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The study of cellulosic fabrics impregnated with porphyrin compounds for use as photo-bactericidal polymers

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ABSTRACT

In the present work, we report on the preparation of cellulosic fabrics bearing two types of photo-sensitizers in order to prepare efficient polymeric materials for antimicrobial applications. The obtained porphyrin-grafted cellulosic fabrics were characterized by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, diffuse reflectance UV–Vis (DRUV) spectroscopy, thermo-gravimetric analysis (TG) and scanning electron microscopy (SEM). Antimicrobial activity of the prepared porphyrin-cellulose was tested under visible light irradiation against *Staphylococcus aureus*, *Pseudomunas aeroginosa* and *Escherichia coli*. In addition, the effect of two parameters on photo-bactericidal activity of treated fibers was studied: illumination time and concentration of photosensitizers (PS).

1. Introduction

Cellulose is the most abundant renewable natural polymer in the world and has countless applications, one of which is in the fabrics industry. It is a high molecular weight polymer consisting of D-glucopyranose units linked through β (1–4) linkages with the empirical formula $[C_6H_{10}O_5]_n$ [1–6]. Cellulose has immensely useful properties such as hydrophilicity, biocompatibility, and biodegradability [7].

Natural fabrics such as cellulose, under moist conditions, can provide an excellent environment for growing microorganisms such as bacteria and fungi. This leads to reduced frazzle property and durability of the fabrics, as well as a repellent odor. Antimicrobial activity imparted on natural fibers by their chemical modification is therefore a highly effective method for improvement on clothing for hospital staff and patients, hospital beddings, sports clothing, armbands, underwear, ladies tights, shoe linings, sleeping bags, toys for children, etc. [2,8,9].

The antibacterial property of the fibers can be imparted either at the manufacturing step by trapping antibacterial chemicals, or by coating the finished product with antibacterial compounds [8].

In Table 1, a number of synthesized cellulosic surfaces and their antimicrobial activities are shown [2,8,10–12].

During the last 20 years, photosensitizers (PS), such as porphyrin compounds, have been intensively studied for their use as photo-bactericidal agents against both Gram negative and Gram positive bacteria in photodynamic antimicrobial chemotherapy (PACT) [9,13–18].

PACT relies on the intracellular accumulation of a photosensitizing agent upon illumination with visible light. The photodynamic process involves energy absorption by the photosensitizer which, brought to its excited triplet state, either activates ground state molecular oxygen into singlet oxygen or generates free radicals. Phototoxicity primarily relies on the formation of singlet oxygen ($^1O_2$) after illumination; and this molecule is able to react with almost every cellular ingredient, bringing about irreversible damage that ultimately leads to cell death [13]. This method has recently been studied against a wide range of clinically important bacteria, yeasts, fungi and viruses [19–23].

A number of water-soluble cationic porphyrins and phthalocyanine complexes of biocompatible metals (zinc, gallium and silicon) have been employed as efficiency agents against oral pathogens in PACT [24,25]. Porphyrin compounds such as meso-tetrakis-(4-aminophenyl)-porphyrin, meso-tetrakis-(*N*-methylpyridyl) porphyrin and other photoactive dyes were used as biocompatible porphyrins [25].

In many studies, photo-bactericidal cellulosic surfaces have been synthesized from cellulose and natural or synthetic porphyrins [9,13,26–30]; some of these polymers are listed in Table 2.

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Table 1
Antimicrobial activities of selected samples on cellulose surface.

Sensitive bacterial strain	Compound	Polymer
S. aureus	Cetylpyridinum chloride [2]	Cellulosic fabric
S. aureus	Benzyldimethylhexadecyl ammonium chloride [2]	Cellulosic fabric
S. aureus *B. cereus*	[2-(Acryloyloxyethyl)]trimethylammonium chloride[8]	Cellulosic fabric
S. epidermidis	Chlorhexidine[10]	Cellulose acetate fiber
E. coli	2-(Dimethylamino)ethyl methacrylate [11]	Cellulosic filter paper
E. coli, *S. aureus*	2-amino-4-chloro-6-hydroxy-*s*-triazine [12]	Cellulosic fabric

Accordingly, cellulose is a perfect support for immobilization of bioactive molecules. In addition, it is a biologically compatible molecule with no cytotoxic properties; and therefore highly amenable for application in medical and biological fields [31,32]. In this study, we applied various concentrations of tetrakis(4-*N*,*N*,*N*-trimethylanilinium)porphyrin and its zinc metal ion complex to the cellulosic surface for the first time and characterized porphyrin-grafted cellulosic fabrics using various analysis methods (ATR-FTIR, DRUV, SEM and TG). In addition, photo-bactericidal activity of these treated cellulosic fabrics was tested against *E. coli*, *P. aeruginosa* and *S. aureus* under illumination with visible light at different times; these bacteria are major pathogenic organisms associated with health care [33]. *E. coli* is mostly found in urinary tract infections [33,34]; *P. aeruginosa* infection in patients with endotracheal and urinary catheters is common [35]; and *S. aureus* infection occurs in patients with prosthetic devices, venous catheters, and peritoneal dialysis catheters, etc. [36].

2. Materials and methods

2.1. Materials

All chemicals were purchased from Merck Company and used without further purification. The porphyrin, tetrakis(4-*N*,*N*,*N*-trimethylanilinium)porphyrin (TAPP) and its zinc ion complex (ZnTAPP) were synthesized as reported previously [37–40].

All fabrics were of plain (woven) construction, weighing 162.5 g/m^2, unfinished 100% cellulosic fabric, laundered and dried. They were then cut along the fiber direction in (2 × 5 cm) strips and pre-washed in hot deionized water. The laboratory temperature was 25 ± 2 °C with relative humidity (RH) of 60 ± 2%.

2.2. Bacterial strain and preparation of cultures

The bacterial strains *S. aureus*, *E. coli* and *P. aeruginosa* were obtained from microbiology laboratory of University of Guilan.

Gram-positive bacterium, *S. aureus* and Gram-negative bacteria, *E. coli* and *P. aeruginosa* were inoculated in liquid culture medium [nutrient broth (source: Merck Company)] and incubated at 37 °C overnight under aerobic conditions in an incubator. The stock suspensions of liquid culture medium were diluted to give a working suspension of approximately ≈10^8 colony forming units/mL (CFU/mL).

2.3. Irradiation system

All the experiments were carried out in a water-jacked reactor irradiated with a 100 W tungsten lamp (1250 lm), as a visible light source with an average intensity of ~0.36 mW.cm^{-2} at a distance of 20 cm from the sample. To avoid light reflection, the reactor was placed in a dark room.

2.4. Preparation of cationic photosensitive cellulosic fabric

For preparation of photoactive cellulosic fabrics, the fabrics were soaked in 10 g/L Na_2CO_3 at 50 °C for 30 min. Afterwards they were squeeze rolled and placed horizontally at room temperature for several minutes. The porphyrin solutions were prepared in distilled water with phosphate buffer at concentrations of 1, 10 and 100 μM and the cellulosic fabrics were impregnated with these solutions. The treatment was prolonged for an additional 30 min at 50 °C. The samples were then washed with hot water several times to remove unbound photosensitizer. These cycles were repeated until washing solutions did not show any trace of porphyrins. After that, the fabrics were dried in an oven at 50 °C. The molar grafting ratio (%) was calculated for each porphyrin-grafted cellulosic fabric by the method of Ringot et al. [9].

2.4.1. Antibacterial activity of photosensitive cellulosic fabric

30 μL of broth culture at a cell density of approximately 10^8 CFU/mL was transferred onto solid culture medium [nutrient agar (source: Merck Company)] plates and spread on the surface with a sterile distributor. Sterile photosensitive textiles (1 × 1 cm) were put on the inoculated Petri dish. The plates were first incubated at 37 °C for 20 min in the dark, followed by illumination for 30, 60 and 90 min, and finally incubated at 37 °C overnight in a humidified incubator.

In one negative control, one plate was treated with the same conditions, except that untreated cellulosic fabric was used. A second control was also employed using un-activated photosensitive cellulosic fabric. After overnight incubation, each photosensitive textile was removed and transferred into 1 mL of sterile water. After 15 min of gentle stirring

Table 2
Photobactericidal cellulosic surfaces with various porphyrin compounds.

Support	Porphyrins	Light source	Sensitive bacterial strains
Cellulosic fabric	TPP-NH_2, TPPS-NH_2, trans-MePy^+-NH_2 [9]	LED	*S. aureus*, *E. coli*
Cellulosic plastic film	5-[4-(3-Carboxypropyloxy)phenyl]-10,15,20-tri(4-methylphenyl) porphyrin); 5-[4-(10-carboxydecanoxy)phenyl]-10,15,20-tri(4-methylphenyl) porphyrin [13]	Four 150 W tungsten bulbs	*S. aureus*, *E. coli*
Cellulosic plastic films	Protoporphyrin IX; 5-[4-(3-propargyloxy) phenyl]-10,15,20- tritolylporphyrin; 5-(4-hydroxy phenyl)-10,15,20-tritolylporphyrin; Protoporphyrinato IX (Zn II) dipropargyl ester; Protoporphyrin IX dipropargyl ester [26]	Ten 23 W bulbs	*E. coli*, *S. aureus* and *P. aeruginosa*
Cellulosic plastic films	Monopyridyltritolylporphyrin [27]	150 W tungsten bulb	*S. aureus*, *E. coli*
Cellulosic fabric	Monohydroxyphenyltritolylporphyrin [28]	White light	*S. aureus*, *E. coli*
Cellulosic plastic film	Protoporphyrin IX [29]	150 W tungsten bulb	*S. aureus*, *E. coli*
Cellulosic paper	5-(4-Nitrophenyl)-10,15,20-(4-pyridyl) porphyrin; 5-(4-aminophenyl)-10,15,20-tri(4-*N*-methylpyridinium)Porphyrin [30]	LED	*S. aureus*, *E. coli*