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Hydrogels containing porphyrin-loaded nanoparticles for topical photodynamic applications

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ABSTRACT

5,10,15,20-tetrakis(1-methylpyridinium-4-yl)-porphyrin tetra-iodide (TMPyP), a potent water-soluble photosensitizer (PS) used in antimicrobial applications, was encapsulated into poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles (TMPyP-PLGA) for topical delivery purposes. Nanoparticles resulted in a mean particle size around 130 nm, narrow polydispersity index (PdI), spherical morphology and association efficiency up to 93%. Free TMPyP and TMPyP-PLGA nanoparticles were incorporated into Carbopol® hydrogels, resulting in controlled TMPyP release of about 60% and 20% after 4.5 h, respectively. Critical properties such as appearance, clarity, viscosity and pH were maintained over time, as hydrogels were stable during 6 months at 4 °C, 25 °C/60% RH and 40 °C/75% RH. For photodynamic applications, the photoproduction of singlet oxygen from these hydrogels was quite efficient being both formulations very photostable after 20 min. No TMPyP permeation through pig ear skin was observed after 24 h, and histological assays did not show relevant damages in surrounding tissues. All these excellent characteristics make them promising platforms for photodynamic applications through topical clinical use.

1. Introduction

The resistance to antibiotics is a worldwide serious issue in terms of public health. Due to the progressive and inevitable resistance to these drugs, other alternative techniques have been developed such as the photodynamic inactivation of microorganisms (PDI) (Wainwright, 1998). This promising approach is based on the combination of molecular oxygen ($^3O_2$) and a PS, which is excited by light at a suitable wavelength in order to produce reactive oxygen species (ROS) capable of destroying microorganisms. Excellent results have been obtained using numerous types of PSs (porphyrins (Alves et al., 2009; Alves et al., 2013, 2014; Gomes et al., 2013), phthalocyanines (Pereira et al., 2012; Vilsinski et al., 2015), chlorins (Costa et al., 2012a; Huang et al., 2011; Ryu et al., 2015), bacteriochlorins (Huang et al., 2010, 2014), phenothiazines (Amaral et al., 2004; Bansode et al., 2009) among many others) and with diverse microorganisms such as bacteria (Edgardo, 2006; Ryskova et al., 2010) yeasts (Stenstrom

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et al., 1980; Strakhovskaya et al., 2002), fungi (Calzavara-Pinton et al., 2005; Donnelly et al., 2008; Pereira Gonzales and Maisch, 2012) and viruses (Costa et al., 2012b, 2011; North et al., 1993; Tomé et al., 2005), including complex structures as biofilms (Beirao et al., 2014; Pereira et al., 2011; Vilela et al., 2012; Wainwright and Crossley, 2004). In fact, some of these formulations are marketed, for example, Photogem®, Radachlorin® or Verteporfin® although their use is mainly in photodynamic therapy (PDT). The development of this kind of pharmaceuticals for antimicrobial use is still in its infancy (Perni et al., 2011) being an interesting field of study in terms of skin diseases caused by microorganisms such as psoriasis, acne vulgaris or mycosis (González-Delgado et al., 2016). Many authors have reported very good antimicrobial results (Xing et al., 2009) but nowadays only Visonac® is being tested for severe acne vulgaris in phase III in Europe and Russia (Ormond and Freeman, 2013) whereas Levulan® Kerastick® is being used off-label for the treatment of moderate acne. Due to the phase II results failed, no more studies were developed (Gold, 2009).

A well-known PS used widely in inactivation of microorganisms is the 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)-porphyrin tetra-iodide (TMPyP) due to its cationic character and excellent physicochemical properties, such as water solubility and high singlet oxygen quantum yield ($\Phi_\Delta = 0.74$ in water) (Wilkinson et al., 1995). TMPyP in solution has been successfully used in gram-positive and gram-negative bacteria, yeast and fungi including biofilms as well (Jori, 2006). This photoactivity is due to the electrostatic interactions between the positively charged PS and the negatively charged cell membrane producing a photodamage and consequently, the inactivation of microorganisms (Nitzan et al., 1995). Unfortunately, this therapeutic method is not highly selective and the photosensitivity can occur by accumulation of PS in normal cells. For this reason, the use of pharmaceutical nanotechnologies such as nanoparticles, may be an excellent approach for a controlled and selected release of the drug on the target (Ganguly et al., 2014; Li et al., 2012; Mundargi et al., 2008).

Semisolid pharmaceutical formulations are excellent drug delivery platforms for topical administration due to easy-handling, biocompatibility, no toxicity and easy-removing. Moreover, semisolids as gels, creams and ointments may improve drug topical penetration, uptake and selectivity. The topical use of a PS requires that the drug migrates from the matrix to the target. This transfer depends on several factors as the solubility of the PS, the retention of the drug by the matrix, and the tissue uptake capacity. Hence, we designed a Carbopol® hydrogel incorporating both free TMPyP and TMPyP-poly(lactic-co-glycolic acid) (TMPyP-PLGA) nanoparticles and characterised their rheological and physicochemical properties, with preliminary biological studies for further antimicrobial assays. These hydrogels should be capable of maintaining the shape and structure onto a wound and release the TMPyP or TMPyP-PLGA efficiently and selectively in a suitable time frame.

2. Materials and methods

TMPyP (5,10,15,20-tetrakis(1-methylpyridinium-4-yl)-porphyrin tetraiodide) was prepared as previously described (Gomes et al., 2011). PLGA 50:50 (PDLG 5004A) and PLGA 75:25 (PDLG 7502A) were kindly provided by Purac (PURASORB®). Ethyl acetate (Merck Millipore, Darmstadt, Germany), ultra-pure water (Synergy 185, Merck Millipore, Darmstadt, Germany), Pluronic F127 (Kolliphor P407 BASF) and Carbopol® 940 were purchased. ADMA (9,10-anthracenediyl-bis(methylene)dimalonic acid), absolute ethanol, propyleneglycol and triethylamine were all from Sigma Aldrich. All other chemicals and solvents were analytical grade and were used without further purification.

2.1. Preparation of TMPyP-PLGA nanoparticles

Both nanoparticles, voided and TMPyP-loaded, were synthesized by evaporation method (Vauthier and Bouchemal, 2009). Briefly, 40 mg of PLGA (75:25) were dissolved in 4 ml of ethyl acetate. 1 ml of TMPyP solution at different concentrations, diluted in ultra-pure water, was added to the previous solution and vortex for 30 s in a full speed. 10 ml of Pluronic F127 0.5% (w/v), prepared the previous day, was added to the w/o emulsion and sonicated for 60 s at 70% intensity in an ice bath. Finally, the w/o/w emulsion was transferred to the glass beaker containing an additional 10 ml of Pluronic F127 0.5% (w/v) under magnetic stirring (vel. 7) in a fume hood at room temperature. TMPyP-loaded nanoparticles formulations were protected from light during the production process.

2.2. Characterization of PLGA and TMPyP-PLGA nanoparticles

The physical-chemical properties of nanoparticles, namely mean particle size, the polydispersity index (PdI) and zeta potential were assessed using a ZetaSizer Nano ZS (Malvern, Worcestershire, UK). Void-PLGA or TMPyP-PLGA (1 mg/ml) suspensions were diluted in ultra-pure water (1:20) and the analysis of each sample conducted at room temperature (25 °C). The external morphology of the void-PLGA and TMPyP-PLGA nanoparticles was examined by scanning electron microscopy (SEM) using High Resolution (Schottky) Environmental Scanning Electron Microscope. Samples were coated with Au/Pd thin film (during 50 s and with a 15 mA current), by sputtering, using the SPI Module Sputter Coater equipment. The percentage of TMPyP encapsulated into the PLGA nanoparticles was determined through the indirect method by measuring the non-encapsulated TMPyP existing in the supernatant (after centrifugation of the nanoparticles at 35.000*g* during 50 min) using an UV–vis spectrophotometer. A calibration curve was used to evaluate the association efficiency (AE, %) by the following formula:

$$\%AE = \frac{total[TMPyP] - non\ \ encapsulated[TMPyP]}{total[TMPyP]} \times 100$$

2.3. Preparation of TMPyP and TMPyP-PLGA hydrogels

10 μM of TMPyP, (void-PLGA or TMPyP-PLGA) was added to a solution of Carbopol 940 (0.5 wt%) in type-II water. Then, propylene glycol (5 wt%), ethanol (3 wt%) and triethanolamine 99.6% (0.7 wt%) were added to complete the formulation up to pH 6.0. All the samples were allowed to equilibrate for at least 48 h at different temperatures prior to performing rheological measurements.

2.4. Singlet oxygen production and photobleaching of TMPyP and TMPyP-PLGA hydrogels

The singlet oxygen generation ability of TMPyP and TMPyP-PLGA hydrogels in PBS after irradiation was evaluated using 9,10-anthracenediyl-bis(methylene)dimalonic acid (ADMA) as a chemical trap (Lindig et al., 1980). ADMA can be photobleached by singlet oxygen to its corresponding endoperoxide, and the process can be monitored by recording the decrease in the absorbance of added ADMA at 378 nm. Briefly, a dispersion of TMPyP hydrogel (10 μM) and ADMA (85 μM) in PBS was placed in a quartz cuvette under constant magnetic stirring. The absorption intensity of ADMA when excited at 378 nm was recorded, and the dispersion was then irradiated using a white LEDs plate for various time periods, up to 25 min with a total light dose of 6.0 J/cm^2.