Materials Science and Engineering C 59 (2016) 333–338

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Polylactide-based microspheres prepared using solid-state copolymerized chitosan and D,L-lactide

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ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 13 July 2015
Accepted 26 September 2015
Available online 30 September 2015

Keywords:
Chitosan
D,L-Lactide
Graft copolymers
Solid-state reactive blending
Microparticles
Oil/water emulsion
Tissue engineering

ABSTRACT

Amphiphilic chitosan-g-poly(D,L-lactide) copolymers have been manufactured via solid-state mechanochemical copolymerization and tailored to design polyester-based microspheres for tissue engineering. A single-step solid-state reactive blending (SSRB) using low-temperature co-extrusion has been used to prepare these copolymers. These materials have been valorized to stabilize microspheres processed by an oil/water emulsion evaporation technique. Introduction of the copolymers either in water or in the oil phase of the emulsion allowed to replace a non-degradable emulsifier typically used for microparticle preparation. To enhance cell adhesion, these copolymers were also tailored to bring amino-saccharide positively charged segments to the microbead surface. Size distribution, surface morphology, and total microparticle yield have been studied and optimized as a function of the copolymer composition.

1. Introduction

Polylactide (PLA) based microspheres are attractive microcarriers for tissue engineering [1,2]. However, in spite of several PLA advantages, such as biocompatibility, biodegradability, and satisfying mechanical properties, PLA suffers from several drawbacks. For instance, PLA hydrophobicity and lack of functionalities, needed to promote cell adhesion and proliferation, limit its usage to design scaffolds for tissue regeneration [3]. Additionally, acidic byproducts released during polymer degradation are known to induce a local inflammatory response [4].

Due to the presence of specific molecular signals promoting cell attachment and growth, natural polymers are more attractive than synthetic ones. In particular, surface coating of polyester microspheres with collagen has been successfully used to enhance cell attachment, proliferation and viability of chondrocytes [5]. Chitosan coating to modify PLA microsphere surface with clear benefits for cartilage repair has been also reported by Lao et al. [6]. A wide usage of this functional polysaccharide for design of scaffolds with mimetic features of the extracellular matrix can be explained by its unique physicochemical and biological properties. Chitosan, which can be degraded in a human body by lysozyme, is considered as temporary replacement for tissue reconstruction [7]. The ability of the positively charged chitosan amino groups to interact with negatively charged cell membranes promotes tissue formation and host tissue integration. Moreover, easy conjugation of the chitosan and PLA degradation products which are glucosamine and lactic acid, respectively, can decrease the inflammation of tissues.

Therefore it is not surprising that surface modification of preformed polyester microparticles through physical sorption or chemical grafting of this bioadhesive polymer has been proposed earlier [2,8]. If adsorption is the most simple methodology to apply, this procedure would lack reproducibility and control of surface characteristics with a risk of polymer desorption. Conversely if a covalent grafting could guarantee the stability of the surface functionalities, this methodology would be particularly tedious to apply. Indeed, the generation of functional groups on polyesters involves several steps with a concurrent risk of polymer degradation.

As an alternative to the abovementioned procedures, but also to exclude the non-degradable emulsifiers which are typically used to stabilize microparticles, we focused our study on the preparation of polylactide-based microspheres using chitosan-g-poly(L,D-lactide) copolymers. These new copolymers were synthesized by the solid-state mechanochemical copolymerization. Due to a solubility difference between the hydrophobic monomer and the hydrophilic polysaccharide, multi-step synthetic schemes for grafting lactide onto chitosan backbone have been reported [9–14].

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http://dx.doi.org/10.1016/j.msec.2015.09.094
0928-4931/

In the current study, to cope with complex chemical schemes which could also raise concerns regarding harmful residues of catalysts and solvents, a single-step mechanochemical approach has been optimized by us for preparation of chitosan-g-poly(D,L-lactide). This reaction was performed through solid-state reactive blending (SSRB) of chitosan with D,L-lactide by co-extrusion to provide conditions for mechanochemical reactions [15]. SSRB in a twin-screw extruder provides shear deformation of co-reactants and their mixing on molecular level that are two key factors to allow chemical reactions in solid-state to occur. Mechanochemical reactions under the action of shearing forces and local pressures have been described earlier [16–18]. This approach to polymer modification has been successfully applied for the preparation of a number of chitosan-based copolymers and derivatives with high yield of the desired products [19–21]. Since the entire process is carried out in a solid state and does not require either melting or dissolution, there is no need in any catalysts but the mechanical energy to promote the chemical interaction. This approach has a great potential for preparation of various biomaterials for biomedical applications.

In the current study, to produce and stabilize microspheres, chitosan-g-poly(D,L-lactide) copolymers were tailored made according to this mechanochemical procedure. Amphiphilicity of these new macromolecules has been adjusted in order to assure their anchorage to the microparticle surface, but also to guarantee their effectiveness and efficiency for the stabilization of the liquid/liquid and solid/liquid interphases generated during the formation of the microparticles produced by emulsion/evaporation process.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide) (PDLLA) with M_w of 76 kDa, was synthesized as described in [22,23]. Chitosan (M_w 60 kDa; acetylation degree 0.1) was prepared by mechanochemical solid-state synthesis as previously reported [24]. D,L-Lactide (Purasorb DL, cis-(±)-3,6-dimethyl-1,4-dioxane-2,5-dione) was purchased from PuracBiochem (The Netherlands).

2.2. Synthesis of chitosan-g-poly(D,L-lactide) copolymers

The reactive mixtures of chitosan and D,L-lactide were blended using a semi-industrial co-rotating twin-screw extruder Berstorff ZE40 (Germany) at lactide molar ratio of 0.5, 1, or 3 per chitosan unit. This reaction was performed at temperature range from 90 to 120 °C, just below the D,L-lactide melting range (124–128 °C). It should be pointed out that the configuration of the screws has been fixed, in order to combine different mixing zones to generate a high shear strain and powerful dispersive action. The reaction process was run on 300-g batches; operating screw rotation speed of 60 rpm.

2.3. Fractional analysis

Chitosan-g-poly(D,L-lactide) (CL) obtained by the SSRB technique was purified as follows: a sample (1 g) was dispersed in 25 ml of chloroform for 3 h at RT under magnetic stirring. After dissolution of unreacted lactide, the insoluble fraction was collected by filtration, washed several times on paper filter with chloroform, before drying in a vacuum oven. The percentage of lactide linked to chitosan has been derived from the difference in weight observed between lactide in CL sample after purification with chloroform and lactide initially taken for synthesis.

The aqueous medium fractionalization of CL sample (~1.5 g) was conducted by stirred in 80 ml of water at RT for 2 h. The insoluble fraction was separated by centrifugation, repeatedly washed with deionized water, lyophilized and weighted. Water soluble fractions were precipitated in 1 M NaOH and were recovered under centrifugation. The sediments were washed with deionized water and dried by lyophilization.

The degree of chitosan amino group substitution was determined by CNH analysis of the samples subjected to neutralization and long-term dialysis: the sample probe (~1 g) was dissolved in 70 ml of deionized water for 2 h. After addition of 4% solution of NaOH to reach pH of 10, the resultant suspensions were dialyzed for 2 weeks against deionized H_2O with membrane of a cut-off of 3500 Da. The purified fractions were dried by lyophilization.

2.4. CHN elemental analysis

CHN elemental composition of the fractions subjected to the long-term dialysis was obtained using a FLASH-2000 Organic Elemental Analyzer (Thermo, UK). The percentages of carbon (±0.05), hydrogen (±0.08) and nitrogen (±0.07) were estimated. Lactic acid unit content (X_L) was calculated using Eq. (1).

$$X_L = 100 - X_{chs} = 100 - A_N \cdot MW/A_N, \quad (1)$$

where X_{chs} represents percentage of nitrogen contained units (chitosan units), A_N — percentage of nitrogen measured by the elemental analysis, A_N— atomic weight of nitrogen, and MW — molecular weight of chitosan unit.

2.5. FTIR

FTIR spectra of KBr/copolymer pellets were acquired with recorded on a Digilab FTS-40 spectrometer (Bio-Rad). All spectra were obtained in absorbance mode at a resolution of 4 cm^{-1}. All spectral manipulations were performed using Win-IR (Version 4) software supplied by Bio-Rad (Digilab Division). For spectral comparisons the spectra were normalized to the same intensity of a well resolved band 1075 cm^{-1} of C–O stretching vibrations of pyranose cycle.

2.6. Preparation of microspheres

Microspheres were prepared by oil/water emulsion solvent evaporation technique [25,26] dissolving CL samples either in the water or oil phases.

In the first case, the oil phase was obtained by dissolving PDLLA in a CH_2Cl_2:acetone (9:1 v/v) mixture to achieve a 8 wt.% concentration. This

Table 1
List of chitosan-g-poly(D,L-lactide) batches: conditions of treatment and the copolymer's yield[a].

Sample	Conditions of synthesis		Macromolecular features of the copolymer batches		
	Lactide/chitosan molar and weight ratio, mol (g/g)	Temperature, °C	Relative amount of the reacted lactide, %	Grafting degree, %	Molar content of lactic acid units
CL-0.5	0.5 (31:69)	120	57.1	26	0.58
CL-1	1 (47:53)	90	85.3	76	1.69
CL-3	3 (73:27)	120	59.3	160	3.58

[a] Percentage of the reacted lactide amount was estimated as a ratio to initial lactide quantity taken for synthesis. Grafting degree (%) was calculated as follows: [(graft-copolymer weight − chitosan weight) / chitosan weight] × 100. Molar content of lactic acid units per chitosan unit equals to grafting degree × 161 / 72.