Role of Carboxyl Pendant Groups of Medium Chain Length Poly(3-hydroxyalkanoate)s in Biomedical Temporary Applications

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ABSTRACT: The poly(3-hydroxyoctanoate) (PHO) is a biodegradable polyester containing hydrophobic side chains. One way to obtain more hydrophilic polyester consisted in the introduction of polar groups in the side chains. Carboxyl groups ($PHO_{75}COOH_{25}$) were introduced by chemical modifications. The role of carboxyl groups was investigated in the first part as potential support for cell seeding by studying the cell adhesion and proliferation, and in the second part as potential drug carrier by comparing the abilities of PHO and $PHO_{75}COOH_{25}$ to form degradable particles. Measurements of human bladder RT112 cells adhesion were done with or without collagen IV. Adhesive RT112 cells were counted by a colorimetric MTT test. The results showed that the COOH pendant groups of $PHO_{75}COOH_{25}$ films promoted cell adhesion after 4 h of incubation. The proliferation of cells is not improved after 4 days of incubation because of a reorganization of macromolecular chains and reorientation of COOH groups. This surface restructuration when the film was in contact with water was showed by contact angle measurements. We showed that the presence of COOH groups modified the hydrophobic/hydrophilic balance and enhanced the formation of particles. Stable lyophilisable particles were then obtained with diblock copolymer P($HO_{75}COOH_{25}$-*b*-CL); the caprolactone block (CL) was necessary to improve particles stability. The results showed that the release of doxorubicin from the particles is enhanced in presence of hydrophilic and degradable block ($PHO_{75}COOH_{25}$). It was possible to obtain a degradable functional polyester based on PHO with carboxyl pendant groups to improve degradation rate by simple hydrolysis required for drug delivery systems. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 1888–1896, 2010

Key words: mcl-poly(3-hydroxyalkanoates); PHO; PHO-COOH; bacterial polyester; cell adhesion; hydrolytic degradation

INTRODUCTION

Poly(3-hydroxyalkanoate)s are polyesters produced by microorganisms under unbalanced growth conditions.[1] Many PHAs monomer structures have been reported.[2] PHAs are classified into two types: short chain length, scl-PHAs ([R]-hydroxyalkanoates of C_3—C_5), and medium chain length mcl-PHAs ([R]-hydroxyalkanoates of C_6—C_{14}) (Scheme 1). They are generally biodegradable, with good biocompatibility, making them attractive for biomedical devices. In fact, they have been considered as polymers with a wide range of potential applications, such as scaffold in tissue engineering,[3,4] for long term release of bioactive molecules,[5–7] or for implants materials.[8–10] The choice of poly(3-hydroxyoctanoate) noted PHO, was based in this study on its physical properties; this bacterial polyester is a thermoplastic elastomer. The elastomeric materials are currently thought to be well suited to tissue engineering applications of cardiovascular tissue. In these types of applications, the tissue scaffolds, particularly in the form of tubes or films, must have good flexibility and must be able to resist to the mechanical forces exerted either from the surrounding tissue or from the body fluids contained within.[11,4] Sodian et al.[12] have constructed a heart valve scaffold from porous PHO. Moreover, PHO is biocompatible to 3T3 fibroblasts human bone marrow cells (HBMCs).[13] But the PHO hydrolysis in aqueous solution is a very slow process.[14–16] To enhance hydrophilicity and degradability which are very important properties in the case of temporary biomedical applications, the creation of tailor-made products has been carried out by chemical modification of functional substituents. Thus, unsaturated groups of poly(3-hydroxyoctanoate-co-3-hydroxyundecenoate) $PHO_{75}U_{25}$ have been turned in carboxyl groups leading to a functionalized polyester noted $PHO_{75}COOH_{25}$. This chemical modification was previously described.[17–19]

The purpose of these research was to investigate the role of the carboxyl groups on the ability for the $PHO_{75}COOH_{25}$ to be used in different biomedical

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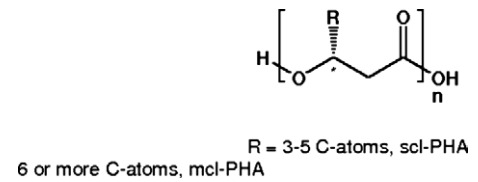


Scheme 1 General formula of poly(3-hydroxyalkanoate)s.

applications as drug delivery systems or/and as scaffold in tissue engineering. To promote tissue formation, the biomaterial should provide a highly biocompatible substrate to enable cell adhesion, migration, proliferation, and differentiation.[20,21] Here, we presented preliminary results concerning interaction between human bladder carcinoma RT112 cells and $PHO_{75}COOH_{25}$ films. In the first stage, it was necessary to consider the *in vitro* ability of RT112 cells to adhere and proliferate on $PHO_{75}COOH_{25}$ films. Then, the ability to use these new polymers in drug delivery systems has been studied. To improve the stability of the particles after lyophilization, block copolymers were prepared from $PHO_{75}COOH_{25}$ and ring opening polymerization of caprolactone. Copolymers have been prepared[22] for the encapsulation and release of doxorubicin used in the field of cancer chemotherapy. We report the feasibility of submicrometer particles tailor-made from diblock copolyesters with a well defined macromolecular architecture. The particles containing doxorubicin have been prepared and studied, in regard to the bioactive molecule release.

MATERIALS AND METHODS

Materials

Poly(3-hydroxyoctanoate), PHO, and poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate), $PHO_{75}U_{25}$, were produced using *Pseudomonas sp* GPo1 (CNRS, Cermav, Grenoble, France) as described in a previous report.[23] $PHO_{75}U_{25}$ (PHOU) was oxidized according to the method described by Renard et al.[19] The obtained product, named PHO_{75}-$COOH_{25}$, was then recovered by evaporation of the water by freeze drying. Diblock copolymers P(HO-*b*-CL) and P((HO_{75}-$COOH_{25}$)-*b*-CL) were synthesized according to the method described in a previous paper.[22] Doxorubicin hydrochloride (for fluorescence, purity >98%) were purchased from Fluka and used without further purification.

Cell culture

The films aimed at the cell culture were prepared by the solvent cast method. The different polymers were dissolved in chloroform and poured onto glass microscope slides. Films were dried overnight at room pressure and then dried in vacuum to remove the residual solvent. Some of the films were used without further modification. The others were pretreated by depositing proteins: collagen IV. Films of the different polymers were placed in a 12-wells plate. A total of 1 mL of the solution of collagen IV with a concentration of 12.5 μg mL^{-1} was deposited on the film surface and placed at 37°C for 1 h. Microscope glass slides were used as blank. A total of 1 mL of human bladder RT112 cells suspension (300,000 cells) was put in each well. Then the plates were incubated at 37°C. At different times, adhesive RT112 cells were counted by a colorimetric MTT test as described by Li et al.[25] A solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) at 1 mg mL^{-1} was added in each well containing cells fixed on polymer after removing the medium of culture with the non attached cells. Plates were placed at 37°C for 1 h. The aqueous solution of tetrazolium salt was converted by mitochondrial succinate dehydrogenase into formazan suspension. The intensity of this suspension was proportional to the quantity of living cells. By dissolving formazan with isopropanol, cells burst and release the coloured solution whose absorption was measured by UV spectrophotometer (λ= 550 nm). Cell adhesion was observed at 4 h and cell growth was measured for up to 4 days of culture. Experiments were repeated three times.

Submicrometer particles

Submicrometer particles were prepared by a solvent displacement/evaporation METHOD. Copolymer (75 mg) was dissolved by stirring in 15 mL of acetone at 50°C. 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 EtOH), equivalent of 200 μg of doxorubicin were added. The mixture was added drop wise in 45 mL of an aqueous solution of Pluronic F-68 (1% w/v) by stirring. Concentration of doxorubicin was adjusted to obtain fluorescence intensity of the assays (weight ratio of entrapped doxorubicin into polymeric submicrometer particles and doxorubicin delivery) in the range of internal standards of 1–10 μg equivalents. The solution was stirred at room temperature in a flux of air during 4 h to completely remove the acetone. The suspension of submicrometer particles was centrifuged at 900 g during 10 min at 10°C to eliminate particles with a diameter higher than 1 μm. The supernatant was centrifuged at 3,500 g during 30 min at 10°C to recover the submicrometer particles. About 2 mL of the solution of Pluronic F-68 (1% w/v) was added to the precipitate containing submicrometer particles to prevent aggregation. The suspension was kept frozen or lyophilized.

Release of doxorubicin from particles

Solution of Pluronic F-68, 5 mL (1% w/v), was added to the precipitate of submicrometer particles. A total of about 2 mL of the suspension was transferred into a regenerated cellulose ester dialysis membrane (12–14,000 Da cut off) and put in 10 mL of a solution of Pluronic F-68 ((1% w/v), pH = 6) in a beaker, covered by parafilm to avoid evaporation of water and placed at room temperature in dark. The 10 mL solution was taken after different times, lyophilized and replaced by 10 mL of Pluronic F-68. The lyophilized solution was analyzed by spectrofluorescence compared with assay with no doxorubicin-loaded submicrometer particles.

Hydrolytic degradation

Films (30–40 mg) were cast out by compression at 80°C. The degradation studies were done in 50 mL of a phosphate buffer at pH 7.3 containing 7.54 g L^{-1} NaCl, 1.83 g L^{-1} Na_2HPO_4, and 0.24 g L^{-1} Na_2HPO_4 at 37°C. Two samples were used for each data point.

Molecular weight measurements

Average molecular weights were determined by SEC using a Kontron 420 pump with 2 styragel columns connected in series which type is PL gel (mixte C, 5 μm) from polymer laboratories, and a Shodex RI-71 model refractive index detector. THF was used as eluent at a flow rate of 1.0 mL min^{-1}. The sample concentration was 10 mg mL^{-1} and the injection volume was 50 μL.

Thermal analysis

Thermal analysis was conducted by differential scanning calorimetry using a DSC-2010 TA instrument. The samples were scanned from 20 to 100°C with a heating rate of 10°C/min then cooling at –120°C, and scanned again from –120°C to 20°C with a heating rate of 10°C/min.

Water contact angle

The water contact angle of polymer films was measured in air using the sessile droplet method using Easy Drop KRUSS instrument. The volume of deionised water (MilliQ water) droplet was 10 μL.

Nanosizer

The size of the resulting particles was measured by light scattering using a zetasizer Nano-ZS Nanoseries (MALVERN Instruments).

Spectrofluorescence

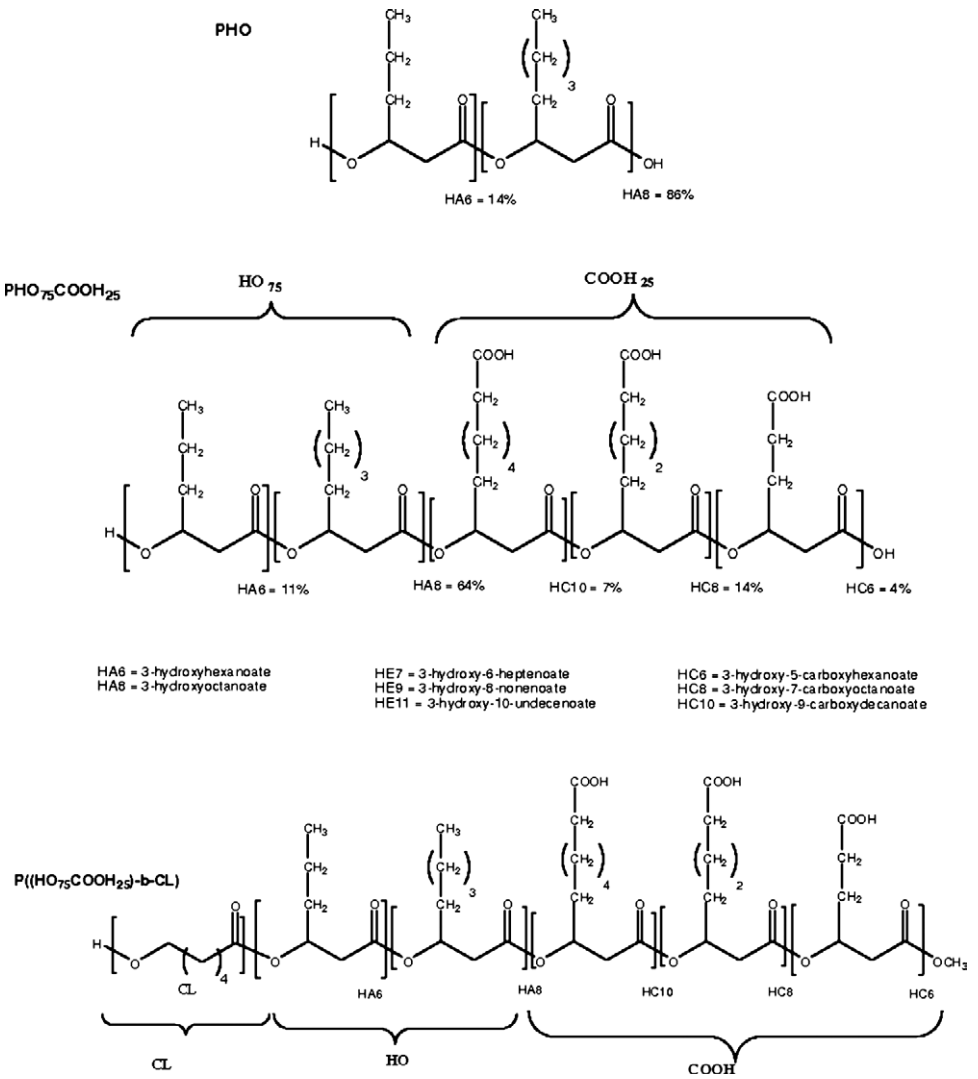
The amount of doxorubicin in filtrates was measured by a SLM Amico 8 100 SLA INSTRUMENTS INC (slit 8× 10, λ_{exc} = 480 nm, λ_{em} = 590 nm). All the samples were dissolved in a mixture EtOH/THF (20/80) before being analyzed.

RESULTS AND DISCUSSION

Poly(3-hydroxyoctanoate) (PHO) is a hydrophobic bacterial polyester containing 85% of 3-hydroxyoctanoate and 15% of 3-hydroxyhexanoate monomer units, and its hydrolytic degradation rate is very slow.[16] To introduce polar groups in the side chains, we used an unsaturated polyester, the poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate) ($PHO_{75}U_{25}$) containing 25% of unsaturated units. The unsaturated units were converted to carboxyl groups by total oxydation as previously described.[17,19] The obtained polyester $PHO_{75}COOH_{25}$ contained 25% of carboxyl groups (Scheme 2). Its molar mass is smaller than the initial $PHO_{75}U_{25}$ because of a treatment involving some chain degradation (Table I). The glass transition temperature (T_g) of $PHO_{75}COOH_{25}$ is significantly higher than that of $PHO_{75}U_{25}$ and PHO. This may be linked to the formation of intermolecular hydrogen bonds stiffening the polymer. PHO and $PHO_{75}U_{25}$ are semicrystalline, in contrast to $PHO_{75}COOH_{25}$ which is an amorphous polymer.

Surface characteristics of $PHO_{75}COOH_{25}$ films

Films of PHO and $PHO_{75}COOH_{25}$ were prepared by solvent casting and wettability was determined by contact angle measurements. High values of contact angles showed that both polymers are very hydrophobic (Table II). As observed, water contact angle increases with the thickness of PHO films. Higher values for thick films in comparison with thin one indicate the higher hydrophobicity of thick films polymer surface. Alkyl pendant groups of PHO are preferentially oriented at polymer/air interface. When the thickness is smaller (1 μm) the segregation of alkyl side chains is less important. The $PHO_{75}COOH_{25}$ films are more hydrophobic (107°) than PHO (98°). We do not see here the influence of the presence of COOH groups. This observation is explained by the internalization of the polar groups COOH inside the films when the thickness of the films is 80 μm. The situation is different for the thin films because the contact angle of $PHO_{75}COOH_{25}$ films is equal to 77°. In this case the polar groups are oriented at the polymer/air interface. We noticed here a particular segregation of COOH groups depending on the film thickness.



Scheme 2 Structure of PHO, $PHO_{75}COOH_{25}$ and the copolymer P($HO_{75}COOH_{25}$-b-CL).

The evolution of the contact angle of water with time was monitored for $PHO_{75}COOH_{25}$ films (80 μm) and was reported on Figure 1. As the surface comes into contact with water its hydrophobicity decreases with time. The COOH groups are coming to the surface of the films. Contact angle relaxation with time indicated that the surface undergoes restructuration when exposed to water. Reorientation of COOH groups was thus considered as the main motive for the surface restructuration.

These PHO and $PHO_{75}COOH_{25}$ films were then totally immersed in water at room temperature and contact angles were measured at different times of immersion (Fig. 2). During the first period, the contact angle increased for both polymers. This is because an important increase of surface roughness

TABLE I
Physical Characteristics of PHO, $PHO_{75}U_{25}$ and $PHO_{75}COOH_{25}$ Determined by SEC and DSC

Sample	M_n (g/mol)	M_w (g/mol)	T_g (°C)	T_m (°C)	ΔH_m (J/g)
PHO	80,000	160,000	−39	50	18
$PHO_{75}U_{25}$	80,000	158,000	−39	40	6
$PHO_{75}COOH_{25}$	48,000	90,000	−19	–	–

TABLE II
Contact Angle Measurements of PHAs Films with Different Thickness

Sample thickness (μm)	Contact angle (°)
PHO 1	90
PHO 80	98
$PHO_{75}COOH_{25}$ 1	77
$PHO_{75}COOH_{25}$ 80	107

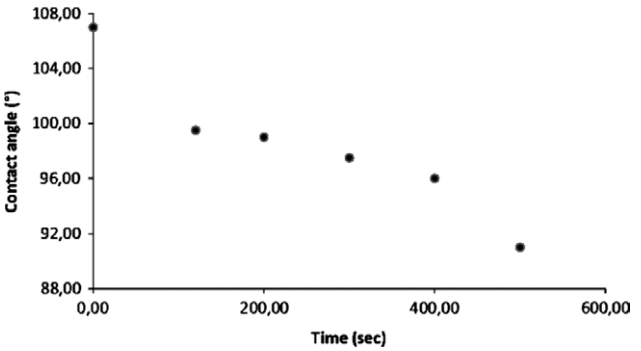


Figure 1 Evolution of contact angle of $PHO_{75}COOH_{25}$ film (80mm).

promoted by water diffusion into the film. The $PHO_{75}COOH_{25}$ films became white. After 72 h of immersion, the surface of $PHO_{75}COOH_{25}$ is more hydrophilic and contact angle is equal to 98°. The reorganization of COOH groups to the surface lead to a surface restructuration.

Cell adhesion and proliferation on $PHO_{75}COOH_{25}$films

Cell adhesion and proliferation studies were done on films based on PHO and $PHO_{75}COOH_{25}$ to evaluate the role of COOH groups. The films were prepared by the solvent cast method. Once dried, they have been used without further modification (no protein) or after pretreatment with collagen IV. Collagen IV is a glycoprotein, ubiquitous component of basement membranes which provides the major structural support for this matrix. It can be used as a thin coating on tissue culture surfaces to promote cell attachment and proliferation. The cell line RT112 used in this study came from human bladder carcinoma. Carcinoma cells are usually used by biologists because of their rapid proliferation. These epithelial cells required some support to adhere and proliferate.

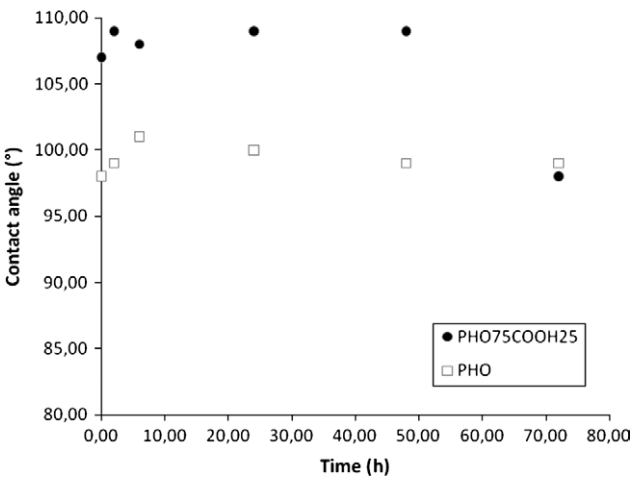


Figure 2 Evolution of contact angle of $PHO_{75}COOH_{25}$ and PHO films after immersion in water.

Cell adhesion is the first necessary step to allow cell growth. The physicochemical properties of the surface affect cell attachment and cellular behavior.[13] They also govern cellular adhesion, subsequent cellular viability and performances. Results of cell adhesion are displayed on Figure 3 which represents the quantity of living cells adhered on PHO and $PHO_{75}COOH_{25}$ films after 4 h of incubation at 37°C in the different conditions. Results showed that the adhesion of cells was better on $PHO_{75}COOH_{25}$ films than in PHO films. This result can be explained by the presence of carboxyl groups at the films surface which promoted cell adhesion. Some authors also showed that the presence of negative charges brought by carboxyl groups favoured Protein adhesion as collagen.[24,25] The PHO_{75}-$COOH_{25}$ copolymer is sticky and completely amorphous, with a low glass temperature (19°C), whereas the PHO was semicrystalline (T_m = 50°C). This sticky character is due to the presence of polar groups could also explain the good cell adhesion. For both polymers, the pretreatment is favourable to cellular adhesion which confirmed the influence of proteins in the cellular adhesion promotion.

Cell growth was measured after 4 days of incubation (Fig. 4). Proliferation is demonstrated because the number of cells was near 1.05×10^5 after 4 h of immersion and after 4 days the number of cells is 2×10^5 on $PHO_{75}COOH_{25}$. Nevertheless, the number of cells is more important on the PHO surface, and on the glass surface. This difference could be due to the presence of carboxyl groups in PHO_{75}-$COOH_{25}$ which could inhibit the cell growth. This result is explained by the reorganization of the macromolecular chains at the surface of the films, as we have seen in the previous study by contact angle measurement. After 4 days, a decrease of the contact angle was observed for the $PHO_{75}COOH_{25}$ films containing 25% of carboxyl groups because of the rearrangement of macromolecular chains leading to

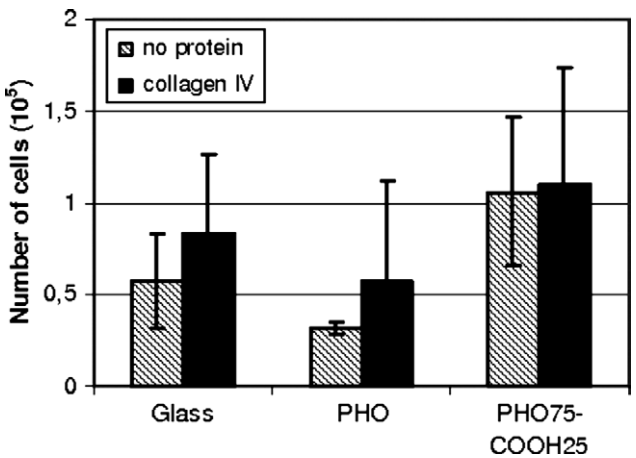


Figure 3 Cell adhesion on PHAs films after 4 hours of incubation at 37°C.

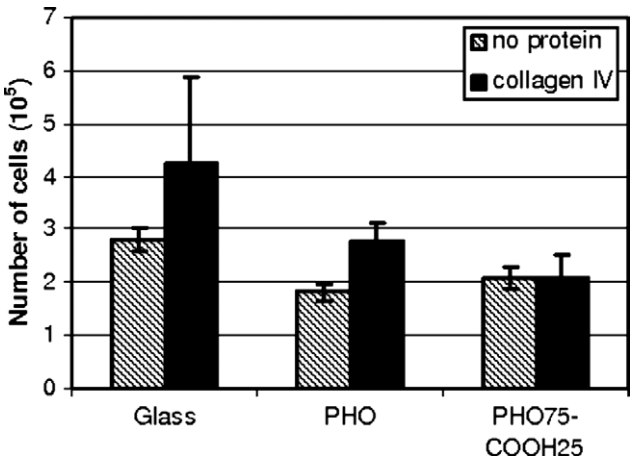


Figure 4 Cell proliferation on PHAs films after 4 days of incubation at 37°C

the migration of polar COOH groups toward the aqueous medium. However, interactions between carboxyl groups of the support, the protein layer and cells are very complex, so it is very difficult to precisely conclude on the eventual cytotoxic effect of $PHO_{75}COOH_{25}$.

Particles based on $PHO_{75}COOH_{25}$ as system for drug delivery

Particles formation and stability

Particles based on PHO or $PHO_{75}COOH_{25}$ have already been prepared by Kurt et al.[26] using a solvent displacement/evaporation method. Because of its nature as an elastomeric material, particles based on PHO collapsed during the particles synthesis On the contrary, when the particles are made of $PHO_{75}COOH_{25}$, the presence of carboxyl groups avoided their aggregation (Fig. 5). Even if particles of $PHO_{75}COOH_{25}$ are successfully prepared the lyophilization process is not possible without aggregation. To prepare particles that can be further lyophilized, copolymers based on $PHO_{75}COOH_{25}$ and a more crystalline material, poly(ε-caprolactone) (PCL), were synthesized. PCL was chosen because of its rigidity, biocompatibility, and biodegradability. Diblock copolymers with strictly controlled segment lengths and narrow molecular weight distribution have been prepared by a two-step synthesis.[23,27] The synthesized copolymers are semicrystalline contrary to previous $PHO_{75}COOH_{25}$ that was completely amorphous (Table III). The semi crystalline feature of all copolymers allows preparing submicrometer particles from these new materials, which have been lyophilized without any problem.

Doxorubicine release from particles

Like many drugs used to treat cancer, doxorubicin is a potent vesicant that may cause extravasations and necrosis at the injection site. Doxorubicin encapsulation reduced toxic effects against normal cells whilst increasing its therapeutic effect. Preparation of particles containing doxorubicin is obtained by precipitation from a solution containing doxorubicin, diblock copolymer in 1% (%w/v) Pluronic F-68 under stirring. This method is often used to prepare colloidal carriers.[28,29] The resulting particles were characterized by light scattering allowing their size to be determined (Table IV). Particle sizes are similar for each type of copolymers and PCL (150 nm). The

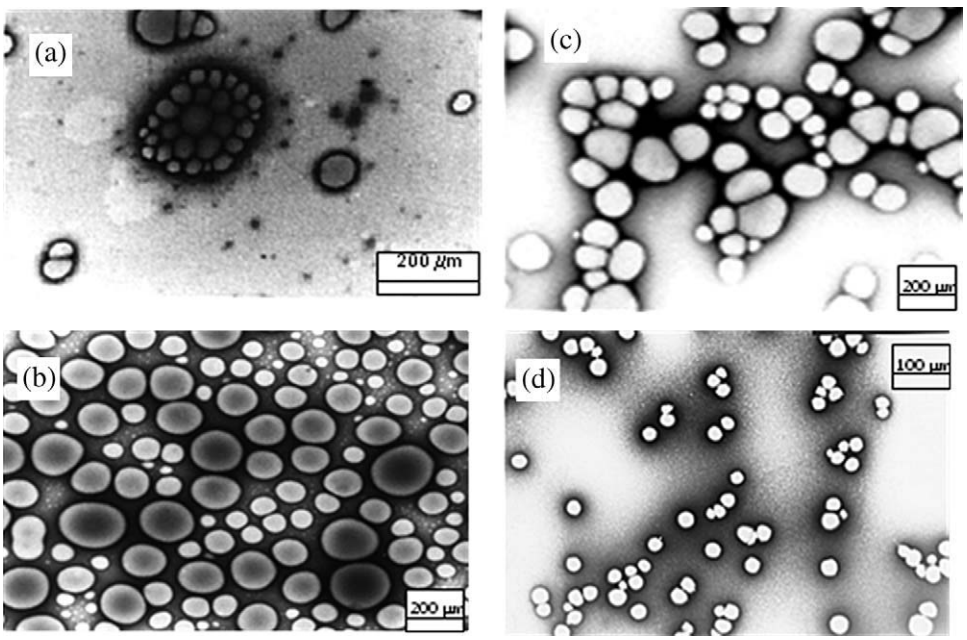


Figure 5 Sub-micrometer particles on PHO (A), $PHO_{75}COOH_{25}$ (B) P($(HO_{75}COOH_{25})$-b-CL) (C) and PCL (D).

TABLE III
Characteristics of Polymers

Nature of polymers		$M_{n\ 1st\ block}$ (g mol^{-1})	$M_{n\ exp\ 2nd\ block}$ (g mol^{-1})	T_g (°C)	T_m (°C)	ΔH_m (J g^{-1})
PCL	1	20,000[b]		−59	62	80
$PHO_{75}COOH_{25}$	2	48,300		−19	–	–
P(($HO_{75}COOH_{25}$)-*b*-CL)	3	4800[a]	26,800[a]	−58	61.5	70
	4	20,500[a]	35,300[a]	−57	61.5	80.5

[a] Determined by SEC (THF, polystyrene standards), 1st block = PHO or PHO-COOH, 2nd block = PCL.
[b] Determined by ^{1}H NMR.

encapsulation efficiency (EE) is based on the percentage of encapsulated doxorubicin compared with the initial doxorubicin quantity. The encapsulation efficiency (EE) was calculated according to eq. (1).

Equation 1:

$$\text{Encapsulation efficiency(EE)} = \frac{n_{\text{encapsulated doxorubicin}}}{n_{\text{initial doxorubicin}}} \times 100$$

$$\text{Entrapment mass percentage} = \frac{m_{\text{encapsulated doxorubicin}}}{m_{\text{polymer}}} \times 100$$

Whatever the block lengths of the copolymers, EE are larger than 50%, due to the hydrophobicity of both doxorubicin and polyesters. Best results were obtained with pure PCL (EE = 77%). It is known that physical entrapment of hydrophobic drug in particles is driven by hydrophobic interactions between the drug and the hydrophobic segments of polymers. Hydrophobic interactions and also hydrogen bonds between carbonyl groups of amorphous polyesters and polar groups of doxorubicin (amino and hydroxyl groups) explained the ability of this polymers family to entrap the bioactive molecule. We have then tested the kinetic of drug release to confirm the choice of these copolymers as a doxorubicin loaded carrier system. The *in vitro* release behaviours of doxorubicin-loaded particles at pH = 6 (bladder pH) and room temperature were studied. A typical two phase release profile was observed. It corresponds to release profiles obtained in the case of matricial type particles[22] (Fig. 6). These results are coherent with the method of particles preparation. In this case, the doxorubicin is co-precipitated with the polymer. The relatively rapid release in the first stage can be explained by a fast release of doxorubicin adsorbed at the particles surface. This burst effect is inferior to 10% for each copolymer. This first stage is followed by a sustained and slow release over a prolonged time up to some days, which corresponds to the drug diffusion. After 5 days, the percentage of released doxorubicin is comprised between 12% (PCL) and 25% (Copolymer 4: P((HO-COOH)-*b*-CL), $Mn_{\ block\ PHO\text{-}COOH}$ = 20,500 g mol^{-1}). This result can be explained by hydrophobic interactions between PCL and doxorubicin which limit the drug diffusion kinetic. PCL is a higher semi crystalline polymer than the copolyesters and its crystallinity also decreased the doxorubicin diffusion.

Moreover, the observation of doxorubicin release from P((HO—COOH)-*b*-CL) particles is particularly interesting. We can observe that doxorubicin release kinetic corresponding to particles with a PHO—COOH segment length of 20,500 g mol^{-1} is the highest. Doxorubicin content released reached 25% after 118 h. This result can be explained by an increase of the device hydrophilicity due to the presence of pendant carboxyl groups in the copolymer. When the PHO—COOH block length is 4,800 g mol^{-1}, the contribution of this hydrophilic group is less

TABLE IV
Characteristics of the Submicrometer Particles

		Encapulation of doxorubicin (%)		nanosizer	
Nature of polymers	polymers	EE	Mass percentage	Ø (nm)	PDI
PCL	5	77	0.18	150 ± 6	0.10
P((HO-COOH)-*b*-CL)	3	52	0.14	140 ± 5	0.06
	4	50	0.13	130 ± 6	0.08

EE = Efficacy of encapsulation, PDI = Dispersion index.

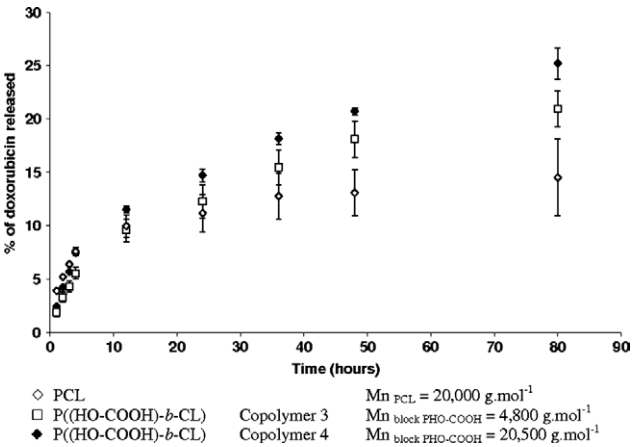


Figure 6 Doxorubicin release from different copolymers.

important, and the content of doxorubicin release reached only 20%. The proportion of carboxylic groups has to attempt a certain proportion to have a visible effect on drug release.

Degradability of $PHO_{75}COOH_{25}$

PHO films are known to be very hydrophobic and do not degrade at pH 10 at 37°C. The $PHO_{75}COOH_{25}$ is actually the only mcl PHA easily hydrolysable (few hours at pH 10)[14] because of the presence of COOH groups. Degradation under moderate conditions (pH = 7.3 and 37°C) of films made from different copolymers is shown on Figure 7. Only $PHO_{75}COOH_{25}$ and P((HO—COOH)-*b*-CL) are subject to hydrolysis at pH 7. The variation of the average number of bond cleavages per original polymer molecule ($N_{(t)}$) with the degradation time was calculated according the following equation.

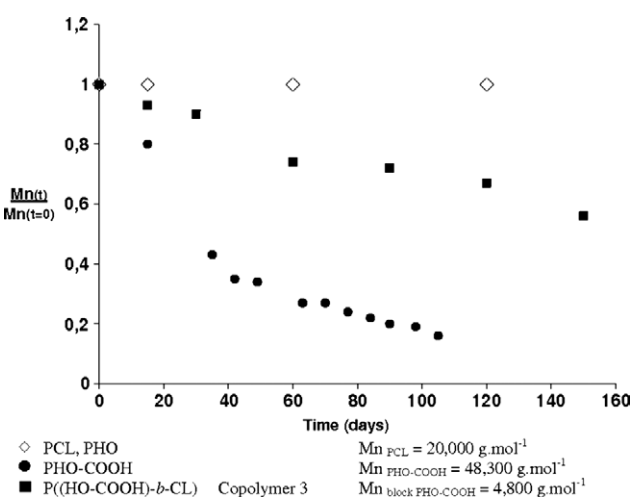


Figure 7 Hydrolytic degradation of the different polymers at pH 7.3 at 37°C.

$$N_{(t)} = k_d\, Pn_{(0)} t$$

with:

$$N_{(t)} = \left(\frac{Mn_{(0)}}{Mn_{(t)}}\right) - 1$$

where k_d represents the kinetic constancy of the hydrolytic degradation, $P_{n(0)}$ the degree of polymerization at $t = 0$ and M_n, the molar mass.[30]

It has been demonstrated that the average number of bond cleavages per original polymer molecule (N) linearly increased with the degradation time,[30] indicating that M_n decrease is because of random chain scissions. As expected, PHO and PCL films are not degraded at 37°C at pH 7.3 during 275 days. In the case of $PHO_{75}COOH_{25}$ films and copolymers P((HO-COOH)-*b*-CL), the presence of carboxyl group promote water penetration into the polymer and participate to ester group hydrolysis through better water penetration and catalysis.

CONCLUSIONS

Natural polyesters from the group of PHAs have emerged as promising products particularly for biomedical applications. Adhesion and proliferation of specific cells on natural and artificial biopolyesters are very complex. Moreover, the type, conformation, and amount of proteins adhered to the surface affect also the cellular adhesion. Bacterial polyesters are very versatile and can be considered as materials for tissue engineering, but the couple cell/biopolyester has to be tested for each application. PHOCOOH is convenient for adhesion with human bladder cancer RTT112 and PHO for cell proliferation. We have also observed that the segregation of COOH groups at the surface of the films is well influenced by the thickness. Thick films exhibit surface restructuration when they come into contact with water. The COOH groups come to the surface, that is, in contact with water and change the properties of the films.

The potential of the copolyesters family in the drug release area has been displayed through the study of doxorubicin release and it is important to note again the copolymers versatility. This study has shown the benefit of introducing carboxyl pendant groups to better control the drug release; a high content of carboxyl groups leading to a faster release of the bioactive molecule. They can be adapted to other biomedical applications through tailor making of both segments (length, chemical structure) to fit prerequisites corresponding to a specific device. Versatility of the PHAs family and its derivatives is a major reason to test novel compounds. These polymers will be further tested in drug eluting stents.

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