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Chlorin-PEI-labeled cellulose nanocrystals: Synthesis, characterization and potential application in PDT

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ABSTRACT

This Letter reports the synthesis and characterization of a new series of water-stable and soluble photosensitizers (PS-CNCs) composed of cellulose nanocrystals (CNCs) bearing polyaminated chlorin *p6*. With a view to improve cancer cell targeting, these photosensitizers were assayed for their antitumor activity against HaCat cell line. IC_{50} values fell within the nanomolar-range, making these photosensitizers promising for further in vitro and in vivo investigations.

Photodynamic therapy (PDT) is an established modality for treatment of neoplastic diseases. PDT involves selective accumulation of a photosensitizer into cancer cells, followed by in situ photoactivation of the photosensitizer by visible light and subsequent death of the treated cells.[1] Two types of photoreaction mechanisms have been proposed to explain photosensitizer action: light-activated photosensitizers in their triplet state can either generate free radicals by hydrogen abstraction from H-donor substrates (type I photochemical reactions) or transfer energy to molecular oxygen and produce singlet oxygen ($^1O_2$) (type II reactions). Singlet oxygen seems to be the major mediator of photochemical damages to cell components but its mechanism of action is not well understood.[2] The therapeutic outcome greatly depends on the behavior of the photosensitizers, including their efficiency in generating singlet oxygen and other photophysical properties. Introduction of 660–800 nm lasers that emit light in the near-infrared spectral tissue window has stimulated the development of compounds absorbing in this region of the electromagnetic spectrum. Nevertheless, lack of selectivity is the main concern with this technique and considerable efforts are being devoted to improve targeting of proliferative tissues. For example, photosensitizers can be covalently attached to sugars, peptides, folic acid, hormones or polyamines, leading to receptor-mediated drug targeting.[3–9] Indeed, vectored or targeted drugs, which possess enhanced affinity for cancer cells, would constitute an important advance in cancer therapy. Moreover, recently, with the goal to combine the photodynamic action of photosensitizers and chemotherapeutic properties of ruthenium complexes, pyridyl-porphyrin have coordinated arene ruthenium moieties and shown good photocytotoxicities toward human melanoma cancer cells.[10]

Nanoparticle carriers represent a breakthrough for drug targeting and delivery and stand at the forefront of nanomedicine research.[11,12] Owing to slacked junctions between endothelial cells, solid tumors suffer from leaky vasculature and poor lymphatic drainage. These characteristics allow the passive accumulation of nanoparticles in the 50–200 nm range, named 'enhanced permeability and retention' (EPR) effect.[13] To reach the targeted tumoral tissue, nanoparticles must be able to stay intact in the bloodstream for sufficient time lapses.[14] According to the literature, for a prolonged blood circulation time, carriers have to be small, constituted of natural compounds and must present a neutral and hydrophilic surface.[15] To this end, we have focused our attention on cellulose nanocrystals (CNCs). Indeed these nanomaterials possess several favorable characteristics like renewability, low cost and biodegradability.[16] Furthermore, the presence of surface hydroxyl groups allows chemical modifications for applications in various fields.[17] So, cellulose nanocrystals based on these size and hydrophilicity requirements were chosen as carriers.[18] Currently, only few reports deal with cellulose nanocrystal derivatization with a fluorescent probe, for use in cell bioimaging or with unnatural porphyrins derivatives for antibacterial applications.[19,20]

In connection with our research program on anticancer photodynamic therapy,[21] we have devised a synthetic route to obtain

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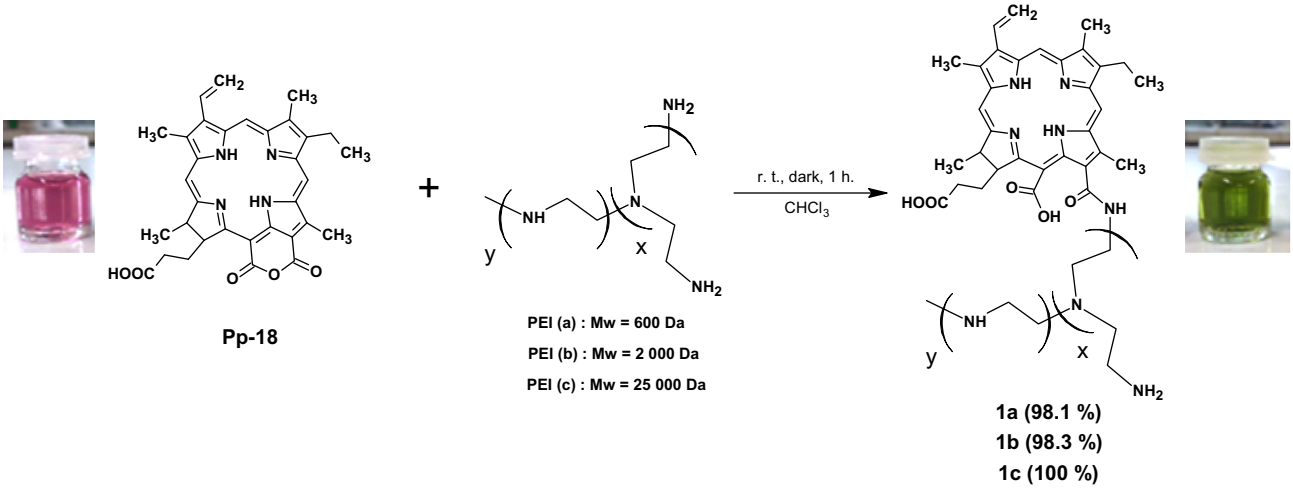

Scheme 1. Synthesis of photosensitizers bearing PEI 600 Da **1a**, 2000 Da **1b**, 25,000 Da **1c**.

porphyrin derivatives designed for selective uptake by tumor cells, and it occurred to us that CNCs bearing natural photosensitizers could appear as promising candidates for an application in PDT. So, in the present work, we have chosen to use purpurin-18, a natural chlorin derivative which presents several advantages, including good ionic stability at 4 °C, neutral pH, during several weeks, strong absorption at visible and near infrared wavelengths and easy access to large amounts through easy synthesis from the cyanobacterium *Spirulina maxima*.[22] Polyethyleneimine (PEI) is usually employed as multifunctional drug and dye nanocarrier in order to optimize cellular internalization for in vitro and in vivo studies.[23] Three PEI of different molecular weights (600 Da, 2000 Da, 25,000 Da) were tested in order to build the more efficient photosensitizers for PDT application.

In this Letter, we report the design, synthesis and characterization of a new vehicle for delivery of photodynamic agents constituted by PEI-chlorin *p6* derivatives covalently attached to cellulose nanocrystals. Phototoxicity and cellular uptake of this new natural platform were evaluated against a human keratinocyte cell line (HaCaT); preliminary studies are also reported in the aim to characterize the cell death pathway induced by this treatment.

The synthetic route followed for the preparation of photosensitizers bearing PEI units is depicted in Scheme 1. Purpurin-18 (Pp-18) was synthesized from chlorophyll *a* extracted from *S. maxima*, as previously described.[23] Then, it was allowed to react with different PEI (600 Da, 2000 Da and 25,000 Da) in chloroform during 1 h, resulting in a quick color change from purple to green, characteristic of chlorin *p6* absorbance at 665 nm. After solvent evaporation, polyaminated chlorin *p6* **1a–c** were quickly obtained in a quantitative way as water-soluble compounds. Indeed, the presence of a fused anhydride ring confers a great reactivity especially towards amine functions.[24] Light absorption spectra (Fig. 1) and mass spectrometry of these compounds showed the expected signals.[25] To evaluate the photosensitizing properties of PEI-chlorin *p6* derivatives **1a–c**, quantum yield of $^1O_2$ production was determined by direct analysis of the $^1O_2$ near-infrared luminescence at 1270 nm (see Supplementary data). The value of Φ_Δ 0.75 (EtOH) showed that the three PEI-chlorin *p6* derivatives **1a–c** share a good potential for PDT application. Moreover, in order to establish whether PEI-chlorin *p6* derivatives **1a–c** could undergo photobleaching, photostability studies were performed by using an EdmundOptics Inc. H-series 660 nm red laser developing an output power of 100 mW. Figure 2 shows the absorption spectra obtained for **1a** in presence of 0.3% HSA (Human Serum Albumin), for different time intervals after laser illumination. It can be seen that the chlorin suffers from photobleaching which can be attributed to reaction of **1a** with singlet oxygen or another reactive oxygen species. Similar results were obtained with **1b–c**. However, the total amount of energy delivered to the photosensitizer is 720 J. Knowing that the medical dose generally does not exceed 75 J/cm^2, this molecule can be considered stable enough to be used in PDT. On the other hand, photobleaching, if correctly controlled, can be useful for photosensitizer elimination from the body, reducing post-treatment photosensitization.

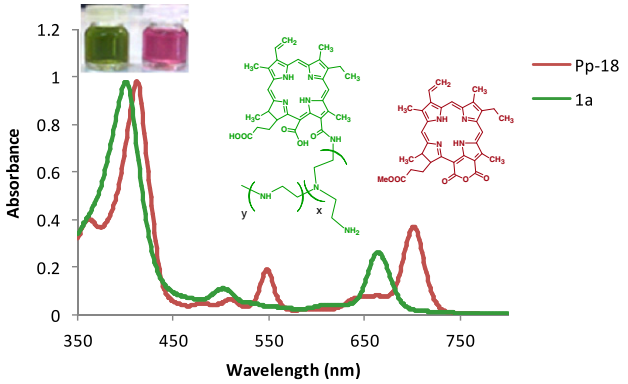

Figure 1. UV–vis spectra of Pp-18 and compound **1a**.

In parallel, CNCs were obtained by hydrolysis of cellulose with sulfuric acid solution (64% w/w) followed by several washings, centrifugations and by thorough dialysis against distilled water until neutrality (Scheme 2).[26] This acid hydrolysis consists in breaking cellulose chains within the amorphous domain, thus leaving free nanocrystals. Cotton was chosen as the source of cellulose for its high crystallinity (73%).[27] The obtained bluish opalescent suspension is very stable (several months) due to the repulsive interactions between negative charges of grafted sulfate functions onto nanocrystals which represents 0.8% of dry matter.[28] A transmission electronic microscopy (TEM) image of cellulose nanocrystals is shown in Figure 3a. Ellipsoidal nanoparticles (100–200 × 10–20 nm) were observed after uranyl acetate staining. X-ray diffraction pattern of CNCs, typical of native crystalline cellulosic materials is also shown in Figure 3b. More precisely, and according to the monoclinic