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Synthesis and photobactericidal properties of a neutral porphyrin grafted onto lignocellulosic fibers

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ABSTRACT

Photodynamic antimicrobial chemotherapy (PACT), as one of the promising alternative antimicrobial treatment, has received great attention in recent years. In this work, a new antimicrobial material has been elaborated by grafting a neutral porphyrin, the metallated 5-(4-azidophenyl)-10,15,20-triphenylporphyrin, onto lignocellulosic fibers by using the Copper (I)-Catalyzed Alkyne-Azide 1,3-dipolar Cycloaddition (CuAAC) reaction. The cross-linked porphyrin-Kraft pulp material was characterized by infrared and by XPS spectroscopy analyses, which proved the covalent linkage between the porphyrin and propargylated Kraft pulp fibers. The antimicrobial activity of this material was tested under visible light irradiation with a low light dose (9.5 J/cm^2) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The two bacterial strains deposited on the resulting photosensitizing Kraft pulp are efficiently killed after illumination. Such materials could find applications in industrial, household and medical environments as an alternative to overcome the widespread microbial multiresistance to classical treatments.

1. Introduction

Bacterial surface contamination constitutes a major public health problem encountered in many domains such as hospitals, environment and food industry [1]. This contamination consists in the adhesion of pathogenic or opportunistic bacteria leading to the formation of a biofilm [2–4]. This biofilm is a potential source of bacterial growth and development [5]. Biofilm formation is often considered to be the underlying reason for the failure of antimicrobial treatments. Because an estimated 65–80% of all infections are thought to be biofilm-related, this represents a serious challenge [6,7]. Many reports have been focusing on the fabrication of antibacterial surfaces, or the improvement of the performance of existing antibacterial surfaces, in order to eliminate or substantially reduce bacterial attachment and biofilm formation [8,9]. Indeed, a variety of surface modifications are possible, for example by coating or grafting chitosan [10], quaternary ammonium salts (QAs) [11], N-halamines [12], polybiguanides [13], or compounds releasing bactericidal moieties such as metal ions [14]. The focus of the present work is the development of antibacterial materials (Fig. 1) by grafting porphyrins onto Kraft pulp fibers through covalent bonds [15].

Porphyrins are used as photosensitizing agents in photodynamic antimicrobial chemotherapy (PACT) (Fig. 2). This therapy utilizes the ability of these drugs, in combination with visible light, to generate cytotoxic reactive oxygen species (ROS) that are lethal to the target pathogen [16–18].

The link between the photoactive molecule and the cellulosic support was realized using the Copper(I)-Catalyzed Alkyne-Azide Cycloaddition (CuAAC), which consists in a 1,3-dipolar cycloaddition between an azide and a terminal or internal alkyne to give 1,2,3-triazole [19]. The modified Kraft pulp sheets were tested for their antibacterial properties against two pathogenic bacterial strains frequently found on surfaces and able to form a biofilm: *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

2. Experimental

2.1. Materials

All solvents and reagents were commercially available and, unless otherwise stated, were used as received. Benzaldehyde (99%), pyrrole (98%), copper(II) sulfate pentahydrate (98%), *p*-anisaldehyde (98%) and sodium ascorbate (98%) were purchased from Aldrich, propargyle bromide (97%) and sodium azide (99%) were purchased from Alfa Aesar. Reactions were monitored by thin-layer chromatography (TLC) on 0.2 mm silica gel precoated 60 F254 (Merck) plates and revealed with an ultraviolet light source at 254 nm. The bleached

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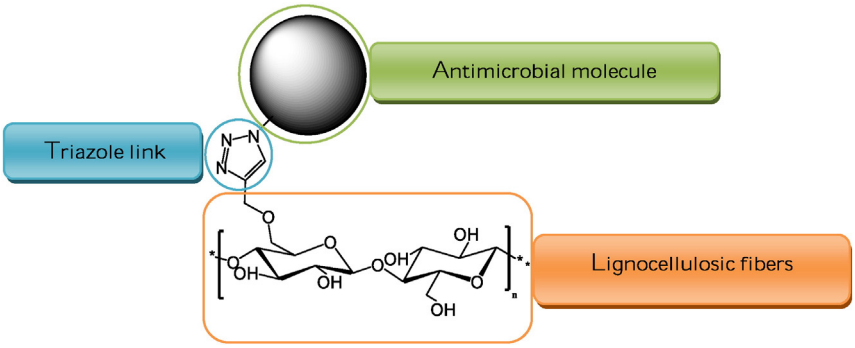

Fig. 1. General procedure for the grafting of antimicrobial molecules onto Kraft pulp fibers.

hardwood Kraft pulp was received in wet-laps from a Notrheastern Canada mill.

2.2. Instrumentation

2.2.1. 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 acquired in an adequate concentration range (10^{-5}–10^{-6} M).

2.2.2. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

A Perkin–Elmer 1000 FTIR spectrometer equipped with the Spectrum software was used to perform FTIR analysis. The spectra were obtained by preparing dried KBr powder pellets containing 5% *w*/*w* of the investigated sample.

2.2.3. X-ray photoelectron spectroscopy (XPS)

XPS experiments were carried out using a Kratos Axis Ultra spectrometer that provided elemental composition information (atomic percentage) within a depth of a few nanometers from the sample surface. A 225 W monochromatic aluminum source (Al Kα) was used. Survey scans were taken with 1.0 eV step and 160 eV analyzer pass energy, while the high-resolution regional spectra were recorded with 0.1 eV step and 40 eV pass energy. The pressure was typically 10^{-9} Torr. An area of 2 mm^2 at three different spots was analyzed in order to average over the sample, to avoid any bias due to any eventual heterogeneity. The position of the detector was at an angle of 90° to the sample surface. Deconvolution analysis was performed with a SUN Sparc Station IPX computer. The spectrum analysis was done with Casa XPS 2.3.9.

2.2.4. Microwave irradiations

Microwave irradiations were performed with an Ethos 1600 MicroSynth reactor from Milestone. The temperature was measured with an optic-fiber thermometer (ATC-FO)/Ethos.

2.2.5. ^1H NMR (^{13}C NMR) spectra

^1H and ^{13}C NMR spectra were recorded at 400.13 MHz (100.62 MHz) with a Bruker DPX-400 spectrometer using $CDCl_3$ as solvent at room temperature. The chemical shifts (δ) are expressed in ppm with Me_4Si as the internal standard (δ_0).

2.3. Synthesis

2.3.1. Synthesis of 5-(4-nitrophenyl)-10,15,20-triphenylporphyrin: TPP–NO_2

378 mg of 4-nitrobenzaldehyde (2.5 mmol; 0.25 equiv.) were dissolved in 100 mL of dichloromethane in the presence of benzaldehyde (760 μL, 0.75 equiv.) under magnetic stirring. Molecular iodine (1.1 equiv.) and pyrrole (700 μL, 10 mmol) were sequentially added. After a first activation by microwave irradiation (3 × 2 min, 100 W, 30 °C), TLC showed the total conversion of benzaldehyde. Then, *para*-Chloranil (0.75 equiv., 1.84 mg) was added and a second activation was performed (1 min, 100 W, 30 °C). The reaction mixture was evaporated on 60 g of florisil and purified by flash chromatography (eluent gradient: EP/$CHCl_3$, 8/2–1/9). 362 mg of porphyrin 1, purple solid, was obtained (22%). R_f: 0.65 ($CHCl_3$/EP; 7/3; *v*/*v*); UV–vis ($CHCl_3$)(λ_{max}, nm): 420, 517, 554, 592, 647; ATR-FTIR: $\nu(NO_2)$ 1516.14 and 1345.57 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$) δ_{ppm}: 8.89 (d, J = 4.7 Hz, $H_{\beta\text{-pyrrolic}}$); 8.86 (s, 4 H, $H_{\beta\text{-pyrrolic}}$), 8.73 (d, J = 4.7 Hz, 2 H, $H_{\beta\text{-pyrrolic}}$); 8.62 (d, J = 8.5 Hz, 2 H, $H_{3,5\text{-aryl}}$); 8.40 (d, J = 8.5 Hz, 2 H, $H_{2,6\text{-aryl}}$); 8.21 (d, J = 7.4 Hz, 6 H, $H_{2,6\text{-phenyl}}$); 7.76 (m, 9 H, $H_{3,4,5\text{-phenyl}}$); −2.78 (s, 2 H, NH_{int}); ^{13}C NMR (400.13, $CDCl_3$) δ_{ppm}: 116.61, 120.67, 121.07, 121.85, 126.76, 127.89, 134.5, 135.13,

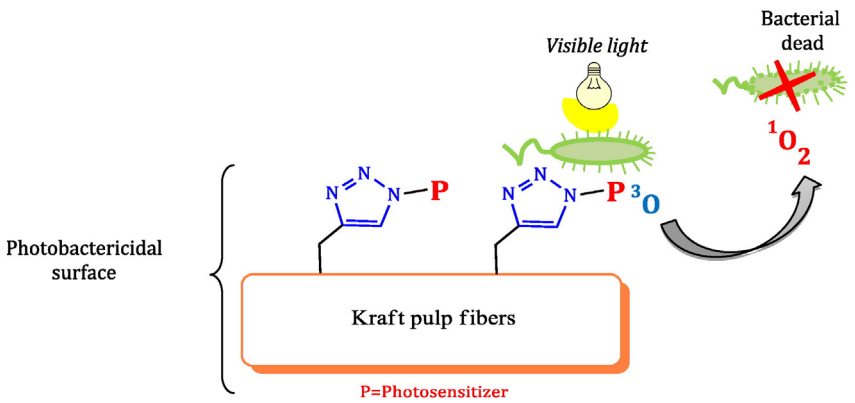

Fig. 2. Photodynamic antimicrobial chemotherapy (PACT) process.