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Engineered polyester-PEG nanoparticles prepared through a "grafting through" strategy and post-functionalization via Michael type addition

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ABSTRACT

Free radical polymerization (FRP) is widely used in industrial processes as an efficient and versatile method to engineer polymeric nanoparticles (PNPs) of controlled size, narrowly distributed, and of well-defined surface properties. Functional Poly(ε-caprolactone) (PCL) and poly(lactic acid) (PLA) can be utilized as macromonomers in FRP in combination with a *co*-polymerizable poly(ethylene glycol) (PEG), to achieve aqueous dispersions of PNPs composed of a hydrophobic polyester core and a hydrophilic PEG shell of tuneable size. For several industrial and biological applications, PNPs also need surface functionalization to provide specific physicochemical characteristics, including stimuli-responsiveness, and bioactivity.

In this work, a flexible "grafting through" strategy based on Ring opening polymerization (ROP) and FRP was proposed to obtain engineered polyester-PEG nanoparticles functionalized with acrylate groups on the hydrophilic shell. The presence of acrylates allows a versatile surface functionalization through Michael-type addition with a thiolated ligand (peptide), in aqueous solution under physiological pH, with the advantage of high conversion and absence of reaction side products. A cysteine-containing cyclic RGD was used as model peptide for conjugation, due to its potential application as ligand for endothelial cells. Results indicated that active cell targeting can be achieved by using this surface functionalization approach.

1. Introduction

The field of polymeric nanoparticles (PNPs) has reached a significant importance in different industrial and biomedical applications during the last decades [1, 2]. A wide spectrum of areas, ranging from adhesives to coatings, additives for paper and textiles, medicine to biotechnology, food and nutraceutics, environmental technology, exploited the unique physicochemical characteristics of PNPs in terms of size, surface properties, dye/drug loading and release, biocompatibility and biodegradability [1, 3, 4].

Among different polymerization techniques, Free radical polymerization (FRP) is widely used in industrial processes as efficient and versatile method to synthesize polymer colloids of controlled size distribution, and well-defined surface properties [5]. In these processes, FRP is carried out either in a continuous phase or in emulsion, where high conversions of monomers in a short reaction time are generally obtained [2]. FRP in a continuous phase is generally followed by a nanoprecipitation of the polymer in an aqueous solution to obtain the final suspension, whereas in free radical emulsion polymerization (FREP), the monomers are polymerized in combination with reactive surfactant (surfmer) to obtain stable PNPs dispersed in water phase [5]. Compared with more sophisticated controlled-living polymerization techniques [6, 7], FRPs are relatively cheap and can be easily scaled-up, with a large amounts of dispersed polymers produced every year under safe conditions [4].

FRP techniques have recently been proposed to produce biocompatible and biodegradable polymeric nanoparticles based on a hydrophobic polyester core and a hydrophilic poly(ethylene glycol) (PEG) shell [5]. Custom made Poly(ε-caprolactone) (PCL) and poly(lactic acid) (PLA) functionalised with terminal vinyl groups (2-hydroxyethyl methacrylate (HEMA) or N-(2-hydroxypropyl) methacrylamide (HPMA) were used as macromonomers in combination with a *co*-polymerizable surfactant such as methoxy methacryloyl PEG, to obtain aqueous dispersions of PEGylated polyester nanoparticles of controlled

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size [5, 8–11].

In several applications, PNPs need surface functionalization to provide specific physicochemical characteristics, such as stimuli-responsiveness, bioactivity, etc. [12, 13]. In the expanding field of nanomedicine, PNPs have been widely investigated as carriers for drug delivery, with the ability of loading therapeutic molecules and releasing them in a specific site such as a (tumor) cell, a tissue or an organ [14, 15]. Therefore, the surface of PNPs should be engineered in a very precise manner as it needs to be modified with suitable ligands (peptides, proteins, positively/negatively charged moieties, hydrophilic/hydrophobic/amphiphilic molecules) which should specifically interact with the target cell/tissue [16]. This surface functionalization requires conjugation reactions that are highly efficient, applicable under aqueous conditions, and stable in biological environments [17].

Among different surface modification strategies, a very versatile conjugation method based on Michael type addition can be proposed [18]. The conjugate addition reaction between electron-poor olefins and nucleophiles, such as thiols, has been widely used as a convenient tool for functionalization of biomaterials [19]. In fact, when acrylate groups are displayed by a polymeric (nano)material in an aqueous environment, they are able to react quantitatively with thiolated groups, under mild conditions and at a physiologically acceptable pH, and without generation of side products [18].

In this work, functional polymeric nanoparticles were obtained through a “grafting through” strategy based on FRP of polyester-based and PEG-based macromonomers, and followed by an acrylation of the therminal –OH end groups of the PEG side chains, as summarized in Scheme 1. These brush copolymers were nanoprecipitated to generate PNPs with a (polyester-based) hydrophobic core and hydrophilic (PEG) shell functionalized with electron-poor olefins (acrylate groups), which can act as Michael-type acceptors for a final surface modification. In the post-polymerization functionalization, acrylation was preferred to methacrylation, as methacrylates generally perform poorly as Michael acceptors under physiological conditions [20].

Poly(lactic acid) (PLA)-methacrylate and poly ε-caprolactone (PCL)-methacrylate were synthesized through Ring Opening Polymerization (ROP), and used as the polyester macromonomers for FRP. Commercial poly(ethylene glycol) was methacrylated at a fixed degree of functionalization to obtain hydroxy -terminated PEG methacrylate, which was also and used as co-mononomer.

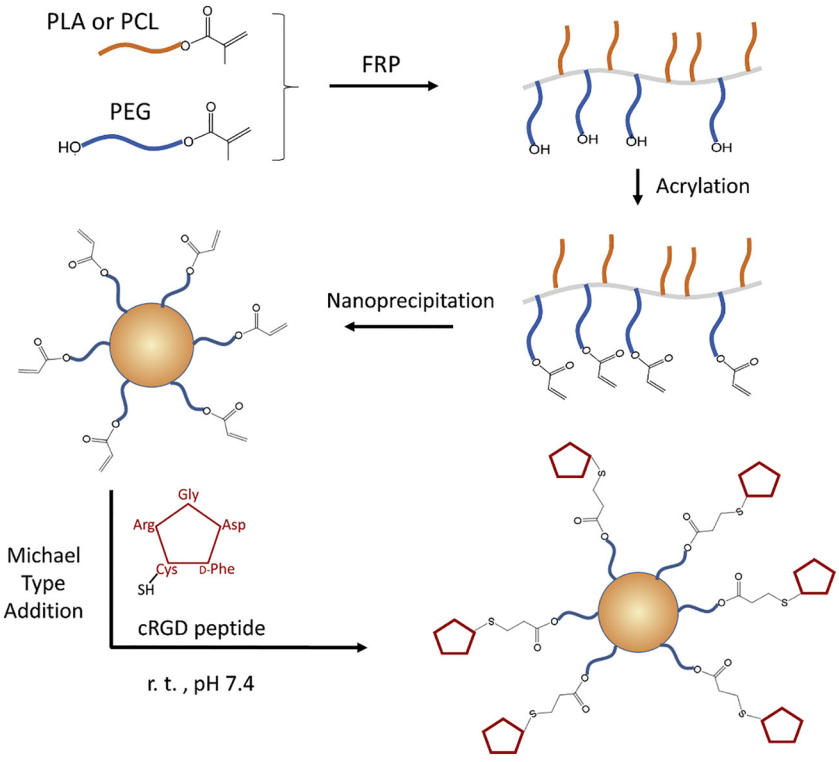
In order to assess the feasibility of the Michael-type addition for the final surface modification of the PNPs, a conjugation step was performed with a cyclic arginine-glycine-aspartate (cRGD) peptide containing a single cysteine, which provided the thiol group for a covalent attachment to the acrylates displayed by the PNPs.

Cyclic RGD was used as model peptide due to its easy availability and its potential application in nanomedicine, as ligand for endothelial cells of tumors, the placenta, and the kidney glomerulus [21–24]. Through this synthetic approach, engineered PNPs may be developed as functional nanocarriers to deliver therapeutic agents to the target site, for effective treatment of different diseases [5, 6, 22–24].

2. Experimental

2.1. General procedures

Chemicals were purchased from commercial sources (Sigma-Aldrich) and used without further purification, unless otherwise indicated. When anhydrous conditions were required, the reactions were performed under nitrogen atmosphere and solvents were degassed prior use for 10 min. Deionized water (18.2 MΩ) was obtained from a Millipore Milli-Q purification unit. NMR experiments were recorded on a Bruker AVANCE 400 MHz instrument at 298 K, using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts (δ) are reported in ppm downfield from TMS as internal standard, coupling constants (J) are in Hz. The ^{1}H resonance of compounds were assigned with the assistance of COSY and HSQC experiments. The number-average molecular weight ($M_{n,GPC}$) values and molecular weight distributions (M_w/M_n) values of the polymers were evaluated using a Jasco LC-2000Plus gel permeation chromatograph (GPC) equipped with a refractive index detector (RI-2031Plus, Jasco) using 3 Agilent PLgel columns, 5×10^{-6} M particle size, 300 × 7.5 mm (MW range: 5×10^2 to 17×10^5 g mol^{-1}). THF was chosen as eluent at a flow rate of 0.5 mL min^{-1} at 35 °C. The GPC samples were injected using a Jasco AS-2055Plus autosampler. The



Scheme 1. A polyester (PLA or PCL) macromonomer was copolymerised by FRP with hydroxyl- terminated PEG methacrylate. The resulting amphiphilic brush copolymer was acrylated and then nanoprecipitated in aqueous solution to obtain size-controlled PNPs. Surface modification was finally obtained through Michael-type addition of a cRGD peptide.