HARNESSING BIOPOLYESTERS IN THE DESIGN OF FUNCTIONAL MATERIALS FOR BIOMEDICAL APPLICATIONS

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Abstract

The present contribution illustrates the versatility of poly(3-hydroxyalkanoate)s (PHAs) in the design of a wide variety of biodegradable and/or biocompatible macromolecular architectures with controlled degradability. Firstly, functionalized PHAs were prepared from unsaturated PHAs. Pendant double bonds have been turned into carboxyl, hydroxyl, alkyne or epoxy groups. These reactive functions were used for further grafting hydrolyzable polylactide (PLA) or poly(ε-caprolactone) (PCL) as well as hydrophilic poly(ethylene glycol) (PEG). Additionally, block copolymers with a PLA, PCL or PEG segment have been prepared by ring-opening polymerization or "click" chemistry from a PHA oligomeric macroinitiator. Functional PHAs represent biodegradable aliphatic polyesters with many possibilities to tune physico-chemical characteristics, such as hydrophilicity and degradation rate, thus making the resulting materials suitable as devices for drug delivery or as scaffolds for tissue engineering. Herein, we address the recent trends in the synthesis of these polymeric materials and their applications in controlled drug delivery and tissue engineering.

Keywords: *poly(3-hydroxyalkanoate)s, block and graft copolymers, drug delivery, tissue engineering*

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Introduction

Poly(3-hydroxyalkanoate)s (PHAs) represent a class of natural aliphatic polyesters accumulated by many bacteria as intracellular energy and carbon storage materials when they are subjected to stress conditions [1,2]. They constitute an enlarged family of bacterial polyesters that can be considered as promising biopolymers for biomedical applications, due to their biodegradability and biocompatibility. Using various substrates, a wide variety of PHAs can be synthesized, differing notably by the length of their side chains [3]. Two main types are distinguished, i.e one type with short-chain length (scl-PHA) that possesses alkyl side chains up to two carbon atoms, and a second type with medium-chain length (mcl-PHAs) that displays between three and eight carbon atoms on its side chains (FIG. 1). The length of side chains strongly affects the physical properties of PHAs.

In this contribution, the versatility of PHAs in the design of a wide variety of biodegradable and/or biocompatible macromolecular architectures will be illustrated. First, unsaturated PHAs were chemically modified via the transformation of pendant double bonds into epoxy, carboxyl, hydroxyl or alkyne groups. Moreover, these reactive functions could further be used for grafting hydrolyzable polylactide (PLA) or poly(ε-caprolactone) (PCL) as well as hydrophilic poly(ethylene glycol) (PEG) through two distinct mechanisms, namely either a "grafting from" method or a "grafting onto" approach (direct esterification or "click" chemistry). PHA-b-PEG block copolymers were also synthesized by "click" chemistry, while totally degradable block copolyesters were generated by ring-opening polymerization of D,L-lactide or ε-caprolactone applying either conventional thermal heating or microwave dielectric activation. Finally, mucoadhesive degradable nanoparticles having a great potential for drug delivery applications were prepared from block copolymers. Such copolymers were also tested as biomaterials for tissue engineering, e.g. as degradable coatings in drug eluting stents.

Materials and Methods

PHB (M_w=330 000 g·mol^{-1}), PHBHV (14 mol.% HV, M_w=240 000 g·mol^{-1}), PHBHHx (9 mol.% HHx, M_w = 330 000 g·mol^{-1}) were respectively purchased from Biomer, Goodfellow, and Procter & Gamble. PHOU samples were obtained from EMPA, Swiss Fed Labs Mat Testing & Res, Lab Biomat, CH-9014 St Gallen, Switzerland.

Synthesis of PHA oligomers

PHA oligomers with a terminal carboxyl group (PHB, PHBHHx, PHOHHx) were prepared by thermal degradation at 190°C for a determined time. Oligomers were purified by precipitation in ethanol. PHA oligomers with a terminal hydroxyl group were prepared by methanolysis as previously described [4].

Functionalization of PHAs and oligomers

Native PHOU samples were oxidized with $KMnO_4$ to introduce terminal carboxylic acid functions in side chains (PHOD-COOH), following a previously reported procedure [5].

The COOH groups in side chains or at the terminal position were also esterified with propargyl alcohol in the presence of EDC hydrochloride as a coupling agent [6,7].

Synthesis of graft copolymers

Different strategies were used to build PHA-g-PCL, PHA-g-PLA, and PHA-g-PEG graft copolymers. PHA-g-PCL were synthesized according to a "grafting from" method described in a previous paper by ROP of ε-caprolactone [8]. PHA-g-PLA were synthesized by direct esterification as previously reported [5,9]. PHA-g-PEG were prepared by "click" chemistry using the copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) [6].

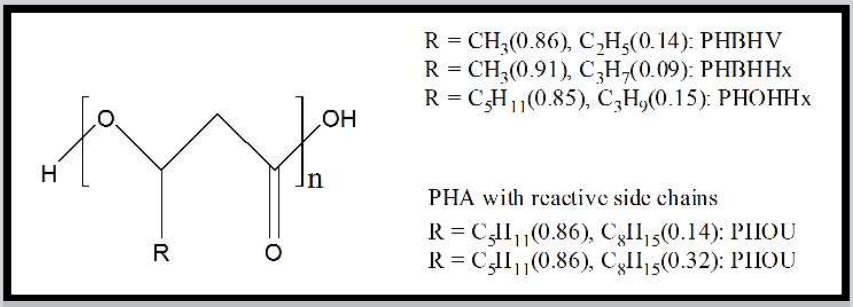

FIG. 1. Chemical structures of PHAs under investigation.

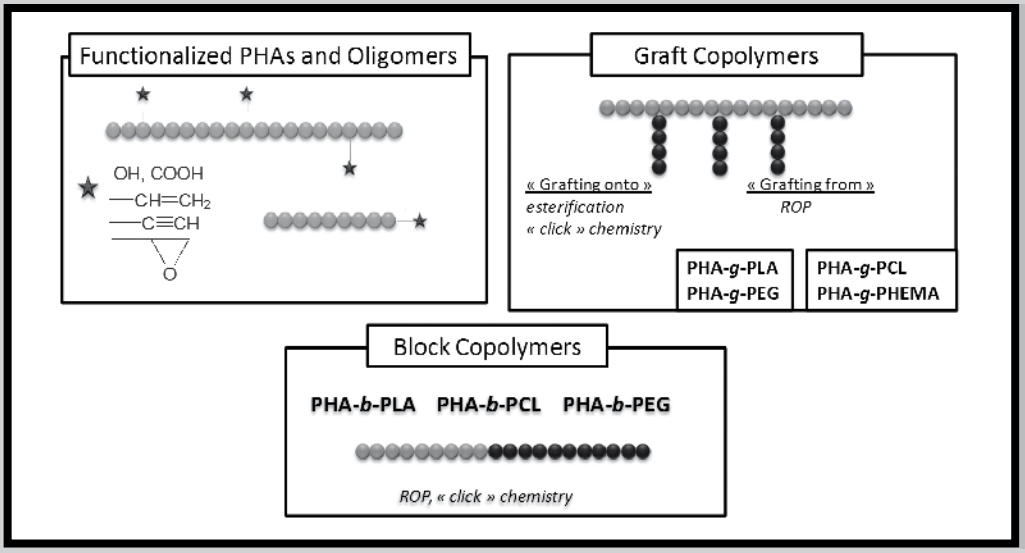

FIG. 2. Well-defined macromolecular architectures based on PHAs.

Synthesis of block copolymers

PHA-b-PEG diblock copolymers with controlled size and easy purification were prepared using the copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) [7]. PHA-b-PLA and PHA-b-PCL copolymers were prepared by ROP of D,L-lactide or ε-caprolactone, respectively [4].

The chemical structures and the physico-chemical features of the copolymers were investigated by NMR, SEC, DSC, and TGA analyses.

Cell adhesion

1mL of human bladder carcinoma RT112 cells suspension containing 300000 cells and RPMI culture medium supplemented with 10% fetal calf serum, 0.05% streptomycin, and 0.05% penicillin was put on each well. Then the plates were placed at 37°C. The medium was changed every day. At different times, adhesive RT112 cells were counted by a colorimetric MTT. Briefly, a solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) 1mg·mL^{-1} was added in each well containing cells fixed on polymer after removing medium of culture with non-attached cells. Plates were placed at 37°C for 1 h. The intensity of the suspension was proportional to the quantity of living cells. By adding isopropanol, cells burst and released the colored solution whose absorption was measured by UV spectrophotometry. Cell adhesion was observed at 4 h and cell growth for 3 days. Experiments were repeated three times.

Nanoparticle preparation

Sub-micrometer particles were prepared by a solvent displacement/evaporation method. A copolymer (75 mg) was dissolved in 15 mL of acetone at 50°C under stirring. The solution was cooled down to room temperature. In the case of doxorubicin-loaded particles, 1.2 mL of a solution of doxorubicin (1 mg/6 mL ethanol) was added. The mixture was added dropwise in 45 mL of an aqueous solution of Pluronic F-68 (1% w/v) under stirring. The solution was stirred at room temperature in a flux of air during 4 h to completely remove the acetone.

Stent coating

A chloroform solution containing 0.5 wt.% of polymer and sirolimus (purity 99.9%, Biocon), in proportion 80/20 wt.% respectively, was sprayed on a stent. Drug Eluting Stents (DES) were dried at room temperature for 12 h. In the case of bilayered coatings, a solution of PHBHV 0.5 wt% in chloroform was sprayed in a second step, and the solvent was evaporated. The total weight of coating was about 600 µg for monolayered coatings and 900 µg for bilayered coatings, with a content of 120 µg ± 10 µg sirolimus per stent.

Results and Discussions

Block and graft copolymers

Compared to other degradable polyesters, poly(3-hydroxyoctanoate-co-3-hydroxyundecenoate) (PHOU) has the major advantage to be alkene-functionalized on its side chains, allowing for post-modifications. By taking advantage of the reactivity of pendant double bonds, novel functionalized PHAs were successfully prepared via the chemical transformation of (C=C) terminal unsaturations into carboxyl [5], alkyne [6], hydroxyl [8] or epoxy [10] groups (FIG. 2).

Moreover, these functional side groups can be used to further conjugate bioactive or targeting molecules, as well as reactive oligomers. Therefore, biocompatible oligomers based on hydrolyzable PLA or PCL as well as hydrophilic PEG were grafted to PHO through two distinct mechanisms, namely either a “grafting from” method [8] (PCL grafts) or a “grafting onto” approach via direct esterification [5,9] or “click” chemistry (PLA or PEG grafts). The latter mechanism turned out to be more efficient in the synthesis of PHO-g-PEG graft copolymers. Such amphiphilic architectures could yield stable nanoparticles in aqueous media. Furthermore, totally biodegradable PHA-based block copolymers were synthesized by ring-opening polymerization of D,L-lactide or ε-caprolactone initiated by hydroxy-terminated PHA oligomeric macroinitiators [4]. The latter macroinitiators were previously prepared by methanolysis of native PHAs. Alternatively, “click” chemistry was implemented to generate well-defined amphiphilic block copolymers based on PHA and PEG [7].

Biomedical applications

Tissue Engineering

Attachment and growth of human bladder carcinoma RT112 cells were investigated in vitro on biopolyesters films in a view to use them as scaffolds in tissue engineering [11]. The effect of the chemical structure of different bacterial polyesters on cell adhesion and proliferation was studied. Measurements of cell adhesion were carried out in the presence of collagen IV or fetal calf serum. The best results for cell attachment were obtained with PHOD-COOH, whatever be the experimental conditions (FIG. 3). The hydrophobic surface of PHO films also induced a good adhesion density of RT 112 cells. PHOD-g-PEG had a contrasted behavior, due to the presence of PEG grafts. Proliferation of human bladder carcinoma RT112 cells was observed on the same polymers. PHOD-COOH did not improve the cell proliferation and did not seem to be a favorable support. This preliminary study showed that PHO has a good potential to induce regeneration of a functional bladder wall.

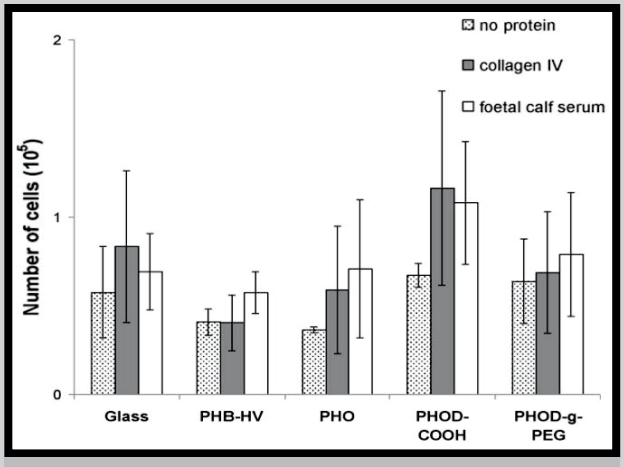

FIG. 3. Cell adhesion on PHA-based films after 4 h of incubation at 37°C.

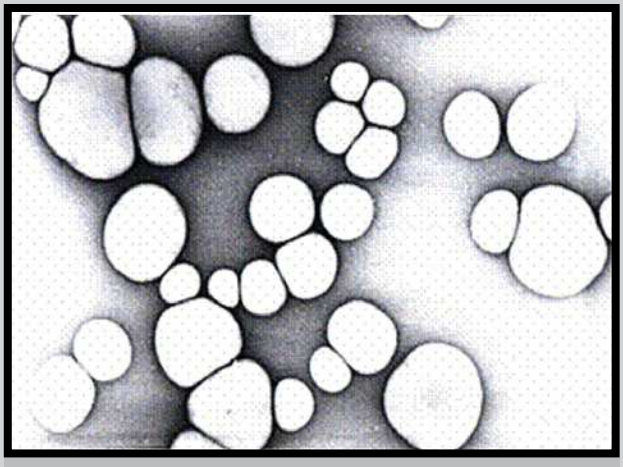

FIG. 4. Sub-micrometer particles of PHO-b-PCL (x 20 000).

Drug delivery systems

Particles based on PHO, PHOD-COOH, PHO-b-PCL and PHODCOOH-b-PCL were prepared using a solvent displacement/evaporation method (FIG. 4). The encapsulation efficiency of doxorubicin was larger than 50% due to the hydrophobicity of both doxorubicin and polyesters. The in vitro release behavior of doxorubicin-loaded particles at pH=6 (bladder pH) was investigated [12]. A typical two-phase release profile was observed (case of matricial type particles). These results were consistent with the method of particle preparation: doxorubicin was co-precipitated with the polymer. The burst effect was inferior to 10%. Interestingly, these nanoparticles were also mucodhesive (FIG. 5).

The association of a hydrophilic segment in conjunction with a hydrophobic block is a classical way to increase the stability of colloidal particles. In this investigation, PEG was used as a model molecule for the hydrophilic part in amphiphilic copolymers. PHBHV-PEG copolymers were unstable colloidal suspensions, whereas PHO-based copolymers resulted in very stable colloidal suspensions. They form stable micelles in aqueous media, with low critical micelle concentrations (FIG. 6). They can be envisioned for biomedical applications as drug delivery systems able to transport bioactive hydrophobic molecules.

Drug eluting stents are of great interest in the field of interventional cardiology by promising a long-term prevention of restenosis. An adequate drug release control, mechanical response to stent expansion, and degradability of the coating are of major importance. The present approach described the potential use of PHAs as biodegradable and compatible coatings. Rates of drug release from different monolayers of PHAs were very fast, and the maximum period of drug release observed was clearly too short to be of practical use (burst effect). The development of a new PHBHV-b-PLA copolymer as a coating enhanced the drug release profile by limiting the release of sirolimus. The extension of the drug release period is very promising by resorting to the bilayared system composed of PBHV-b-PLA containing the drug and PHBHV without drug (FIG. 7). Moreover, this bilayered coating had good mechanical properties in terms of flexibility and adherence to the metallic stent. Further studies are necessary to confirm their performances under in vivo conditions.

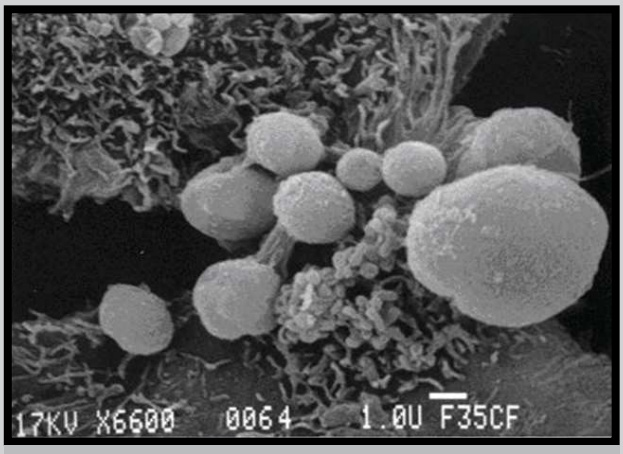

FIG. 5. SEM image showing mucoadhesivity of PHO-b-PCL particles containing 24% of PHO (x 6000).

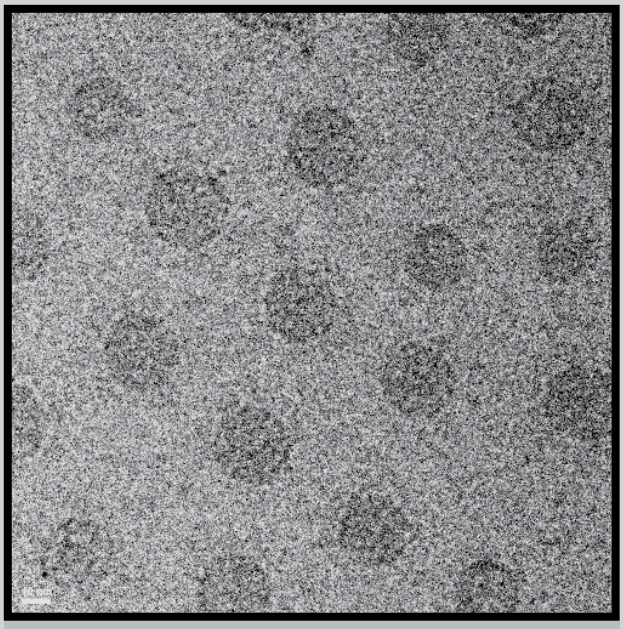

FIG. 6. Cryo-TEM of micelles formed in water by PEG-b-PHO copolymers (scale bar is 10 nm).

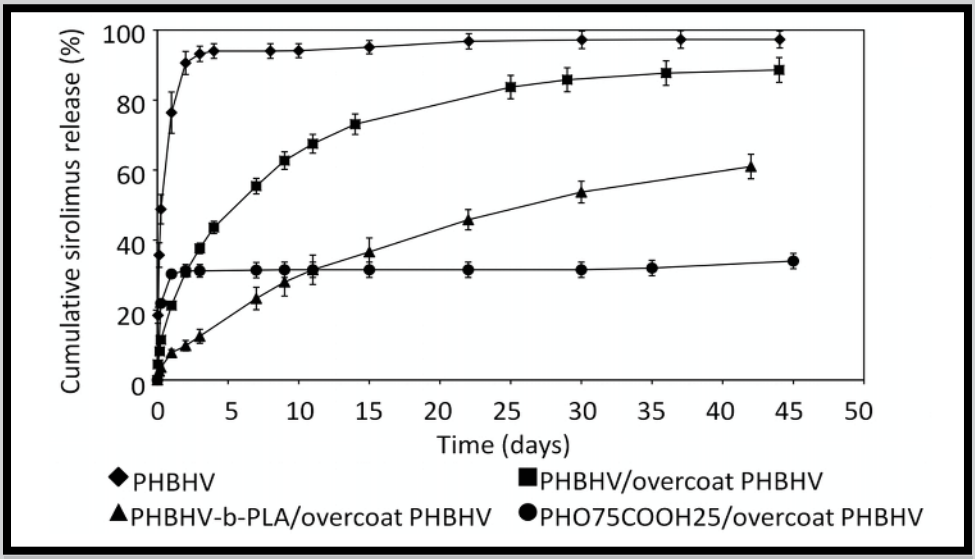

FIG. 7. Sirolimus release from different copolymer systems.

Conclusion

The potential of functional PHAs and relative copolymers in the drug release area and tissue engineering has been illustrated through the investigation of cell adhesion on functionalized PHA films, doxorubicin release from stable nanoparticles, performance of drug eluting stents, and micelle formation. These studies have shown the potentialities of introducing functional groups or segments either on side positions or on a terminal position of PHAs to fine-tune their physico-chemical properties, so as to reach specific applications.

The PHA-based frameworks can be adapted to other biomedical applications through tailor-making topology, composition, and functionality of macromolecular architectures to fit prerequisites corresponding to a specific device. The versatility of PHAs and their derivatives is a major motivation for developing new polyester-based frameworks and scaffolds.

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