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Biocompatibility of poly(D,L-lactic-*co*-hydroxymethyl glycolic acid) microspheres after subcutaneous and subcapsular renal injection

F. Kazazi-Hyseni [a], J. Zandstra [b], E.R. Popa [b], R. Goldschmeding [c], A.A.R. Lathuile [d], G.J. Veldhuis [d], C.F. Van Nostrum [a], W.E. Hennink [a], R.J. Kok [a,*]

[a] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584 CG, Utrecht, The Netherlands
[b] Department of Pathology and Medical Biology, Medical Biology Section, University Medical Centre Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands
[c] Department of Pathology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, The Netherlands
[d] Nanomi B.V., Zutphenstraat 51, 7575 EJ, Oldenzaal, The Netherlands

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ABSTRACT

Poly(D,L-lactic-*co*-hydroxymethyl glycolic acid) (PLHMGA) is a biodegradable copolymer with potential as a novel carrier in polymeric drug delivery systems. In this study, the biocompatibility of PLHMGA microspheres (PLHMGA-ms) was investigated both *in vitro* in three different cell types (PK-84, HK-2 and PTECs) and *in vivo* at two implantation sites (by subcutaneous and subcapsular renal injection) in rats. Both monodisperse (narrow size distribution) and polydisperse PLHMGA-ms were prepared with volume weight mean diameter of 34 and 17 μm, respectively. Mono and polydisperse PLHMGA-ms showed good cytocompatibility properties upon 72 h incubation with the cells (100 μg microspheres/600 μL/cell line). A mild foreign body reaction was seen shortly after subcutaneous injection (20 mg per pocket) of both mono and polydisperse PLHMGA-ms with the presence of mainly macrophages, few foreign body giant cells and myofibroblasts. This transient inflammatory reaction diminished within 28 days after injection, the time-point at which the microspheres were degraded. The degradation profile is comparable to the *in vitro* degradation time of the microspheres (*i.e.*, within 35 days) when incubated at 37 °C in phosphate buffered saline. Subcapsular renal injection of monodisperse PLHMGA-ms (10 mg) in rats was characterized with similar inflammatory patterns compared to the subcutaneous injection. No cortical damage was observed in the injected kidneys. In conclusion, this study demonstrates that PLHMGA-ms are well tolerated after *in vivo* injection in rats. This makes them a good candidate for controlled delivery systems of low-molecular weight drugs as well as protein biopharmaceuticals.

1. Introduction

Poly(D,L-lactic-*co*-glycolic acid) (PLGA) is a biodegradable aliphatic polyester that has been investigated for controlled delivery of low molecular weight drugs (Kim et al., 2011), peptides (Shmueli et al., 2013; Xuan et al., 2013), proteins (Menon et al., 2014; Reguera-Nuñez et al., 2014; Wink et al., 2014) and vaccine antigens (Huang et al., 2014; Joshi et al., 2013). PLGA is degraded by hydrolytic cleavage of ester bonds that connect the monomer units, and the final degradation products are lactic and glycolic acid, both endogenous compounds (Spenlehauer et al., 1989; Vert et al., 1994). An important drawback of PLGA matrices, however, is the formation of acidic degradation products which are detrimental for the stability and integrity of entrapped (therapeutic) proteins (Estey et al., 2006; Park et al., 1995). Denaturation of the formulated protein or structural modifications due to acid-catalyzed reactions will affect both therapeutic efficacy and can cause potential immunological responses to the formulated protein (Hermeling et al., 2004; Patten and Schellekens, 2003).

A novel copolymer, poly(D,L-lactic-*co*-hydroxymethyl glycolic acid) (PLHMGA) (Leemhuis et al., 2006) has a similar molecular

Abbreviations: α-SMA, alfa-smooth muscle actin; BMMG, 3*S*-(benzyloxymethyl)-6*S*-methyl-1,4-dioxane-2,5-dione; DCM, dichloromethane; ED-1, mouse anti rat CD68 monoclonal antibody; FBGCs, foreign body giant cells; HK-2, human proximal tubular cells; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; PBS, phosphate buffered saline; PK-84, human skin fibroblasts; PLGA, poly(D,L-lactic-*co*-glycolic acid); PLBMGA, poly(D,L-lactic-*ran*-benzyloxymethyl glycolic acid); PLHMGA, poly(D,L-lactic-*co*-hydroxymethyl glycolic acid); PLHMGA-ms, poly(D,L-lactic-*co*-hydroxymethyl glycolic acid) microspheres; PTECs, human primary tubular epithelial cells; PVA, polyvinyl alcohol; SEM, scanning electron microscope; T_g, glass transition temperature.

* Corresponding author. Tel.: +31 6 20275995.
E-mail address: r.j.kok@uu.nl (R.J. Kok).

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structure as PLGA with additional pendant hydroxyl groups on the polymer backbone (Fig. 1). The degradation of this co-polymer and the release of entrapped proteins can be tailored by its copolymer composition (Ghassemi et al., 2010; Leemhuis et al., 2007; Samadi et al., 2013b). Furthermore, PLHMGA based microspheres are peptide and protein friendly (Ghassemi et al., 2010, 2012; Samadi et al., 2013b). Owing to the more hydrophilic nature of PLHMGA compared to PLGA, it has been demonstrated that the water-soluble acidic degradation products of PLHMGA are rapidly released from degrading microspheres into the external medium (Liu et al., 2012). As PLHMGA is intended for use of delivering drugs *in vivo*, characterization of the *in vivo* biodegradation as well as biocompatibility properties of these copolymeric microspheres is required.

The aim of this study is to evaluate the *in vitro* cytotoxicity and *in vivo* biocompatibility of PLHMGA microspheres (PLHMGA-ms). These tests are mandatory according to the International Organization for Standardization (ISO) guidelines for biological evaluation of implantable medical devices (ISO Guidelines April 23, 2013). PLHMGA-ms were prepared with two different methods, a conventional single emulsion solvent evaporation method for preparation of polydisperse microspheres and by membrane emulsification method for generating uniform size microspheres. Previously, we have shown that microspheres prepared by this method of emulsification have high batch-to-batch reproducibility in terms of particle characteristics and release kinetics (Kazazi-Hyseni et al., 2014). Moreover, due to the uniform size, monodisperse microspheres also have better injectability and hence allows the use of smaller needles for the administration of microsphere suspensions. This is of special attention in the present study, in which we investigated the feasibility of injecting PLHMGA microspheres under the renal capsule. Subcapsular renal injection is a relatively new method for local delivery of therapeutics to the kidneys which was earlier tested for the injection of hydrogels (Dankers et al., 2012). We created a small pocket between the capsule and the soft cortex tissue with a small blunt needle and used the same needle to inject a concentrated dispersion of the microspheres, to study their biocompatibility at this injection site. In addition, we studied the biocompatibility of PLHMGA microspheres after subcutaneous injection.

The *in vitro* cytocompatibility was assessed in three different cell types, namely dermal fibroblasts (PK-84), proximal tubular epithelial cells (HK-2) and primary tubular epithelial cells (PTECs). For the *in vivo* biocompatibility assessment, both monodisperse and polydisperse PLHMGA-ms were injected subcutaneously in rats. The inflammatory response was studied along with the influence of particle size and polydispersity on the foreign body reaction. Furthermore, the degradation profile of PLHMGA-ms was studied *in vitro* and correlated to the *in vivo* degradation as observed in histopathology tissue samples.

2. Materials and methods

2.1. Materials

O-benzyl-L-serine was purchased from Senn Chemicals AG (Dielsdorf, Switzerland). Tin(II) 2-ethylhexanoate ($SnOct_2$), poly (vinyl alcohol) (PVA; M_w = 13,000–23,000 g/mol), palladium 10 wt % (dry basis) on activated carbon, hematoxylin solution and dimethylsulfoxide (DMSO) were obtained from Sigma–Aldrich (Germany). 1,4-Butanediol, 99+% was obtained from Acros Organics (Belgium). Carboxymethylcellulose (CMC, with viscosity of 2000 mPa s of a 1% solution in water) was obtained from Bufa B. V. (255611, The Netherlands). Sodium phosphate dibasic (Na_2HPO_4) and sodium azide (NaN_3) were purchased from Fluka (The Netherlands). Dichloromethane (DCM) and tetrahydrofurane were purchased from Biosolve BV (The Netherlands). Sodium dihydrogen phosphate (NaH_2PO_4), sodium hydroxide (NaOH) and sodium chloride (NaCl) were supplied from Merck (Germany). Mouse anti rat CD68 monoclonal antibody (clone ED-1) was obtained from AbD Serotec (MCA341R, Germany). Monoclonal mouse anti-human Actin (α-SMA) was obtained from Dako (Clone 1A4, Denmark).

2.2. Polymer synthesis and characterization

Poly(D,L-lactic-*co*-hydroxymethyl glycolic acid) (PLHMGA) was synthesized as previously described (Leemhuis et al., 2006), using butanediol as an initiator, to obtain a hydroxyl terminated co-polymer. In brief, BMMG (3*S*-(benzyloxymethyl)-6*S*-methyl-

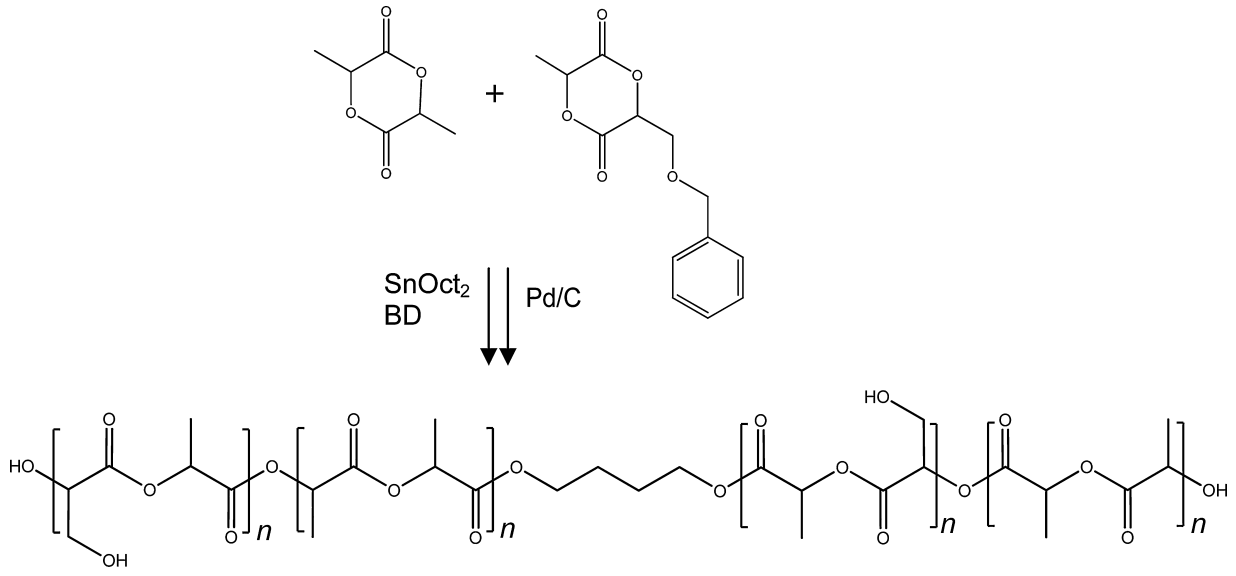

Fig. 1. Synthesis of poly(lactic-*co*-hydroxymethyl glycolic acid) (PLHMGA) from D,L-lactide and 3*S*-(benzyloxymethyl)-6*S*-methyl-1,4-dioxane-2,5-dione (BMMG) by melt copolymerization with $SnOct_2$ as catalyst and 1,4-butanediol (BD) as initiator. The protective benzyl groups were removed by hydrogenation using palladium on activated carbon (Pd/C) as a catalyst.