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Poly((*R*,*S*)-3,3-dimethylmalic acid) derivatives as a promising cardiovascular metallic stent coating: Biodegradation and biocompatibility of the hydrolysis products in human endothelial cells

R. Belibel [a], N. Marinval [b], H. Hlawaty [b], C. Barbaud [a, *]

[a] INSERM U1148, Laboratory for Vascular Translational Science (LVTS), Institut Galilée, Université Paris 13, Sorbonne Paris Cité, 99 Avenue Jean-Baptiste Clément, Villetaneuse, F-93430, France
[b] Inserm U1148, Laboratory for Vascular Translational Science, UFR SMBH, Université Paris 13, Sorbonne Paris Cité, Groupe Biothérapies et Glycoconjugués, Bobigny, F-93430, France

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ABSTRACT

In-stent restenosis is currently treated with drug eluting stents based on biodegradable polymers which can deliver a therapeutic agent and be degraded in a few months preventing the risk of thrombosis. Poly([*R*,*S*]-3,3-dimethylmalic acid) (PDMMLA) is a new and original biodegradable and biocompatible polymer which contains a carboxylic acid functional group in its side chain. This gives it the particularity to be chemically modified and custom-synthesized to meet an adequate degradation time. It was prepared in order to develop new coating exhibiting different groups in its side chain and give natural and non-toxic primary products after a complete degradation. Herein we present the study of hydrolytic degradation of PDMMLAs under physiological conditions for a 6-month period. The most important factors that influence the kinetic degradation of polymers (molecular weight, nature and stability of functional groups, natural biological enzymes, pH and temperature) were studied in order to understand the behavior of PDMMLAs hydrolysis. It has been shown that the different PDMMLA polymers were degraded according to a bulk or erosion-surface profiles. Therefore, a hydrophilic loaded side chain, high temperature, high pH and the presence of specific enzyme accelerated the degradation rate of PDMMLAs with an erosion-surface profile. Since these new biomaterials as promising coating-stent will be in direct contact with the arterial wall, their biocompatibility was evaluated in this study in human vascular endothelial cells which are essential for the repair of the arterial wall to inhibit multiple processes leading to in-stent restenosis. The products of long-term degradation of PDMMLA polymers were non-cytotoxic.

1. Introduction

In the last decade, synthetic biodegradable polyesters were widely used in the biomedical field [1–4]. They can be tailored by chemical modification to meet the requirements of some applications unlike natural ones. Indeed, the chemical modification of biomaterial adjusts on the one hand, the mechanical and biological properties of biomaterial and, on the other hand, the grafting of drug and the control of polymer kinetic degradation and drug-release. Among the family of synthetic and biodegradable polymers, we are interested in side-chain-functionalized polymers, especially poly(malic acid) (PMLA) [5] and [6]. Therefore, it is the most frequently used synthetic polyester which can be chemically modified through to the presence of acidic function in its side chain. In addition, it is known for its water-solubility, good biocompatibility, non-cytotoxicity, non-immunogenic properties, stability in the bloodstream and human cells affinity [5,7–12]. A variety of PMLA derivatives were prepared through the synthesis of a large family of monomers (malolactonates) or by copolymerization with other biodegradable polyesters such as poly(L-lactic acid) (PLA), poly(glycolic acid) (PGA) and poly(ε-caprolactone) (PCL). These copolymers have been mainly used in tissue engineering, as drug delivery systems and as vascular prostheses [13].

Currently, biodegradable polyesters-coated stents with these polyesters are the essential cardiovascular implants used in

* Corresponding author.
E-mail address: barbaud@univ-paris13.fr (C. Barbaud).

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modern medicine to treat the intra-stent restenosis. This pathology is the result of endothelium damage and the proliferation and migration of vascular wall cells, smooth muscle cells (SMCs), after stent implantation. This is also associated with thrombotic risk. Therefore, polymer-coating should initially favor the re-endothelialization, minimize SEM cells migration and avoid the late thrombosis [14] and [15]. For this reason, synthetic and functional biodegradable polyesters before mentioned are the promising family which displays high relevance to satisfy the requirements of this cardiovascular application. In contrast, they have not proven themselves. This is mainly associated to their hydrophobicity and their slow degradation rate. Indeed, PLA which is the most used synthetic polymer as a cardiovascular implant, it displays a limited cell response and poor interaction with body tissue and fluids, poor hydrophilicity, slow degradation and poor ductility (higher glass temperature) which requires its copolymerization to improve its properties (PLGA [16] and [17], PLMA [10,11,18–20]).

The development of the appropriate biomaterial which has good mechanical properties, accelerates re-endothelialization, delivers a drug and degrades completely is the challenge of scientific and medical research in the last decade. To this end, poly([*R*,*S*]-3,3-dimethylmalic acid) (PDMMLA) derivatives which are part of the PMLA family will be prepared for an eventual use as stent-coating. They can be custom-synthesized to meet an adequate degradation time. In addition, its structural design was chosen to give natural and non-toxic primary products after a complete degradation. The hydrolysis of amorphous PDMMLAs gave the corresponding [*R*,*S*]-3,3-dimethylmalic acid (diacid). Indeed, the final product of hydrolytic degradation of chiral PDMMLA ((*R*)-3,3-dimethylmalic acid) is a natural and non-toxic product that enters the biosynthetic pathway of pantothenate. This metabolite present in the synthesis of Coenzyme A gives ketovaline by enzymatic oxidative reaction catalyzed by β,β-dimethyldehydrogenase (EC.1.1.1.84) [21] and [22]. (Fig. 1). Therefore, it was noted that these polymers are bioassimilable.

Functional groups in the side-chain of PDMMLA systems are the alcohol group (−OH), carboxylic acid group (−H) and hexylic group (−He), incorporated during the polymer synthesis. The degree of these groups may be adapted to modify hydrophilic/hydrophobic balance and thus changes the degradation rate, solubility and mechanical and biological properties. First, −OH and −COOH groups provide the neutral and the acid hydrophilic character, respectively. They allow also the chemical modification of polymer. Moreover, −OH functions were often used for its opsonization phenomenon, and their excellent blood compatibility and resistance to thrombus formation [23] and carboxylic groups for its favor cell attachment and proliferation. Then, the hexyl group provides hydrophobic character for PDMMLA polymers.

If the systems studied in this work are used as a stent-coating, they will be in contact with the physiological medium. The study of their degradation *in vivo* is a very important point to understand their kinetic of degradation and the controlled release of drugs once grafted. We report in this work their degradation *in vitro* to screen the candidate polyesters. On the one hand, *in vitro* degradation of a series of PDMMLA homopolymers and statistical copolymers with different hydrophobic/hydrophilic groups in their side-chain was performed in phosphate buffer under physiological conditions. The effect of pH, temperature, specific enzymes and molecular weight of polymers on the degradation rate was evaluated. These materials will be in direct contact with the artery wall containing endothelial cells that repair and regenerate the treated artery wall and thus accelerate reendothelialization. On the second hand, the biocompatibility of degradation products was investigated on human vascular endothelial cells (HUVEC) using two methods: MTT assay and Live/Dead cytotoxicity assay kit. The objective of the present investigation was therefore to understand the degradation way and time of different polymers and finally select the appropriate and best candidate biomaterial.

2. Material and methods

2.1. Polymer synthesis and characterizations

Amorphous PLA (M_n = 20 000 g/mol) was purchased from Sigma Aldrich (France). Anhydrous tetrahydrofuran (THF) was distilled on sodium-benzophenone. In all other cases, the commercially available chemicals were purchased from Sigma Aldrich (France) and employed as received. All reactions, with anhydrous organic solvents were performed under nitrogen atmosphere. Synthetic and amorphous PDMMLAs were prepared in anhydrous THF solution by ROP of racemic β-lactones monomers using the previously reported procedure using tetraethylammonium benzoate as initiator [24] and [25]. The monomers were synthesized according to the literature procedure as well with different functional groups to bring the hydrophilic (acidic or neutral) and hydrophobic characters for polymers. In this study, 3 homopolymers and 3 statistical copolymers having different functional groups in their side chains were prepared. The synthetic homopolymers have different characters: acid hydrophilic (PDMMLA−H), neutral hydrophilic (PDMMLA−OH (HP−OH)) and hydrophobic (PDMMLA−He). At the same time, the copolymers were synthesized with different acidic hydrophilic/hydrophobic percentages: PDMMLAH10-*co*-He90, PDMMLAH20-*co*-He80 and PDMMLAH30-*co*-He70. The polymers with 0, 10, 20, 30 and 100% of acidic groups were named as 0/100, 10/90, 20/80, 30/70 and 100/0,

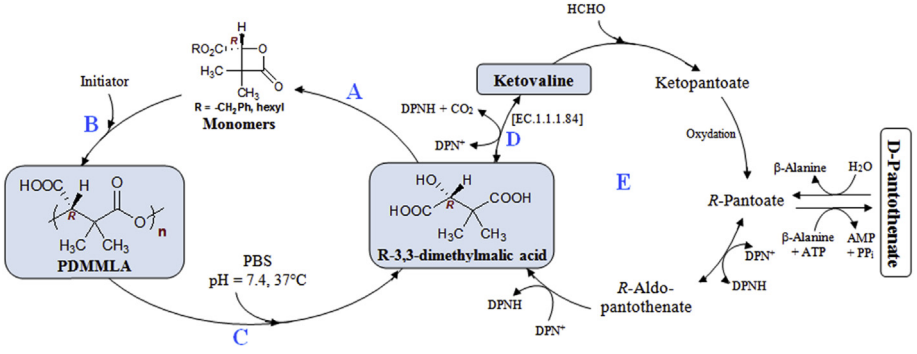

Fig. 1. (A) Synthesis of monomers. (B) Synthesis of PDMMLAs with Ring Opening Polymerization (ROP). (C) PDMMLAs hydrolytic degradation to *R*-3,3-dimethylmalic acid. (D) Enzymatic oxidative reaction catalyzed by "EC.1.1.1.84"of *R*-3,3-dimethylmalic acid to give ketovaline. (E) Degradation cycle of pantothenate.