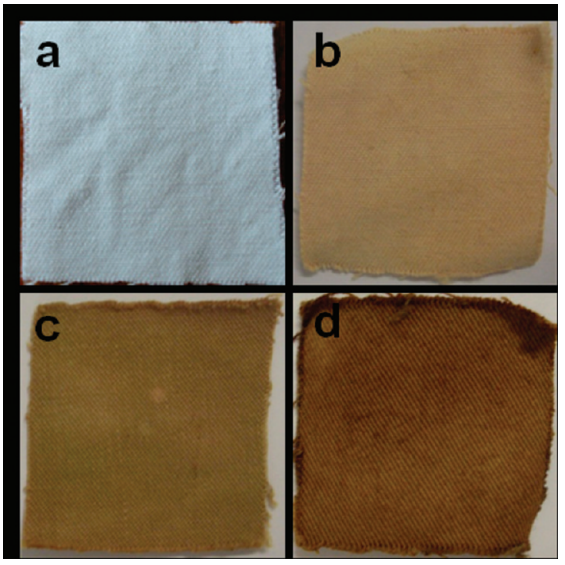


Figure 3. Photographs of (a) native cotton; (b) neutral cotton; (c) anionic cotton; and (d) cationic cotton obtained after treatment.

Molar grafting yield for each amino porphyrin was realized by UV−vis titration (eq 1). Results are presented in Table 1. Significant grafting yields were obtained (54−73%), owing to the proper nature of the cotton fiber and to the heterogeneous reaction conditions. To our knowledge, these molar grafting values between the cellulose surface and a photosensitizer like porphyrin were reported for the first time. There is no correlation between the amount of grafted photosensitizer and the value of grafting yield because the molar absorption coefficient, corresponding to the Soret band, is different for each porphyrin. Amount of grafted porphyrin on the order of 0.03 μmol/mg of cotton shows homogeneity of the grafting system with amino porphyrins of different nature.

The covalent bond between the photosensitizer and cotton surface was confirmed by attenuated total reflectance Fourier transform infrared (ATR-FTIR), diffuse reflectance UV−vis (DRUV) spectroscopies, and thermogravimetric analysis (TGA).

Table 1. Grafting Yield Determination of Photosensitizers by UV−Vis Titration

grafted porphyrin	grafting yield (%)	amount of grafted photosensitizer (μmol/mg of cotton sample)
anionic	73	0.03
neutral	57	0.037
cationic	54	0.028

ATR-FTIR Spectroscopy. Grafted cottons have been analyzed by ATR-FTIR spectroscopy with the aim to characterize the covalent link between cotton fabric and porphyrinic macrocycles. ATR-FTIR spectra of unmodified and modified cotton fibers are presented in Figure 4. Concerning native cotton spectra, classical spectral data have been found: 3340 cm^{-1} (OH stretching), 1325, and 1045 cm^{-1} (C−O stretching). Samples of modified cotton display characteristic signals within the 1750−1550 cm^{-1} zone (amide stretching); 1726 and 1660 cm^{-1} (neutral cotton), 1730 and 1655 cm^{-1} (anionic cotton), 1636 and 1562 cm^{-1} (cationic cotton). Moreover, the presence of peaks in the 900−800 cm^{-1} zone has been assigned to a deformation vibration band of bound aromatic macrocycle (−C═C−), and the intense 1352 cm^{-1} signal observed with cationic cotton corresponds to a stretching vibration of the iminium form (−C═N^+ pyridinium).

The appearance of new bands in the 1750−1550 cm^{-1} zone attests to the presence of a bond corresponding to the amide function. The presence of an amide group comes from the substitution of the last chloride atom on the 1,3,5-triazine ring by the hydroxyl group after treatment of native cotton with sodium hydroxide solution. Introduction of hydroxyl groups onto the triazine ring provides evidence for a tautomeric shift toward the corresponding amide form (Figure 5).[43] The presence of the cyclic amide form was confirmed by the absence of a C−Cl band between 600 and 800 cm^{-1}.

Low intensity of characteristic signals in the ATR-FTIR spectra has complicated the interpretation of the results; however, ATR-FTIR analysis allowed us to better understand the chemical

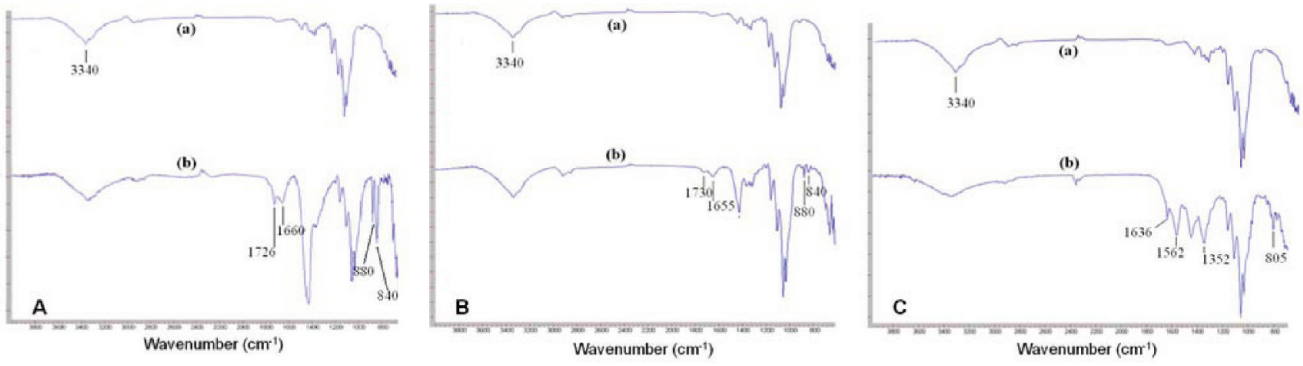


Figure 4. ATR-FTIR spectra (4000−600 cm^{-1}) of (a) unmodified cotton and (b) grafted cotton. A, neutral; B, anionic; and C, cationic cotton.

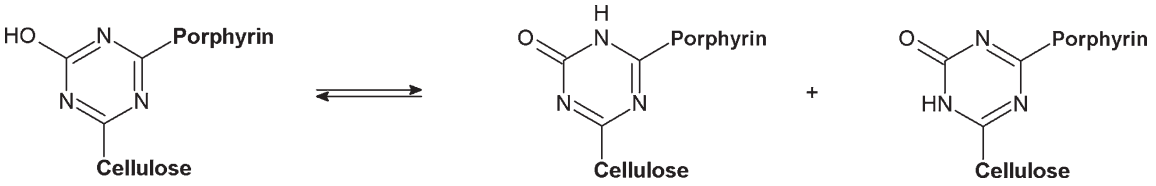


Figure 5. Triazine ring tautomeric shift in the porphyrin−cellulose complex.

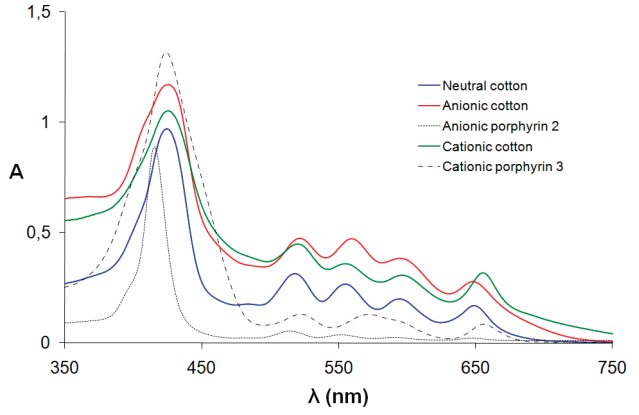


Figure 6. DRUV spectra (350−750 nm) of modified cotton. Red, blue, and green curves refer to anionic, neutral, and cationic grafted cotton, respectively. Black dotted line (···) refers to anionic porphyrin **2** recorded at the concentration of 2×10^{-6} M in MeOH and the dash-dotted line (−·−··) refers to cationic porphyrin **3** at the concentration of 2×10^{-6} M in $CHCl_3$.

structure adopted by the triazine ring and to show the existence of a covalent link between the photosensitizer and cellulosic cotton surface.

DRUV Spectroscopy. In order to observe the presence of porphyrins on the surface of cotton fabric, modified cotton was also analyzed by DRUV spectroscopy and compared to reference molecules as anionic and cationic compound (Figure 6). DRUV analysis led to spectra similar to UV−vis porphyrin derivatives in solution: Soret band near 420 nm and Q bands between 500 and 700 nm clearly show up (see Table 2 for electronic data).

Results showed the Soret band maxima at 422 nm for neutral and 425 nm for anionic and cationic cottons, which were identical to those of free TPP-NH_2 **1**, TPPS-NH_2 **2**, and *trans*-$MePy^+$-NH_2 **3**, respectively. Moreover, the appearance of a Q band implies

Table 2. Electronic Diffuse Reflectance UV−Vis Spectral Data

products	Soret band maxima (nm)	Q bands (nm)
1[a]	421	517, 554, 591, 648
neutral cotton	422	518, 555, 594, 649
2[b]	416	514, 552, 590, 648
anionic cotton	425	521, 559, 594, 650
3[a]	424	523, 572, 595, 656
cationic cotton	425	520, 557, 595, 655

[a] UV−vis spectra in $CHCl_3$. [b] UV−vis spectra in MeOH.

that the surface of support did not bring any distortion of the conjugated plane of grafted porphyrins. However, the following changes were observed in diffuse reflectance UV−vis spectra upon chemical grafting: (i) the ratio of molar absorption coefficients of the Soret to Q bands was reduced; and (ii) Soret bands broadened. The latter was attributed to π-electron interaction with surface hydroxyl groups. Moreover, maximum of absorption shows no change in time, providing evidence of the stability of the grafted cotton surface and that photosensitizers are not leached from the materials in the solid states.

DRUV analyses, where porphyrins are covalently attached onto surface, have not been reported in the literature. Similar observations have been reported by Rahiman et al. for porphyrins encapsulated into mesoporous silica structures.[44]

Thermogravimetric Analysis. Thermogravimetric analysis (TGA) was used to investigate the thermal properties of grafted cotton. TGA thermograms of neutral, anionic, and cationic photosensitizers grafted on cotton fabric are presented in Figure 7 in comparison with unmodified cotton. Initial weight loss was attributed to loss of water of each sample (dehydration phenomenon) which follows the order neutral, cationic > anionic and unmodified cotton. At higher temperatures, cotton displayed better thermal stability than grafted cotton as significant weight

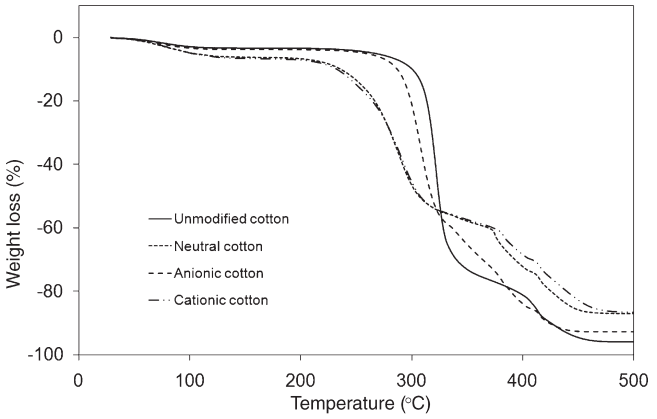


Figure 7. TGA thermograms of cotton fabric before and after grafting.

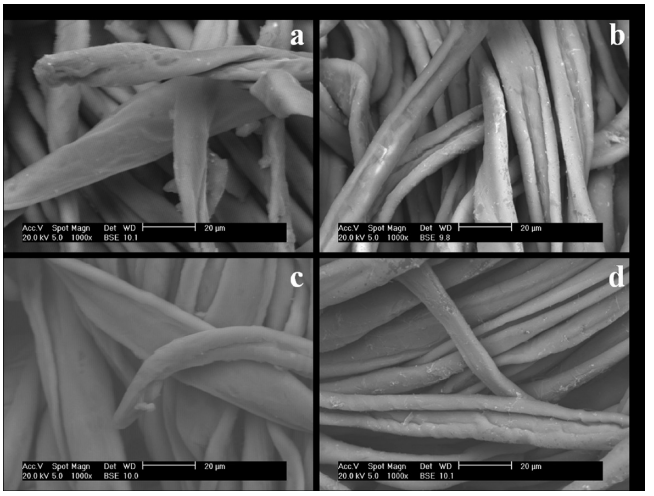


Figure 8. SEM photomicrographs (20 μm scale) of cotton fibers. Unmodified cotton (a) and modified cotton; neutral (b), anionic (c), and cationic (d) porphyrin-grafted cotton.

loss was observed at temperatures of 295, 280, 245, and 240 °C for cotton, grafted cotton with photosensitizers **2**, **1**, and **3**, respectively. At much higher temperatures, grafted cotton samples showed multistep weight loss due to decomposition of photosensitizers, removal of linker groups, or degradation of polymeric material or backbone itself. The lower thermal stability of grafted cotton in comparison to parent cotton was due to faster decomposition of modified cotton fibers in relation to the strong compact pure cotton. Similar results on loss of thermal stability of grafted cotton (quaternary ammonium polymers or quaternary ammonium triazine derivatives, for example) have also been reported recently.[45,46] Moreover, TGA analyses show that the change of thermal properties correlated with the covalently modified cotton surface.

Scanning Electron Microscopy. The surface of unmodified cotton and porphyrin-grafted cotton was examined by scanning electron microscopy (SEM). Typical scanning electron photomicrographs are presented in Figure 8. Photomicrographs did not show any destructuration of cotton fibers (similar diameter and structure). These observations indicated that porphyrin grafting did not affect fiber morphology; no destruction was observed. SEM results show that cotton fiber structures are resistant to chemical treatment.

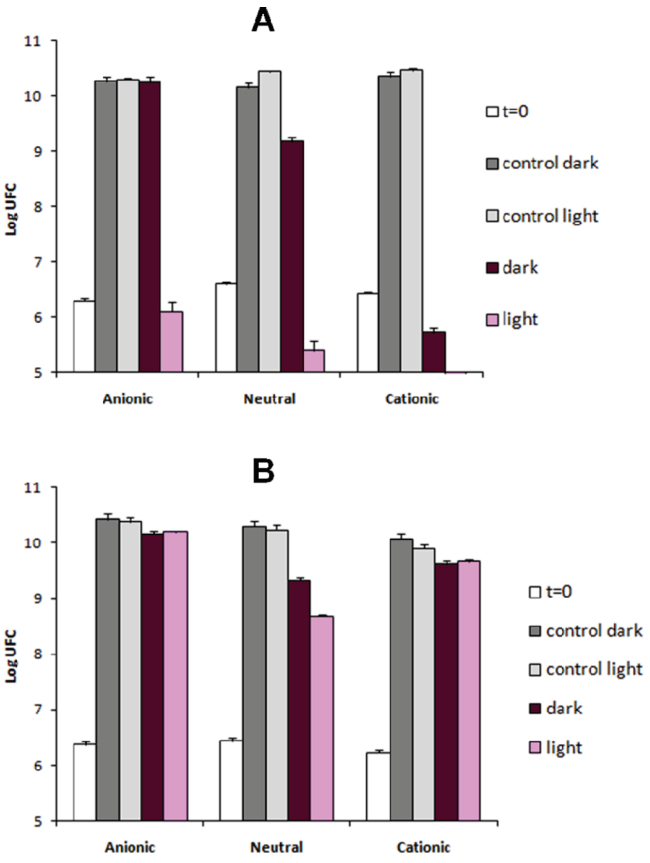


Figure 9. Bacterial counts ($\log_{10}$ CFU) of (A) *S. aureus* and (B) *E. coli*.

Antibacterial Activity of Photosensitive Cotton. Photodynamic activity of modified cotton was evaluated in vitro against *S. aureus* and *E.coli*, used as model Gram-positive and Gram-negative bacteria.

Experimental data, expressed as the number of CFU per cotton square sample, are reported in Figure 9. Untreated control samples (in the dark and under light irradiation) and control treated samples (in the dark) allow bacterial growth of 4 log compared with reference (t = 0, number of bacteria initially deposited on textile square). These controls allowed us to assess that neither the chemical modifications of cotton nor the light dose (9.5 J/cm^2) have any influence on bacterial growth or viability.

After 24 h exposure to light irradiation at a fluence dose of 9.5 J/cm^2 and at a temperature of 37 °C, all modified surfaces cause a photobactericidal effect in Gram-positive bacteria. Nevertheless, observed activities are different according to the kind of modified cotton; in fact, percentages of bacterial growth inhibition are 37% for anionic cotton, 93.7% for neutral cotton, and 100% for cationic cotton. Electric charge of photosensitizers directly influences photoinactivation efficacy, and these results confirm the presence of a structure−activity relationship on photoinactivation of Gram-positive bacteria cells. These data are in accordance with former results showing that bacterial cell photoinactivation by free base porphyrins was also strongly dependent on the global electric charge of the photosensitizers whose photobactericidal powers had the same ranking: cationic > neutral > anionic.[47,48] For cationic cotton fabric, toxicity in the dark (80%) is probably due to the presence of the quaternary ammonium charge, known to disorganize bacterial cell walls without light

Table 3. Photoinactivation of Triazinyl Cotton (%)

	photoinactivation (%)	
porphyrin-grafted cotton	*S. aureus*	*E. coli*
neutral	93.7 ± 3.2	0
anionic	37 ± 1.1	0
cationic	100	0

Table 4. Singlet Oxygen Quantum Yield

amino precursor	Φ ($^1O_2$)
TPPS-NH_2 **1**	0.59
TPP-NH_2 **2**	0.65
trans-$MePy^+$-NH_2 **3**	0.82

irradiation.[49] Concerning Gram-negative bacteria, the three materials did not show any photoactivity. These results are summarized in Table 3.

Owing to the insoluble and immobilized character of these photosensitizers, mechanistic interpretations of our experiments must take into account the generation of a reactive species, such as singlet oxygen, on the material surface,[31−35,38] followed by its diffusion and eventual interaction with the target cell. Midden and co-workers have already shown that such photoinhibition is due to type II photochemical process implying singlet oxygen ($^1O_2$).[50] In fact, we found a good correlation between antibacterial photoactivity and singlet oxygen production (Table 4). The same authors have demonstrated in a photomicrobiocidal experiment that diffusion of singlet oxygen across an air gap is 0.65 mm, but that, in water, owing to its shorter lifetime, diffusion of singlet oxygen is limited to about 100−200 nm (2×10^{-6} s in water versus 7.6×10^{-2} s in air). This distance being smaller than the average size of bacteria, the cell envelope is first encountered by singlet oxygen. It is thus likely that differences in cell wall structures could justify different susceptibilities to singlet oxygen whose vital target seems to be the cytoplasmic membrane.[51] These conclusions could explain the large difference of photoactivity of our cotton−porphyrin materials against *S. aureus* and *E. coli*, more precisely in relation with the differences in cell wall structures (Table 3). The cell wall of *E. coli* is composed of a lipopolysaccharide coat (LPS) and an outer membrane that protects this bacteria from extracellular singlet oxygen. On the contrary, singlet oxygen reaches the cytoplasmic membrane of Gram-positive bacteria by diffusion through the relatively open peptidoglycan layer structure of the cell wall. The three cotton−porphyrin materials showed photoactivity against *S.aureus* and ranked in the following order: cationic > neutral > anionic. Antimicrobial activity of cationic porphyrin-grafted cotton, when used in darkness, that we observed against *S. aureus* is reminiscent of other observations conducted with insoluble cationic polymers, which acted upon bacteria by simple adsorption.[13,52−54] The reason why cationic cellulose only acted upon *S. aureus*, although unexplained, is presently under scrutiny.

CONCLUSIONS

In the present study, photosensitive cotton fabrics were successfully prepared by grafting anionic, neutral, and cationic porphyrins onto cellulose cotton fibers according with the use of cyanuric chloride as linking agent. This strategy has allowed us to avoid chemical modification of the cellulosic material which appears very interesting for further industrial applications. These functionalized cellulose samples displayed an antibacterial activity against *S. aureus*, which was shown to depend on the nature of photosensitizers. These surfaces could be efficiently used in biomedical fields to prevent microbial infections.

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ACKNOWLEDGMENT

We thank the "Conseil Régional du Limousin" for financial support. The authors are indebted to Dr. Michel Guilloton for help in writing the manuscript, and Pierre Carles (SPCTS-Limoges) for MEB analysis.

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