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DPPC:MPOx chimeric advanced Drug Delivery nano Systems (chi-aDDnSs): Physicochemical and structural characterization, stability and drug release studies

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ABSTRACT

Chimeric advanced Drug Delivery nano Systems (chi-aDDnSs) could be defined as mixed nanosystems composed of different biomaterials that can be organized into new nanostructures that can offer advantages as drug carriers. In this work, we report on the self assembly behavior and on stability studies of chi-aDDnSs consisting of DPPC (dipalmitoylphosphatidylcholine) and poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline) (MPOx) gradient copolymer in Phosphate Buffer Saline (PBS). Light scattering techniques were used in order to extract information on their physicochemical and structural characteristics (i.e. ζ-potential, Polydispersity Index (PD.I.), size/shape and morphology), while their stability was also studied as a function of gradient block copolymer content, as well as temperature. The colloidal stability of the chimeric nanovectors and their thermoresponsive behavior indicates that these nanosystems could be considered as sterically stabilized nanocontainers. DPPC:MPOx chimeric advanced Drug Delivery nano Systems were found to be effective nanocontainers for the incorporation of indomethacin (IND). The combination of gradient block copolymers with phospholipids for the development of novel chimeric nanovectors is reported for the first time and appears very promising, mostly due to the fact that the MPOx acts as a modulator for the release rate of the IND.

1. Introduction

Soft Nanotechnology is a relatively young scientific and technological discipline (Hamley, 2003; Nayak and Lyon, 2005; Whitesides and Lipomi, 2009). Pharmaceutical nanotechnology has brought a wide variety of new possibilities into biomedical discovery and clinical practice because nano-scaled carriers have revolutionalized drug delivery (Hughes, 2005; Ravichandron, 2009; Mishra et al., 2010; Souza et al., 2010). The importance of nanotechnology in drug delivery is based on its ability to manipulate supramolecular self-assembled structures in order to produce devices with programmed functions (Whitesides and Grzybowski, 2007).

Over the last years liposomes have been proved as the archetypical nanoscale vectors and represent one of the most thoroughly studied categories of colloidal nanocarries (Bangham et al., 1965; Gregoriadis et al., 1974). On the other hand, polymersomes are a class of artificial vesicles made from synthetic amphiphilic block copolymers. Polymersomes have relatively thick (3–4 nm) and robust membranes formed by amphiphilic block copolymers with relatively high molecular weight (Discher and Eisenberg, 2002; Le Meins et al., 2011; Meng and Zhong, 2011; Lee and Feijen, 2012; Thompson et al., 2012). From a biophysical point of view polymersomes, as well as liposomes, can be considered as interesting cell membrane mimics (Le Meins et al., 2011). Their closed bilayer structure is a first step toward compartmentalization, which is one of the key architectural requirements to reproduce the natural environment of living cells. Interestingly, membrane proteins can be incorporated into such bio-mimetic membranes by reconstitution methods, leading to so-called proteopolymersomes (Nallani et al., 2011).

Additionally, the application of polymers in medicine as components of drug nanocarriers are considered essential for producing and developing new formulations against several human diseases. Amphiphilic block copolymers and vesicle forming surfactants have attracted major scientific interest in recent years due to their intriguing self-assembly behavior in aqueous media, which results in a plethora of nanoassemblies and their potential applications in drug delivery (Pispas and Sarantopoulou, 2007; Pispas, 2011a,b). Lipopolymers self-assembled into biocompatible

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; d_f, mass fractal; MPOx, poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline); PBS, phosphate buffer saline; aDDnSs, advanced Drug Delivery nano Systems.

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nanostructured multifunctional biomaterials offer many potential and attractive applications in drug, protein and nucleotide delivery, nanomedicine and diagnostics, too (Liu et al., 2007; Shi et al., 2010; Angelova et al., 2011). Amphiphilic block copolymers are able to form a range of different nanoparticulate morphologies for pharmaceutical applications (Letchford and Burt, 2007). A large number of studies indicate that it is possible to achieve absorption or penetration of block copolymers onto preformed liposomes or self-assembled superstructures in aqueous media (Stoenescu et al., 2004; Leiske et al., 2011; Nam et al., 2011). The interactions and the insertion mechanism between lipids and block copolymers are of paramount importance due to their pharmaceutical applications (Firestone and Seifert, 2005; Ruysschaert et al., 2005; Amado et al., 2009; Antunes et al., 2009; Kita-Tokarczyk et al., 2009; Leiske et al., 2011; Nam et al., 2011).

Furthermore, Modulatory Controlled Release Drug Delivery nano Systems (MCRDDnSs) are considered as suitable self-assembled nanocarriers that can be composed of more than one biomaterial, *i.e.* lipids producing liposomes and polymers, dendrimers or dendritic structures which can be mixed in order to produce new and effective delivery systems with unique biophysical properties and controlled release profile. Such DDnSs with a Modulatory Controlled Release profile, are denoted as advanced Modulatory Controlled Release Drug Delivery Systems and could be categorized as hybridic or chimeric based on the nature of the mixing elements that could be the same or different, respectively (Demetzos, 2010a,b; Gardikis et al., 2011; Du et al., 2012; Kaditi et al., 2012). These nanovectors can be characterized as mixed nanosystems due to the combination of different in nature materials and are used for biomimetic delivery. The interest in such systems stems from the possibilities for basic understanding of biological behavioral motifs, since biological systems also extensively use mixed materials in order to create "smart" self-assembled nanostructures, (Demetzos, 2010a,b).

Although the Pluronics, composed of poly(ethylene oxide) and poly(propylene oxide) blocks are the most widely studied amphiphilic block copolymers, poly(2-oxazoline)s may present important advantages for clinical applications (Adams and Schubert, 2007; Hoogenboom, 2009; Knop et al., 2010; Barz et al., 2011). Additionally, poly(2-oxazoline)s and their copolymer are characterized as bioinspired materials due to the pseudopeptide nature of the oxazoline segments, while poly(2-methyl-2-oxazoline) is proposed as an alternative to PEG in terms of biocompatibility and stealth properties (Kempe et al., 2009; Schlaad et al., 2010; Barz et al., 2011; Lambermont-Thijs et al., 2011, Bauer et al., 2013). Furthermore, polymers of the poly(2-oxazoline) family, as modifiers of the liposomal surface, are efficient in conveying long-circulating and stealth properties to liposomes in mice (Woodle et al., 1994; Zalipsky et al., 1996).

Controlling the morphology of nanoparticles is of key importance for exploiting their functionality and their properties in several emerging technologies (Henry, 2005). The morphology of nanoparticles is critical to their biological interactions, like protein binding (Semple et al., 1998). The polymers induce significant morphological perturbations when included in lipid mixtures used for preparation of liposomal bilayers. According to the recent literature, the demixing of lipid-rich and polymer-rich membrane domains within the same vesicle bilayer was demonstrated and these morphological and structural differences were discussed as the possible resultant interdomain interactions within the mixed liposomal membrane (Nam et al., 2011). The morphology and the shape of these systems are directly related to their colloidal behavior (Pippa et al., 2012a,b). Validated assays are important for detecting and quantifying nanopharmaceuticals, Drug Delivery Systems or biologically active drug products, and how biophysical characteristics and structure may impact product quality in clinical use (Chen et al., 2007).

The goal of this study is to design and develop novel chimeric advanced Drug Delivery nano Systems (chi-aDDnSs) composed of dipalmitoylphosphatidylcholine (DPPC) and poly(2-methyl-2-oxazoline)-grad-poly(2-phenyl-2-oxazoline (MPOx) at different molar ratios. Prime interest is focused on the determination of the physicochemical and structural characteristics (*i.e.* ζ-potential, Polydispersity Index (PD.I.), size/shape and morphology) of the produced chimeric nanostructures composed of different concentrations of DPPC:MPOx in PBS as well as of the effect of the temperature on their size/shape, PD.I., distribution and their fractal dimension. We also studied the chimeric nanoassemblies formed by the incorporation of indomethacin (IND) and the drug release profile of this lipophilic drug which is correlated with their structural characteristics.

2. Materials and methods

2.1. Materials

The phospholipid used for liposomal formulations was 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC). It was purchased from Avanti Polar Lipids Inc., (Albaster, AL, USA) and used without further purification. Chloroform and all other reagents used were of analytical grade and purchased from Sigma–Aldrich Chemical Co. Indomethacin was supplied by Fluka and was used as received. The MPOx amphiphilic gradient block copolymer was prepared *via* cationic polymerization (Milonaki et al., 2012). In this copolymer phenyl-oxazoline segments comprise the hydrophobic components and methyl-oxazoline segments the hydrophilic ones. The copolymer is considered biocompatible due to the pseudopeptide nature of the oxazoline segments. Poly(2-methyl-2-oxazoline) is proposed as an alternative to PEG in terms of biocompatibility and stealth properties. Molecular weight and molecular weight distribution of the MPOx copolymer was determined by size exclusion chromatography (SEC) using a Waters system, with a Waters 1515 isocratic pump, a set of three μ-Styragel mixed bed columns, having a porosity range of 10^2–10^6 Å, a Waters 2414 refractive index detector (at 40 °C) and controlled through Breeze software. $CHCl_3$ was the mobile phase used, at a flow rate of 1.0 mL/min at 25 °C. The set-up was calibrated with polystyrene standards having weight average molecular weight in the range 1200–900,000 g/mol. Average composition of the copolymer was determined by ^{1}H-NMR spectroscopy in $CDCl_3$, using a Bruker AC 300 spectrometer in $CDCl_3$ at 30 °C.

The gradient copolymer MPOx was characterized by SEC and ^{1}H-NMR and it was found to have the following molecular characteristics: M_w = 3300, M_w/M_n = 1.26, 39 wt% PhO*xz* (hydrophobic component). The structures of the components of chimeric nanostructures are presented in Fig. 1. The neat amphiphilic gradient copolymer was found to form polymersomes in aqueous media (Milonaki et al., 2012).

2.2. Preparation of chimeric aDDnSs

Different liposomal formulations have been prepared using the thin-film hydration method, composed of DPPC:MPOx (9:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:9 molar ratios). Briefly, appropriate amounts of DPPC:MPOx mixtures were dissolved in chloroform/methanol (9:1 v/v) and then transferred into a round flask connected to a rotary evaporator (Rotavapor R-114, Buchi, Switzerland). Vacuum was applied (vacuum 1.0×10^{-2} mbar) and the phospholipid thin film was formed by slow removal of the solvent at 50 °C. The mixed phospholipid film was maintained under