Polymeric matrices based on graft copolymers of PCL onto acrylic backbones for releasing antitumoral drugs

Gustavo A. Abraham,[1] Alberto Gallardo,[1] Julio San Román,[1] Alfonso Fernández-Mayoralas,[2] Mercedes Zurita,[3] Jesús Vaquero[3]

[1]*Instituto de Ciencia y Tecnología de Polímeros, CSIC, Juan de la Cierva 3, 28006, Madrid, Spain*
[2]*Instituto de Química Orgánica General, CSIC, Juan de la Cierva 3, 28006, Madrid, Spain*
[3]*Clínica Puerta de Hierro, Madrid, Spain*

Received 11 November 2001; accepted 24 January 2002

Abstract: Graft copolymers of poly(ε-caprolactone) (PCL) on poly(dimethylacrylamide) (PDMAm), poly(methylmethacrylate) (PMMA), or on copolymers of poly(DMAm-*co*-MMA) have been synthesized and characterized by ^{1}H NMR spectroscopy, differential scanning calorimetry (DSC), and size exclusion chromatography (SEC). These partially biodegradable copolymer matrices have been proposed as drug delivery systems for the release of low-molecular-weight glycosides. Octyl-*N*-acetyl-6-*O*-[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-α-D-glucosamide, a synthetic carbohydrate able to inhibit the proliferation of human malignant glioma cells in culture and transplanted glioma in rats was selected as drug model. The *in vitro* aqueous behavior of four drug-loaded and unloaded graft copolymers of different MMA: DMAm and PCL ratios has been analyzed performing swelling, degradation, and drug release experiments. An intimate dependence of the aqueous behavior with the composition has been found. The higher was the DMAm content, the higher was the hydrophilicity of the synthesized systems as well as the swelling, degradation, and drug release rate. *In vivo* experiments in pigs demonstrated the very good tolerance of drug-loaded implanted polymeric discs, and that >95% of the charged drug is released after 2 months' implantation. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res 64A: 638–647, 2003

Key words: partially biodegradable matrices; graft copolymers; antitumoral drugs; drug release; *in vivo* experiments

INTRODUCTION

During the past years Nieto-Sampedro et al.[1,2] have reported that synthetic carbohydrates related to blood group oligosaccharides are able to inhibit the proliferation of transformed neural cells. *In vivo* experiments showed that a synthetic tetrasaccharide caused not only the inhibition but also the destruction of a malignant rat brain glioma after transplantation of C6 cells.[3] In order to minimize the synthetic work, a second generation of inhibitory compounds with simplified structures was synthesized and tested.[4] One of these compounds, a glycoside derived from the monosaccharide *N*-acetyl-D-glucosamine, inhibited the proliferation of human glioma cell in culture and transplanted glioma in rats.[5] Regarding its possible clinical use, a intracranially implanted drug delivery system able to release this glycoside for long-term appear to be interesting.

Although several systems for the controlled release of therapeutic agents have been reported in the literature,[6–9] one promising and alternative support for controlled drug release of antitumoral agents that involves the use of partially biodegradable matrices implanted within the cavity of a resected brain tumor is described in this article

The proposed matrices are graft copolymers of poly(ε-caprolactone) (PCL) on poly(dimethylacrylamide) (PDMAm), poly(methylmethacrylate) (PMMA), or copolymers of poly(DMAm-*co*-MMA) of different compositions. Poly(ε-caprolactone) is a biodegradable and hydrophobic polyester, broadly investigated in the biomedical field. Because of its low degradation rate it has been proposed as matrix for long-term delivery.[10] The degradation is autocatalyzed by the carboxylic acid end groups of the polymer, eventually forming carbon dioxide and water, which are easily cleared from the body.[11]

On the other hand, DMAm and MMA are two well-known biocompatible acrylic components, very different from a chemical point of view. The PMMA that has

Correspondence to: J. San Román; e-mail: ictsr04@ictp.csic.es

Contract grant sponsor: Comision Interministerial de Ciencia y Tecnologia; contract grant number: MAT 98-0964.

been extensively used as biomaterial is a stiff and hydrophobic polymer, whereas PDMAm is a hydrophilic polymer that is hydrosoluble. The combination of these dissimilar structures can lead to the control on the hydrophobic/hydrophilic balance and on the biodegradation rate of the grafted polyester moieties. Thus, the ratio of the three components will tailor the swelling and degradation rate and finally will modulate the drug release from the matrix to the tumor bed. The systems are partially biodegradable because of the grafted PCL chains, and depending on their composition the residual polymeric system after biodegradation of PCL-grafted chains is composed of MMA, DMAm, and methacrylic acid salts, which becomes hydrosoluble depending on its average composition.

MATERIALS AND METHODS

Materials

ε-Caprolactone (Merck-Schuchardt), methacryloyl chloride (Acros Organics), and *N*,*N*-dimethylacrylamide (DMAm, Aldrich) were distilled under vacuum before use. Methyl methacrylate (MMA, Merck) was purified by being passed over a short column of basic alumina to remove the inhibitor. 2,2′-Azobisisobutyronitrile (AIBN) was purified by fractional crystallization from methanol and used as free-radical initiator. Other reagents were extra pure grade and were used as purchased. The antitumoral drug used was octyl-*N*-acetyl-6-*O*-[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-α-D-glucosamide, whose synthesis is currently under application for patent.[5] The chemical structure of this glycoside is shown in Figure 1.

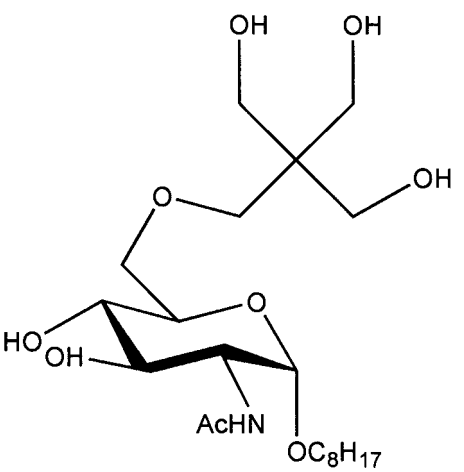

Figure 1. Chemical structure of the antitumoral drug, octyl-*N*-acetyl-6-*O*-[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-α-D-glucosamide.

Synthesis of the macromonomer

The ring-opening polymerization of ε-caprolactone in the presence of numerous catalysts to obtain high-molecular-weight polyesters has been widely investigated.[12,13] In this work we have synthesized PCL by direct polycondensation without using a catalyst, following the procedure described by Fukuzaki et al.[14] The absence of organic catalysts containing metals such as Al, Zn, Sn, lanthanide compounds, or other commonly used assure the nontoxicity of this polymer. This method avoids the presence of residues that can compromise the biocompatibility of the prepared materials. Briefly, aqueous solution of ε-caprolactone (80% wt/wt) was charged into a glass ampule, and then nitrogen gas was bubbled into the solution at a controlled flow rate of 200 mL min^{-1}. The ampule was immersed in an silicone-oil bath maintained at 200°C, being the solution under reflux. After 2.5 h, the water was allowed to be removed from the solution, and the polymerization reaction was carried out during 21 h. The polymer was purified by dissolution in dichloromethane and then precipitated in a large excess of ethanol to remove the residual monomer. Afterward, the solid was filtered and exhaustive dried under high vacuum (10^{-3} mm Hg) during 24 h at room temperature. The reaction yield was 89%.

In order to synthesize the macromonomer, a suitable amount of PCL was dissolved in dichloromethane. Then a calculated amount of triethylamine, in stoichiometric ratio to hydroxyl groups, was added to the solution under stirring. A large excess of freshly distilled methacryloyl chloride in a dichloromethane solution was added dropwise to the flask. The mixture was stirred in an ice bath and then for 10 h at room temperature. The triethylammonium hydrochloride byproduct, which remain suspended as a white powder, was removed by filtration. The unreacted acid halide was neutralized by washing the solution three times with sodium hydroxide solution. The organic phase was finally concentrated and precipitated in a large amount of cold ethanol. After filtration, the methacryloyl-terminated PCL (M-PCL) was extensively dried under high vacuum at room temperature.

Copolymerization and sample preparation

Free radical copolymerization of macromonomers is the most commonly used technique for the graft copolymer synthesis. Although several novel routes for obtaining graft copolymers have been recently described in the literature,[15] in this work traditional free radical polymerization procedure appeared to be suitable for preparing graft copolymers.[16,17] M-PCL was

copolymerized with DMAm and MMA in different weight ratios. Mixtures of the macromonomer, comonomers, and AIBN as free radical initiator were prepared in dioxane and deoxygenated by bubbling nitrogen for 30 min. The concentration of macromonomer was 0.1 g mL^{-1}, comonomers 1 mol L^{-1}, and initiator 0.01 mol L^{-1}. The reaction was thermally induced at 65°C and allowed to react during 24 h. The polymer solutions were precipitated in cold diethyl ether assuring that unreacted macromonomers/monomers were removed, and finally the resulting product was dried under vacuum until a constant weight. Polymer discs (1.3 cm in diameter and 2.2 mm in thickness) for swelling and degradation measurements were prepared by compression molding. Thus, polymer powder was compressed with a stainless steel mold under light pressure at room temperature and 300 psi. Drug-loaded samples were prepared by mixing the polymer powder and glycoside (i.e., 300 mg powder and 25 mg drug) and then shaped as described above.

Characterization of macromonomer and graft copolymers

^1H Nuclear magnetic resonance spectra were performed in a Varian XLR-300 NMR spectrometer operating at 300 MHz. The ^1H NMR spectra were obtained from 5% (w/v) $CDCl_3$ solutions at room temperature. Molecular weights and molecular weight distributions were determined by size exclusion chromatography (SEC) using a Perkin Elmer gel permeation chromatograph equipped with TriSEC detector module model 270 (Viscotek Corp.), which consists of a concentration detector (refractive index detector), a light-scattering detector, and an on-line viscometer. A set of 10^4, 10^3, and 500 Å PL-gel columns conditioned at 25°C were used to elute the samples of 15 mg mL^{-1} concentration at 1 mL min^{-1} HPLC-grade chloroform flow rate. The triple detector combination eliminates the need of SEC column calibration and instrumental band broadening correction. Thus, the number-average molecular weight (M_n) and weight-average molecular weight (M_w) values as well as the polydispersity index (PI; M_w/M_n), could be accurately calculated.

Differential scanning calorimetry (DSC) experiments were carried out in a Perkin-Elmer DSC-7. Sample weights ranged from 5 to 15 mg were sealed in aluminum pans. Two scans were performed by using a 10°C min^{-1} heating rate and a 320°C min^{-1} cooling rate (quenching) between runs. Thermograms were obtained in the range 20 to 200°C under nitrogen purge. The glass transition temperature (T_g) of the polymer systems were determined at the onset of the transition of DSC traces. The degree of crystallinity (X_c) of PCL was calculated taking into account the melting heat (ΔH_m) value of 16.9 kJ/mol of repeating units computed for 100% crystallinity reported for PCL.[18]

Swelling behavior and *in vitro* degradation

The water sorption measurements were obtained from dried discs of known weights that were immersed in phosphate-buffered saline (PBS) solution at pH 7.4 and incubated at 37°C. At appropriate times, the samples were removed, blotted quickly with absorbent paper to remove the water attached on its surface, and weighed. The swelling percentage of the samples was calculated from the following relation:

$$\text{Swelling degree (\%)} = [(W_t - W_0)/W_0] \times 100$$

where W_t is the weight of swollen specimen at time t and W_0 is the initial mass of the specimen. *In vitro* degradation studies were performed by placing the discs in PBS at 37°C. Weight loss was gravimetrically monitored at various time intervals. In all the experiments a minimum of three samples were measured and averaged.

Drug release measurements

Polymer discs were prepared as above. The glycoside-loaded polymer discs were incubated in 0.1*M* phosphate buffer solution (pH 7.4) at 37°C and 30 rpm in an orbital shaker (Adolf Kühner AG CH-4127). The volume of buffer used was 200 mL $mmol^{-1}$ of the glycoside contained in the disc. At different incubation times, 50-μL aliquots were removed, filtered, and injected onto a C_{18} HPLC column (Licosphere-100RP-18) as part of a Waters 600E system with a UV detector (Waters 484). The mobile phase consisted of water: acetonitrile (74:26), and detection was at 230 nm. The amount of glycoside released was determined from previous calibration curves obtained for this compound. When the incubation of the polymer discs was carried out in rat brain extract, the aliquots (50 μL) were treated with methanol (50 μL) at 0°C and centrifuged (15,000*g*, 15 min) to remove precipitated proteins. The supernatant was subjected to HPLC analysis.

In vivo studies

In order to assess the tolerance of the cerebral tissue and the possibility of tissue damage or reactions as a

consequence of the intracerebral discs, an *in vivo* experimentation was carried out. *In vivo* experiments were conducted in pigs weighing approximately 10 kg (n = 5). Drug-loaded and unloaded discs were implanted intracerebrally into pigs during 2 months and then extracted for additional analysis. The reaction around the cerebral tissue was evaluated histologically after performing magnetic resonance imaging (MRI). The explanted discs were analyzed in terms of surface morphology and total drug content. Surface morphology before and after implantation was observed by means of optical microscopy (Nikon, eclipse E400) and environmental scanning electron microscopy (ESEM; Philips XL-30).

Surgical procedure

The disc implantation was performed under general anesthesia following the procedure briefly described as follows. Frontal craniotomy on the right side was carried out. The dura was then cut within the margins of the skull opening. After making a minimal corticotomy the tumor was removed, and the disc was positioned perpendicularly with respect to the cortical surface. Taking into account the disc size, almost the entire of the disc stayed implanted into the white substance.

After the implantation, all bleeding was secured, the dura was sutured closed and the bone flap restored to the skull with sink sutures (10/0). The scalp was then sutured closed.

Histological analysis

After RMI observations, the animals were sacrificed, and the discs were removed. The brain were immersed-fixed in 10% (v/v) formalin in 0.1*M* phosphate buffer (pH 7.4). In the histopathological examination, the brain-tissue fixed samples were embedded in paraffin, sectioned into slices of 5 μm thickness, and then stained with hematoxylin and eosin to assess cerebral tissue response to the implanted discs. The prepared sections were examined using optical microscopy.

RESULTS AND DISCUSSION

Preparation and characterization of the macromonomer and graft copolymers

The synthesis of the macromonomer was carried out by ring-opening polymerization followed by the appropriate functionalization reaction. In order to characterize the PCL and the corresponding macromonomer, ^{1}H NMR measurements were carried out. Figure 2 shows the spectra of the products. PCL exhibited its typical pattern, the signal at 3.62 ppm being assigned to the CH_2–OH end group. After derivatization with trifluoroacetic anhydride this signal shifted downfield to 4.3 ppm. This observation confirms the assignation of the end group. M-PCL displayed two signals at 5.51 and 6.05 ppm, corresponding to vinyl protons of the methacryloyl end group and a peak at 1.90 ppm assigned to the methyl protons of the end group. The signal of the methylene unit of PCL bonded to the methacryloyl end group appeared at 4.10 ppm, partially overlapped with the signal of methylene protons (denoted as a in Figs. 2 and 3) of the PCL chain at 4.02 ppm. The signal of CH_2–OH end group (3.62 ppm) disappeared, confirming the end-capping of the PCL chains by the methacryloyl group. The number-average molecular weight value determined by NMR is in agreement with the calculated by SEC, as quoted

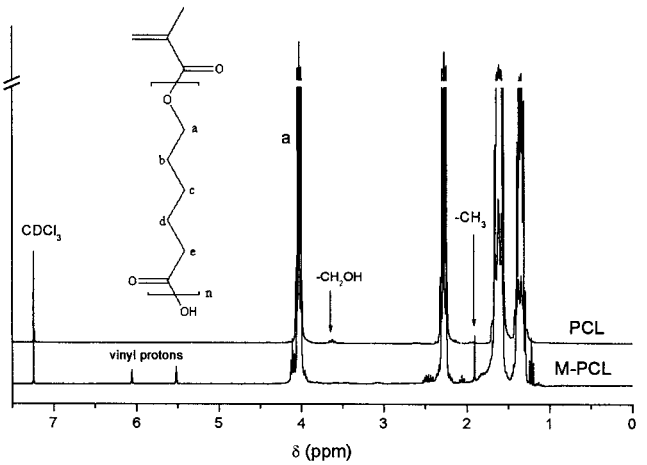

Figure 2. Chemical structure of M-PCL and ^{1}H NMR spectra of PCL and methacryloyl-terminated poly(ε-caprolactone) (PCL).

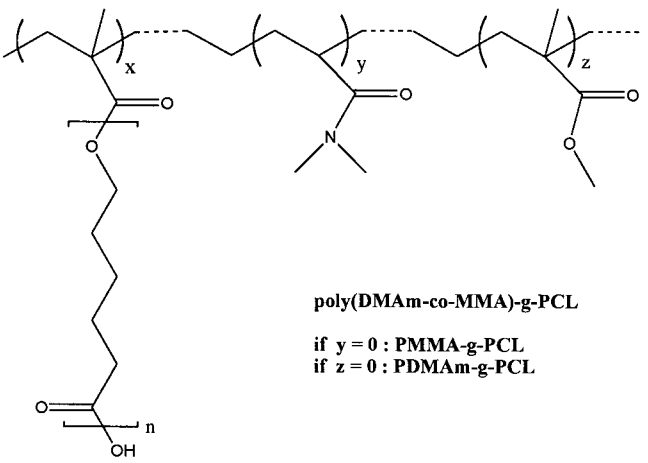

Figure 3. Chemical structure of synthesized graft copolymers.

TABLE I
Composition, Average Molecular Weights, and Thermal Properties of the Macromonomer and Graft Copolymers

Sample	Feed (wt %)			Copolymer Composition (wt %)[a]			M_n[b]	M_w[b]	PI[b]	$T_{m\ PCL}$ (°C)[c]	$\Delta H_{m\ PCL}$ (J/g)[c]	X_c (%)[d]	$T_{g\ PMMA}$ (°C)[c]
	PCL	DMAm	MMA	PCL	DMAm	MMA							
M-PCL	100	—	—	100	—	—	6,000 5,800[a]	7,800	1.3	62.9	96.8	65.4	—
C 48-52-0	50	50	—	48.1	51.9	—	165,300	300,500	1.8	60.7	62.5	42.2	—
C 48-22-30	50	25	25	48.3	21.9	29.8	126,000	218,000	1.7	60.2	62.6	42.3	104
C 50-12-38	50	5	45	50.1	12.1	37.8	86,000	116,000	1.3	58.1	83.8	56.6	104
C 52-0-48	50	—	50	52.3	—	47.7	82,000	105,000	1.3	56.1	93.5	63.2	104

[a]Determined by ^{1}H NMR.
[b]Absolute values determined by SEC.
[c]Estimated by DSC (first scan).
[d]Calculated as mentioned in the text.

in Table I. M-PCL chains of low polydispersity and controlled Mn were obtained.

M-PCL was copolymerized by free radical mechanism with DMAm, MMA, or a mixture of them, in the proportions given in Table I. The macromonomer/comonomer molar ratio was 0.017, which means that approximately one PCL chain is expected to be grafted for every 60 acrylic units. Figure 3 shows the chemical structure of the synthesized graft copolymers prepared in this work. All the systems contained 48 to 52% by weight of M-PCL, and different weight ratios of the comonomers mean that the ratio of incorporation of the macromonomer into the graft structure was close to the feed ratio. The four graft copolymers were identified as follows: PDMAm-*g*-PCL (C 48-52-0), P(DMAm-*co*-MMA)-*g*-PCL (C 48-22-30 and C 50-12-38), and PMMA-*g*-PCL (52-0-48), where the code indicates the copolymer composition by weight percent of PCL-DMAm-MMA, as shown in Table I.

Graft copolymers were characterized by ^{1}H NMR spectroscopy and SEC. Figure 4 shows the NMR spectra of these samples. The composition of the graft copolymers was obtained by integration of the characteristic signals of PCL [3.9–4.25 ppm, methylene protons; Fig. 4(a)], MMA (3.4–3.85 ppm, methoxy protons), and DMAm (2.75–3.2 ppm, *N*-methyl protons). The calculated values, expressed by weight percent of each component, are shown in Table I.

SEC chromatograms of the prepared polymer systems are plotted in Figure 5. The average molecular weight values, M_n and M_w, as well as the PI are summarized in Table I. The synthesized graft copolymers were homogeneous systems with unimodal molecular weight distribution, the values of PI being in the range of 1.3 to 1.8. The comparison of the SEC traces of purified graft copolymers and the starting M-PCL indicates the absence of residual macromonomer. It is clear from the data collected in Table I that M_n of the copolymer decreases with the increasing of MMA content in the polymer chains. This could be ascribed to transfer reactions of MMA growing radicals to the oxymethylene groups of caprolactone units, which has been described for the polymerization of MMA in the presence of PCL[19] as well as PLLA.[20] This also agrees

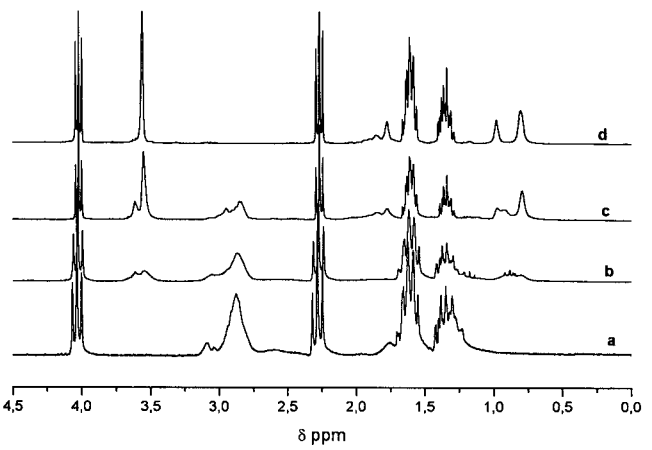

Figure 4. ^{1}H NMR spectra of graft copolymers: (a) C 48-52-0; (b) C 48-22-30; (c) C 50-12-38; and (d) C 52-0-48.

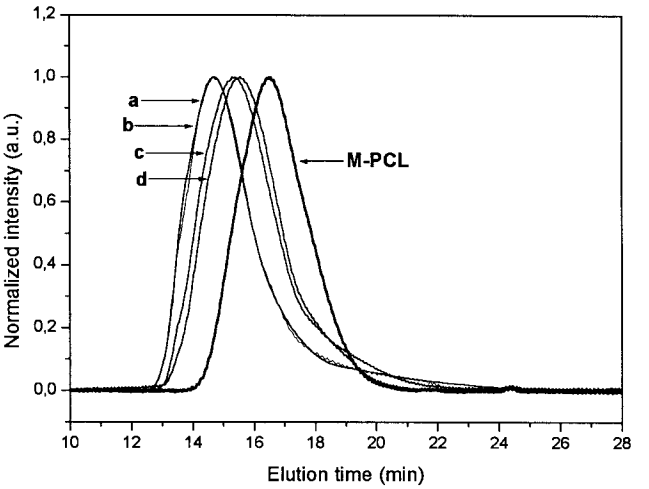

Figure 5. SEC chromatograms of the synthesized macromonomer (M-PCL) and graft copolymers: (a) C 48-52-0; (b) C 48-22-30; (c) C 50-12-38; and (d) C 52-0-48.

with the smaller PI for copolymers prepared in the presence of increasing quantities of MMA.

DSC results are also shown in Table I. The melting endotherm of PCL was observed in the first scan for all the copolymer studied. The melting peak decreased with respect to M-PCL, and on the other hand, the crystallinity degree (X_c) decreased for the copolymers containing PDMAm. The second scan traces, after quenching, exhibited a lower melting endotherm of PCL chains (X_c = 24%) but only could be detectable in the case of PMMA-*g*-PCL (C 52-0-48). P(DMAm-*co*-MMA)-*g*-PCL copolymers did not showed PCL melting, suggesting that the incorporation of PDMAm avoid the rapid crystallization of PCL chains during cooling. The samples containing PMMA displayed a T_g value of PMMA at 104°C, whereas whose containing PDMAm did not showed a T_g of PDMAm clearly discernible.

Swelling and *in vitro* degradation

Figure 6 shows the water uptake curves for these systems. The swelling behavior was controlled by the composition of the acrylic components (DMAm:MMA ratio), ranging from averaged-values of 144% water uptake for the C 48-52-0 to 1.2% for the C 52-0-48. The

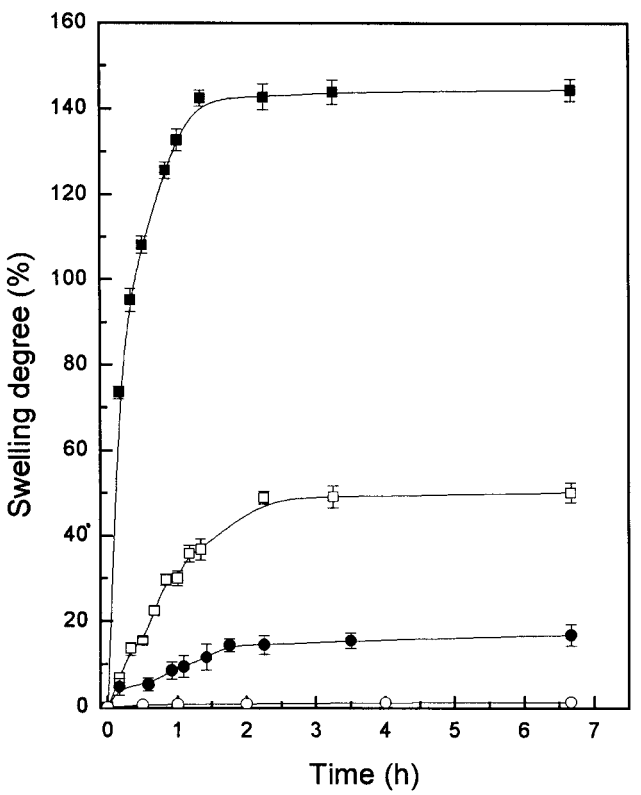

Figure 6. Swelling degree as a function of the immersion time in PBS at 37°C. (■) C 48-52-0; (□) C 48-22-30; (●) C 50-12-38; and (○) C 52-0-48.

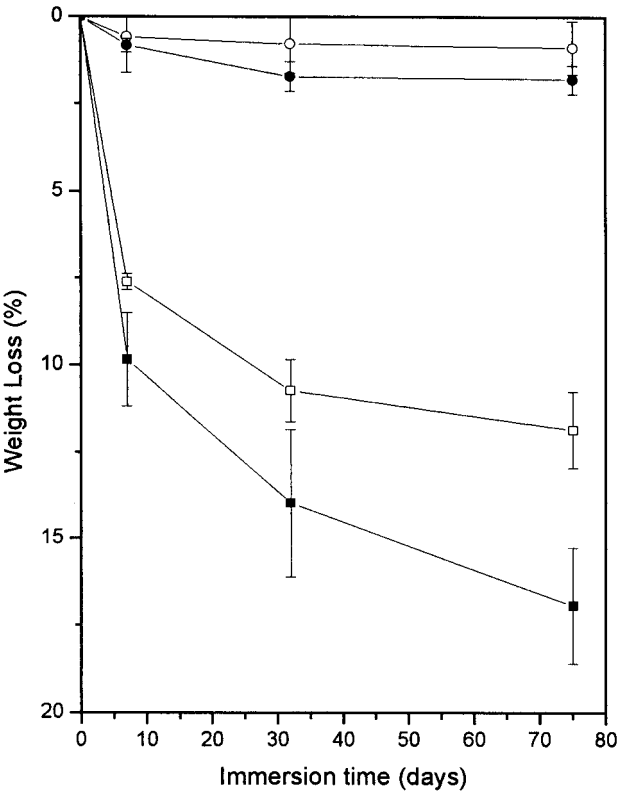

Figure 7. *In vivo* degredation of the graft copolymers in PBS at 37°C. (■) C 48-52-0; (□) C 48-22-30; (●) C 50-12-38; and (○) C 52-0-48.

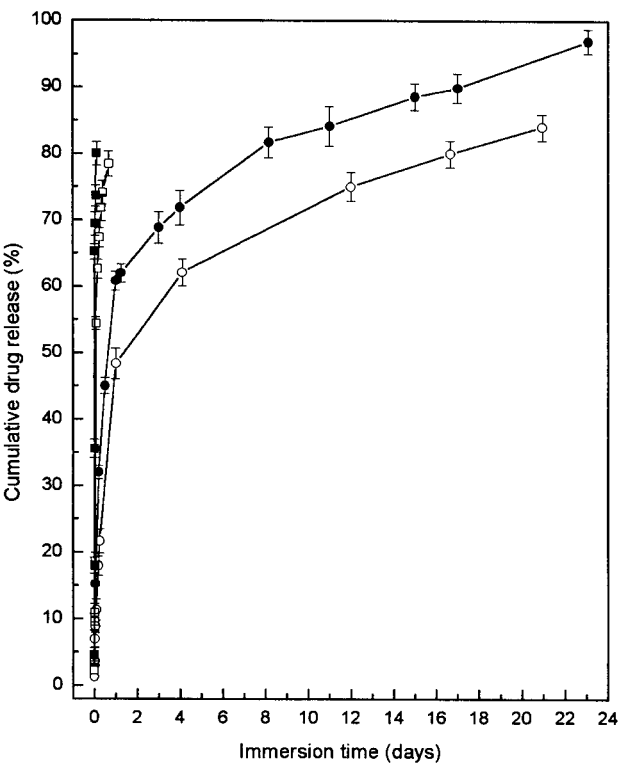

Figure 8. *In vivo* release profiles of drug-loaded polymer discs. (■) C 48-52-0; (□) C 48-22-30; (●) C 50-12-38; and (○) C 52-0-48.

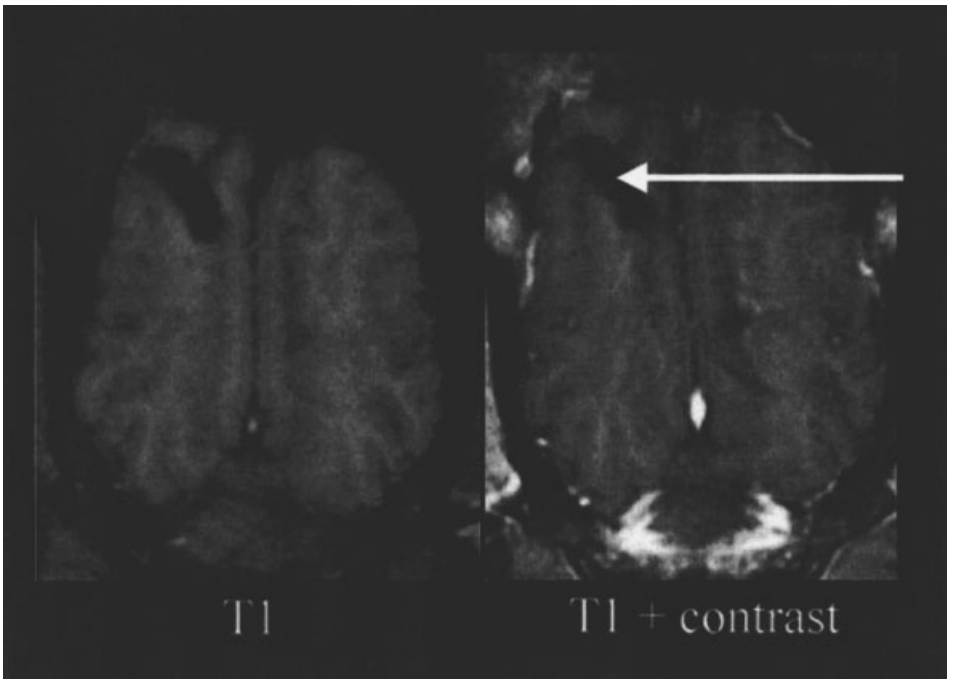

Figure 9. T1-weighted magnetic resonance images obtained after 2 months intracerebral implantation of a drug-loaded polymer disc C 52-0-48 (arrow). Right: After contrast administration.

higher was the DMAm content, the higher was the hydrophilic character of the system and consequently the higher was the swelling degree.

On the other hand, the *in vitro* degradation kinetic was found to be intimately related to the swelling and again controlled by the composition. Thus, the higher was the water uptake, higher was the ester hydrolysis rate (as shown in Fig. 7). Moreover, the higher was the

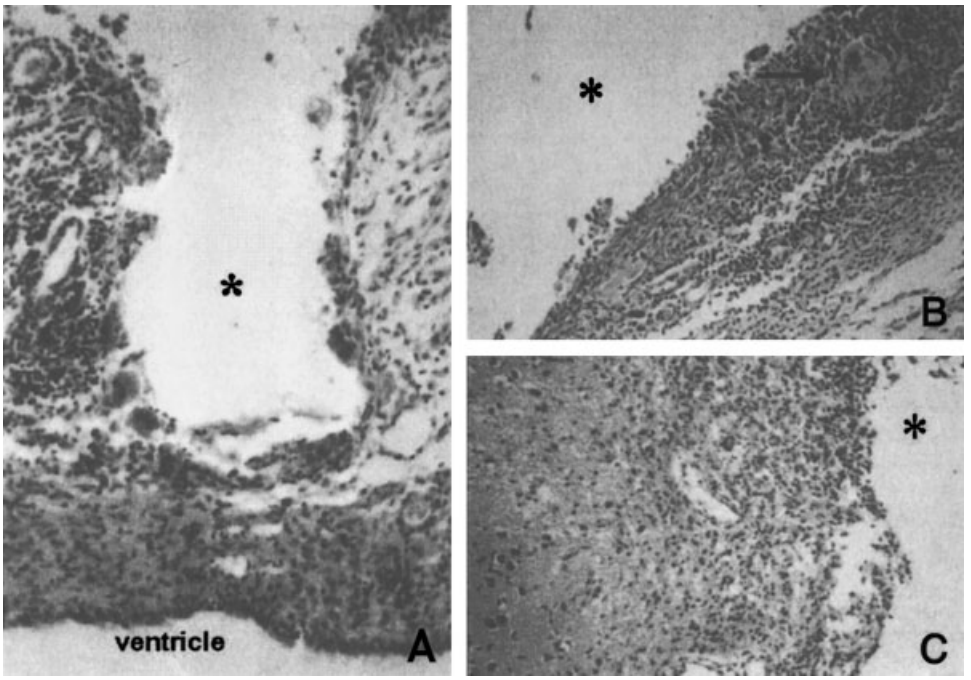

Figure 10. Histological aspects of the cerebral tissue around the implanted discs. (A) The cavity that remains after the retrieval of the disc (*) is surrounded by scarce inflammatory cells, mainly lymphocytes and some macrophages [hematoxylin and eosin (HE), ×100]. (B) Inflammatory tissue with foreign-body cells (arrow) surrounding the cavity where the intracerebral disc was implanted (*). HE, ×75. (C) Inflammatory and conjunctive reaction was observed only in the vicinity of the implant cavity (*). On the left side, normal cerebral tissue is observed. HE, ×100.

DMAm content, the higher was the solubility in water of the methacrylic residues. Therefore, the swelling and the degradation rate of the polyester component can be tailored at some extent by the composition of the acrylic part.

In vitro drug release

Figure 8 shows the cumulative glycoside release as a percentage of total drug initially loaded into the polymer. The drug release depended on composition and swelling, which also was related to the degradation rate as it has been indicated above.

The main factor that controls the release rate was the swelling degree, which determined the characteristics of the diffusion across the polymer matrix. At high water uptakes as for the C 48-52-0 sample (144%) the diffusion of this highly hydrosoluble drug through such a swollen matrix was highly facilitated, and it was released in few hours. As the polymer matrix reduced its water uptake, the diffusion was slowed as well the release rate. In this way, the C 50-12-38 and the C 52-0-48 systems with equilibrium water uptakes of 16.8 and 1.2%, respectively, exhibited a release profile of the glycoside during several days.

PCL has been reported to control the permeability and release of certain type of hydrophobic drugs.[21,22] However, in this work, from the comparison of the results of release profile (Fig. 8) and those of the swelling behavior or degradation (Figs. 6 and 7, respectively), seems that the main factor controlling the kinetic of the drug release was the hydrophilic character of the polymeric support system, which is related to the microstructure or structural distribution of the PCL grafted chains along the copolymer backbone.

In vivo behavior: microscopic observation

The important complication of medical devices are largely based on biomaterials-tissue interactions that include both effects of the implant on the host tissues and effects of the host on the implant.[23] The copolymer C 52-0-48 was selected for a preliminary study of *in vivo* implantation because of both lower swelling degree and drug release kinetics. In order to evaluate the tissue reaction around the implanted disc, MRI studies were performed 2 months postoperatively. Figure 9 shows T1-weighted images (spin-lattice relaxation time) before and after intravenous administration of a paramagnetic contrast agent [Gadolinium (III) complex]. Discs were identified as hypotense material, as much in T1- as in T2-weighted images, without the appreciation of enhancement around the discs after application of the contrast agent. In all cases the formation of images that can suggest demyelinization, edema, or necrosis of the cerebral tissue around the implanted disc was not observed.

Histological results indicate that in all cases an inflammatory response of variable intensity and small amount of fibrous tissue were observed surrounding the implant cavity, but this alterations were only closely to it, and this fact did not affect the adjacent

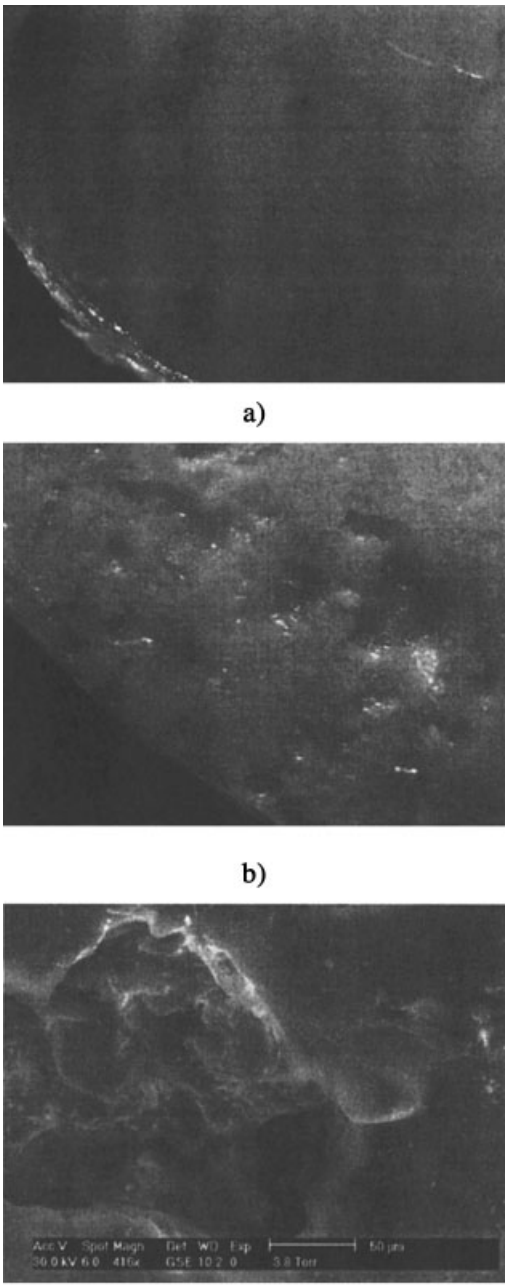

Figure 11. (a) Opticla micrograph of drug-loaded disc C 52-0-48 (original magnification , ×20) before implantation; (b) same sample after 2 months' implantation; (c) ESEM micrograph after 2 months' implantation.

tissue (Fig. 10). Again, as mentioned above for MRI studies, demyelinization, edema, or necrosis of the cerebral tissue were not observed in microscopic images. The results obtained allow to demonstrate the very good tolerance of the cerebral tissue with the implanted disc.

The formation of porous structures in the polymeric matrix of explanted discs was observed by optical microscopy and ESEM (Fig. 11). The porous can be associated to the release of the drug and the biodegradation of the polymeric support. Moreover, the residual drug released *in vivo* after 2 months' implantation was lower than 3% (w/w) as determined in the retrieved discs by HPLC. Further studies of the effect of this controlled delivery system on gliomas produced in rats are in progress.

CONCLUSION

The graft copolymers of PCL onto acrylic backbones prepared in this work could be applied as partially biodegradable supports for antitumoral drugs, with a controlled release of the active compound modulated by the hydrophilic/hydrophobic character of the system. The drug release seems to be carried out mainly by diffusion through the swollen polymeric matrix, in a cooperative way with the biodegradation of PCL-grafted acrylic chains. The average composition of these materials control the biodegradative behavior and therefore the resorption of the whole support.

In vivo experiments demonstrated a good tolerance of implanted drug-loaded polymer discs with the cerebral tissue. Further investigations about the drug release behavior of these systems *in vivo* as well as its potential applications in cancer brain surgery are in progress.

The authors thank G. Domenech for HPLC measurements. G.A.A. is the recipient of a fellowship from the Ministerio de Educación y Cultura of Spain.

References

1. Santos-Benito FF, Fernández-Mayoralas A, Martín-Lomas M, Nieto-Sampedro M. Inhibition of proliferation of normal and transformed neural cells by blood group-related oligosaccharides. J Exp Med 1992;176:915–918.
2. Coterón JM, Singh K, Asensio JL, Domínguez-Dalda M, Fernández-Mayoralas A, Jiménez-Barbero J, Martín-Lomas M, Abad-Rodríguez J, Nieto-Sampedro M. Oligosaccharides structurally related to E-selectin ligands are inhibitors of neural cell division: synthesis, conformational analysis, and biological activity. J Org Chem 1995;60:1502–1519.
3. Nieto-Sampedro M, Bailón C, Fernández-Mayoralas A, Martín-Lomas M, Mellström B, Naranjo JR. Experimental brain glioma: growth arrest and destruction by a blood-group-related tetrasaccharide. J Neuropathol Exp Neurol 1996;55:169–177.
4. Aguilera B, Romero-Ramírez L, Abad-Rodríguez J, Corrales G, Nieto-Sampedro M, Fernández-Mayoralas A. Novel disaccharide inhibitors of human glioma cell división. J Med Chem 1998;41:4599–4606.
5. Bernabé M, Fernández-Mayoralas A, Nieto-Sampedro M, Vaquero J, Zurita M. *N*-acetyl-6-*O*-[2,2-bis(hydroxymethyl)-3-hydroxypropyl]-D-glucosamine glycosides, procedure of obtaining and use for treatment of brain tumors. Applied Spanish Patent No. 200000982, 2000.
6. Rosen HB, Chang J, Wnek GE, Linhardt RJ, Langer R. Bioerodible polyanhydrides for controlled drug delivery. Biomaterials 1983;4:131–133.
7. Leong KW, Brott BC, Langer R. Bioerodible polyanhydrides as drug-carrier matrixes. I: Characterization, degradation, and release characteristics. J Biomed Mater Res 1985;18:941–955.
8. Domb AJ, Langer R. Polyanhydrides: I. Preparation of high molecular weight polymers. J Polym Sci 1987;25:3373–3386.
9. Valtonen S, Timonen U, Toivanen P, Kalimo H, Kivipelto L, Heiskanen O, Unsgaard G, Kuurne T. Interstitial chemotherapy with carmustine-loaded polymers for high-grade gliomas: a randomized double-blind study. Neurosurgery 1997;41:44–48.
10. Pitt CG, Schinder A. Capronor—A biodegradable delivery system for levonorgestrel. In: Zatuchni GL, editor. Long-acting contraceptive delivery systems. Philadelphia: Harper and Row; 1984. p 63–84.
11. Cunxian A, Pengyan W, Hongfan S, Hualian S, Qing Y, Xiumin C, Ruiwen S. The in vivo degradation, adsorption and excretion of poly(ε-caprolactone). J Biomed Eng 2000;17:25–28.
12. Löfgren A, Albertsson A-C, Dubois P, Jérôme RJ. Recent advances in ring-opening polymerization of lactones and related compounds. J Mater Sci Rev Macromol Chem Phys 1995;C35(3):379–418.
13. Johns DB, Lenz RW, Luecke A. Lactones. In: Ivin KJ, Saegusa T, editors. Ring-opening polymerization. London: Elsevier Applied Science; 1984. Vol.1, p 461–521.
14. Fukuzaki H, Yoshida M, Asano M, Minoru K. Synthesis of low-molecular-weight copoly(L-Lactic acid/ε-caprolactone) by direct copolycondensation in the absence of catalysts, and enzymatic degradation of the polymers. Polymer 1990;31:2006–2014.
15. Jérôme R, Mecerreyes D, Tian D, Dubois Ph, Hawker CJ, Trollsas M, Hedrick JL. Synthesis of novel polymeric materials based on aliphatic polyesters by combination of different controlled polymerization methods. Macromol Symp 1998;132:385–403.
16. Schulz GO, Milkovich R. Graft polymers with macromonomers. I. Synthesis from methacrylate-terminated polystyrene. J Appl Polym Sci 1982;27:4773–4786.
17. Eguiburu JL, Fernández-Berridi MJ, San Román J. Graft copolymers for biomedical applications prepared by free radical polymerization of poly(L-lactide) macromonomers with vinyl and acrylic monomers. Polymer 1997;37:3615–3622.
18. Wunderlinch B, Cheng S ZD, Loufakis K. Thermodynamic properties. In: Mark HF, Bikales NM, Overberger CG, Menges G, Kroschwitz JI, editors. Encyclopedia of polymer science and engineering. Vol. 16. 2nd ed. New York: Wiley-Interscience; 1985. p 777.
19. Abraham GA, Gallardo A, Motta A, Migliaresi C, San Román J. Microheterogeneuos polymer systems prepared by suspension polymerization of methyl methacrylate in the presence of poly(ε-caprolactone). Macromol Mater Eng 2000;282:44–50.
20. Avella M, Errico ME, Immirzi B, Malinconico M, Falcigno L, Paolillo L. Radical polymerization of methyl methacrylate in the presence of biodergadable poly(L-lactic acid). Preparation

of blends, chemical-physical characterization and morphology. Macromol Chem Phys 2000;201:1295–1302.
21. Schindler A, Jeffcoat R, Kimmel GL, Pitt CG, Wall ME, Zweidinger R. Biodegradable polymer for sustained drug delivery. In: Pearce EM, Schaefgen JR, editors. Contemporary topics in polymer science. Vol. 2. New York: Plenum Press; 1977. p 251–289.
22. Peppas NA, Korsmeyer RW. Dynamically swelling hydrogels in controlled release applications. In: Peppas NA, editor. Hydrogels in medicine and pharmacy, Vol.III. Boca Raton, FL: CRC Press; 1987. Chapter 6, Properties and applications. p 109–149.
23. Anderson JM, Gristina AG, Hanson SR, Harker LA, Johnson RJ, Merritt K, Naylor PT, Schoen FJ. Host reactions to biomaterials and their evaluation. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, editors. Biomaterials science: an introduction to materials in medicine. London: Academic Press, 1996. p 165–214.