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Functionalized poly(lactic-co-glycolic acid) enhances drug delivery and provides chemical moieties for surface engineering while preserving biocompatibility

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Abstract

Poly(lactic-co-glycolic acid) (PLGA) is one of the more widely used polymers for biomedical applications. Nonetheless, PLGA lacks chemical moieties that facilitate cellular interactions and surface chemistries. Furthermore, incorporation of hydrophilic molecules is often problematic. The integration of polymer functionalities would afford the opportunity to alter device characteristics, thereby enabling control over drug interactions, conjugations and cellular phenomena. In an effort to introduce amine functionalities and improve polymer versatility, we synthesized two block copolymers (PLGA-PLL 502H and PLGA-PLL 503H) composed of PLGA and poly(ε-carbobenzoxy-L-lysine) utilizing dicyclohexyl carbodiimide coupling. PLGA-PLL microspheres encapsulated approximately sixfold (502H) and threefold (503H) more vascular endothelial growth factor, and 41% (503H) more ciliary neurotrophic factor than their PLGA counterparts. While the amine functionalities were amenable to the delivery of large molecules and surface conjugations, they did not compromise polymer biocompatibility. With the versatile combination of properties, biocompatibility and ease of synthesis, these block copolymers have the potential for diverse utility in the fields of drug delivery and tissue engineering.

Keywords: Block copolymers; PLGA; Poly(amino acid); CNTF; VEGF

1. Introduction

The common polyester biomaterials poly(lactic acid) (PLA) and poly(glycolic acid) (PGA), and their copolymer poly(lactic-co-glycolic acid) (PLGA) lack chemical functionalities to elicit specific drug or cell interactions. Furthermore, their potential for the sustained delivery of hydrophilic molecules (i.e. proteins) is often limited [1]. This frequently results in poor encapsulation and a large "burst" release of the encapsulated drug within the first few hours or days [1-3]. This initial burst is due to desorption of surface-associated hydrophilic molecules on devices composed of hydrophobic polymers, not polymer degradation [1]. When introduced to an aqueous environment, these surface absorbed molecules are readily dissociated. These undesirable outcomes are often due to poor associations between the drug and the polymer. In an effort to optimize these phase or electrostatic associations, many utilize alternative materials [4]. However, these alternatives present their own limitations (i.e. immunogenicity) [5]. To circumvent these limitations and establish therapeutic efficacy, large doses or site-specific administration are often required of devices composed of the polyester biomaterials [6,7].

Furthermore, these polymers lack functionalities to promote cellular phenomena such as adhesion and proliferation. Many have achieved cellular interactions or cell-specific drug targeting through the addition of ligands to device surfaces [7-9]. However, a key shortcoming

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hindering this addition to these polyesters is the lack of functional groups on the aliphatic backbones, which limits the number of sites for potential incorporation and conjugation of biologically active molecules [7]. In an attempt to address these inadequacies, numerous groups have introduced functional groups to these degradable polyesters either through direct conjugation [10,11] or with additives during device fabrication [7,12].

Modifications of particular interest have included the addition of poly(amino acids). The incorporation of a poly(amino acid) allows one to tailor device characteristics such as hydrophilicity and surface charge while providing sites for conjugation and cellular interactions [13-15]. Much of this research has previously focused around PLA [10,13,14,16]. Researchers have noted that devices comprising a poly(L-lysine) (PLL)-PLA conjugate have significantly improved protein encapsulation and facilitated surface conjugations over their PLA equivalents [16,17]. However, with the hydrophobicity of PLA, its potential for drug delivery and tissue engineering applications is limited [18,19]. In addition to a relatively complex conjugation scheme (ring-opening polymerization), a simpler technique was desired.

PLGA is a staple of drug delivery and tissue engineering [18,20]. Not only is PLGA used in commercially available and FDA-approved devices, but simple variations can alter the polymer's rate of degradation and release of encapsulated agents [21,22]. Because of these attributes, many have utilized PLGA in their efforts of controlled and site-specific drug delivery [23]. Lavik et al. [24] found that, by using dicyclohexyl carbodiimide (DCC) coupling, one is able to easily and efficiently conjugate PLL and PLGA, thereby circumventing the aforementioned limitations of PLA.

However, a fundamental issue with incorporating PLL into biomaterials is the potential for cytotoxicity [25,26]. It is well established that polycations in general are toxic [25,27]. This risk requires care to be taken when adding or modifying functional groups, as it can compromise the biocompatibility of the material or device [28]. Therefore, the potential toxicity for any inclusion of PLL in a biomaterial must be thoroughly investigated.

In this paper, we have looked to determine the potential of incorporating PLL and PLGA. We synthesized two block copolymers (PLGA-PLL 502H and PLGA-PLL 503H) composed of PLGA (502H: M_n ~10 kDa or 503H: M_n ~25 kDa, respectively) and poly(ε-carbobenzoxy-L-lysine) (M_w ~1000 Da). We hypothesized that the conjugation of PLL with PLGA would not only prove advantageous, as was the case with PLA, but the addition of amine functionalities would not sacrifice the biocompatibility and properties that PLGA is favored for.

Here, we present a functionalized PLGA via synthesis of an amphipathic block copolymer composed of PLGA and poly(ε-carbobenzoxy-L-lysine). Conjugation of poly(ε-carbobenzoxy-L-lysine) to different PLGA polymers introduces amine functionalities that significantly enhance the incorporation of vascular endothelial growth factor A_{165} (VEGF) and ciliary neurotrophic factor (CNTF) in microspheres. These added functionalities enable conjugations, and their availability was explored via surface coupling of fluorescein isothiocyanate (FITC). By developing these block copolymers and establishing their biocompatibility both in vitro and in vivo, we were able to create a construct amendable for numerous biomedical applications while preserving the desired attributes of PLGA.

2. Materials and methods

2.1. Materials

PLGA 502H (50:50 lactic to glycolic acid ratio and an M_n of ~10 kDa) and PLGA 503H (50:50 lactic to glycolic acid ratio and an M_n of ~25 kDa) were from Boehringer Ingelheim (Ingelheim, Germany). H signifies PLGA terminated with a carboxylic acid group. Poly(vinyl alcohol) (PVA) (88 mol.% hydrolyzed) was from Polysciences (Warrington, PA, USA). Poly(ε-carbobenzoxy-L-lysine) (M_w ~1000 Da) was from Sigma (St. Louis, MO, USA). The Micro bicinchoninic (BCA) protein assay kit was from Pierce (Rockford, IL, USA). The enzyme-linked immunosorbent assays (ELISA DuoSet), (VEGF and CNTF were from R&D Systems (Minneapolis, MN, USA). Deuterated dimethyl sulfoxide (D_6-DMSO) was from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). All other chemicals were used as received from Sigma.

2.2. Synthesis and characterization of block copolymer

2.2.1. Conjugation

The block copolymers (PLGA-PLL 502H and PLGA-PLL 503H) were synthesized according to Lavik et al. [24]. Briefly, PLGA 502H or PLGA 503H and poly (ε-carbobenzoxy-L-lysine) (1:1 M ratio) were dissolved in dimethyl formamide (DMF). Two molar equivalents (with respect to PLGA) of DCC and 0.1 M equivalents of dimethylamino-pyridine (DMAP) were added and the reaction was allowed to run for 36 h under argon. Following conjugation of PLGA and poly(ε-carbobenzoxy-L-lysine), the polymer solution was diluted with chloroform and filtered to remove N,N′-dicyclohexylurea (DCU), an insoluble by-product of the reaction. The presence of DCU was indicative of successful conjugation. The block copolymer was then precipitated in methanol, vacuum filtered, rinsed with ether to remove any unconjugated poly(ε-carbobenzoxy-L-lysine) and lyophilized for at least 48 h.

2.2.2. Deprotection

To expose primary amines, ~1.5 g of the block copolymer was dissolved in 30 wt.% hydrogen bromide in acetic acid (HBr/HOAc) and stirred. After 1.5 h, ether was added to the solution and the precipitated polymer was removed. The polymer was washed with ether until an off-white brittle mass was obtained. The mass was then dissolved in

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