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# Development and biodistribution of a theranostic aluminum phthalocyanine nanophotosensitizer

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ABSTRACT

*Background:* Aluminum phthalocyanine (AlPc) is an efficient second generation photosensitizer (PS) with high fluorescence ability. Its use in photodynamic therapy (PDT) is hampered by hydrophobicity and poor biodistribution.
*Methods:* AlPc was converted to a biocompatible nanostructure by incorporation into amphiphilic polyethylene glycol-polycaprolactone (PECL) copolymer nanoparticles, allowing efficient entrapment of the PS in the hydrophobic core, water dispersibility and biodistribution enhancement by PEG-induced surface characteristics. A series of synthesized PECL copolymers were used to prepare nanophotosensitizers with an average diameter of 66.5–99.1 nm and encapsulation efficiency (EE%) of 66.4–78.0%. One formulation with favorable colloidal properties and relatively slow release over 7 days was selected for *in vitro* photophysical assessment and *in vivo* biodistribution studies in mice.
*Results:* The photophysical properties of AlPc were improved by encapsulating AlPc into PECL-NPs, which showed intense fluorescence emission at 687 nm and no AlPc aggregation has been induced after entrapment into the nanoparticles. Biodistribution of AlPc loaded NPs (AlPc-NPs) and free AlPc drug in mice was monitored by *in vivo* whole body fluorescence imaging and *ex vivo* organ imaging, with *in vivo* imaging system (IVIS). Compared to a AlPc solution in aqueous TWEEN 80 (2 w/v%), the developed nanophotosensitizer showed targeted drug delivery to lungs, liver and spleen as monitored by the intrinsic fluorescence of AlPc at different time points (1 h, 24 h and 48 h) post iv. administration.
*Conclusions:* The AlPc-based copolymer nanoparticles developed offer potential as a single agent-multifunctional theranostic nanophotosensitizer for PDT coupled with imaging-guided drug delivery and biodistribution, and possibly also fluorescence diagnostics.

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## 1. Introduction

Photodynamic therapy (PDT) is an emerging noninvasive medical modality for the treatment of certain types of cancer, microbial infections, ophthalmic and periodontal conditions. It is based on the cytotoxic effect of reactive oxygen species (ROS) generated by *in situ* activation of a photosensitizer (PS) using light of appropriate wavelength [1]. Selective PS accumulation in target cells and co-localization of light irradiation, the primary determinants of efficacy and safety of PDT, has been the focus of extensive research in the last few decades.

Three generations of PSs were developed to date to improve performance in clinical use. In this context, limitations of the first generation porphyrinoids PSs were overcome by second generation PSs [2]. Among these, phthalocyanine derivatives have photophysical and spectral characteristics putting them forward as the most efficient PSs for PDT.

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Phthalocyanine PSs are tetrapyrolic aromatic macrocycles capable of forming intensely colored metal complexes for diverse industrial, biological and medical applications [3]. Metal phthalocyanines proved highly efficient in anti-cancer PDT [4] as they accumulate effectively in cancer cells and strongly absorb tissue-penetrating red light with the generation of high quantum yield of ROS. Phthalocyanines were also demonstrated to inactivate microbial pathogens, offering potential in antimicrobial PDT [5]. In addition, metal phthalocyanines exhibit fluorescence, allowing for fluorescence diagnostics and noninvasive *in vivo* spectral analysis of tissues [2,6,7]. This enhances real-time data processing and correction of parameters for treatment optimization.

Despite favorable photophysical and spectral characteristics, phthalocyanine derivatives present limitations due to their hydrophobic nature and limited specific retention at the target site. Phthalocyanine PSs tend to aggregate under physiological conditions with self-quenching and loss of photodynamic activity [8,9]. Further, molecular aggregation hampers intravenous administration and tissue distribution and may lead to rapid clearance by the mononuclear phagocyte system [10,11].

Approaches to overcome the hydrophobicity of phthalocyanine PSs were based mainly on chemical modification [12,13] and pharmaceutical nanotechnology [14,15]. According to the latter approach, presentation of PSs in a conjugated, encapsulated or associated form with water dispersible nanocarriers significantly enhanced photodynamic activity, introducing the third generation PSs or nanophotosensitizers [14,16]. Nanocarriers reduce the tendency of hydrophobic PSs to aggregate in aqueous media, increase circulation time, allow passive and active targeting of tumors and intracellular delivery of the PS payload [16]. Due to the inherent size of nanocarriers, they can be selectively accumulated in the tumor tissue through the “enhanced permeation and retention” (EPR) effect which is characterized by the presence of leaky vasculatures due to having an incomplete endothelial barrier, defective vascular architecture and impaired lymphatic drainage system of the tumor [17,18].

In this framework, AlPc, a highly efficient second generation PS, was formulated as nanophotosensitizer based on lipid, polymer and inorganic biomaterials. These included mainly nanoemulsions [19,20], liposomes [21], solid lipid nanoparticles [22], polymer micelles [23], polymer nanoparticles [24] and gold, titanium dioxide and graphene nanoplartforms [25–27]. These nanocarriers were shown to greatly enhance AlPc photodynamic activity. However, suppression of phthalocyanine fluorescence in a nanoparticulate form [7,28] may necessitate PS liberation at a target imaging site [2,28] or chemical modification of the nanocarrier to prevent fluorescence quenching [27] for fluorescence imaging and diagnostics.

In the design of polymer nanoparticulate systems for the delivery of hydrophobic PSs in PDT, an appropriate polymer matrix should generate water dispersible nanocarriers with relatively high loading capacity, favorable circulation time and biodistribution to disease sites to allow for co-localization of light irradiation. Amphiphilic block copolymers such as polyethylene glycol-polycaprolactone (PECL) copolymer confer highly tunable physicochemical properties to the nanostructures obtained from them [31], making them suitable for PSs delivery [17,32,33]. A hydrophobic core allows for effective encapsulation of water insoluble PSs while a commonly PEG-rich hydrophilic surface protects the nanostructures from rapid clearance by the mononuclear phagocyte system (MPS). This results in prolongation of circulation time [34] and passive targeting of tumors by the EPR effect [17]. Sustained release of the PSs at target disease sites and intracellularly greatly enhances PDT [35].

The objective of the present study was to develop a new nanophotosensitizer formulation based on AlPc both as a photodynamic agent and as a probe for fluorescence bio-imaging purposes. A series of polyethylene glycol/polycaprolactone (PECL) amphiphilic block copolymers were used as a nanocarrier matrix to confer the combined advantages of high AlPc entrapment efficiency, PEG surface characteristics and controlled release of AlPc for enhanced photodynamic activity and post administration fluorescence imaging. However, finding molecules producing fluorescence to have bioimaging properties without lowering the phototoxicity is rare; our developed nanophotosensitizer actually maintained enough fluorescence needed for bioimaging *in vivo* even after 48 h post injection without quenching which provides an additional advantage to our nanophotosensitizer system.

## 2. Materials and methods

### 2.1. Materials

$\varepsilon$-Caprolactone monomer ($\varepsilon$-CL, 99%) and tin (II) 2-ethylhexanoate as a catalyst were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Poly(ethylene glycol) monomethyl ether, molecular weight 5000 Da was purchased from Fluka Chemie (Buchs, Switzerland). Aluminum-phthalocyanine chloride (AlPc, Mw 574.96 Da), TWEEN 80 and all solvents were provided by Sigma-Aldrich Chemical Co. (Munich, Germany).

### 2.2. Experimental animals

Balb/C mice (22 ± 2 g) were purchased from Charles River (Charles River Laboratories, Sulzfeld, Germany) and kept for one week in the animal facility to acclimatize before the experiments. The animals had free access to food and water, *ad libitum*, and were kept in a 12 h light/dark cycle under controlled humidity (55% ± 5%) and temperature (21 °C ± 2 °C). The animal study was approved by the Stockholm Southern Ethical Committee on Animal Research and performed in accordance with Swedish Animal Welfare law.

## 3. Methods

### 3.1. Synthesis of polyethylene glycol-polycaprolactone diblock copolymers (PECL)

Five amphiphilic di-block PECL copolymers (PECL 1–5) with different molecular weight and relative chain length of the hydrophobic and hydrophilic segments were synthesized by ring opening polymerization of $\varepsilon$-CL monomer using tin (II) 2-ethylhexanoate as catalyst and monomethoxy poly ethylene glycol as macro initiator [29,30]. The copolymer yield ranged from 85 to 92%.

Fourier transform infrared (FTIR) spectra of the 5 PECL copolymers obtained using Shimadzu-8400S FTIR spectrophotometer (Tokyo, Japan) indicated copolymerization (Fig. 1A). A strong sharp absorption band that appears in all PECL copolymers at 1729 $cm^{-1}$ corresponds to the (C═O) stretching vibration of the ester carbonyl group of PCL. This was verified by the $^1H$ NMR spectrum of a sample copolymer, PECL-4, acquired with 400 MHz a JEOL JNM ECA 500 nuclear resonance spectrometer (Tokyo, Japan) (Fig. 1B). The spectrum showed a sharp peak at ~3.63 ppm due to the methylene protons of the PEG blocks unit ($-OCH_2CH_2-$), and a small peak at 3.33 ppm corresponds to the methyl protons of the end group of the PEG block. Two multiplets at ~1.4 and ~1.6 ppm are assigned to the methylene protons of $-(CH_2)$ in the PCL units and a weak multiple peak at ~4.1 ppm is assigned to the methylene protons of the ester bond in the block linkage $PCL-CO-OCH_2-CH_2-O-PEG$. Gel permeation chromatography (GPC) data obtained using a Jasco PU-1580HPLC liquid chromatography (Easton, USA) connected to a Jasco 830-RI and a PerkinElmer LC-75 Spectrophotome-

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