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Poly(ester–ether–ester) block copolymers as biomaterials

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Poly(ester–ether–ester) block copolymers, belonging to a class of biodegradable materials, were synthesized from poly(ethylene glycol) and ε-caprolactone by a simple ring-opening mechanism, which avoids the use of potentially toxic inorganic or organometallic initiators. The morphological and mechanical properties of such materials were investigated by gel-permeation chromatography, vapour pressure osmometry, proton magnetic resonance, infrared spectroscopy, differential scanning calorimetry, X-ray diffractometry and stress–strain tensile tests. The biocompatibility was investigated by cytotoxicity and hemocompatibility tests; the cytotoxicity was tested by the Neutral Red uptake assay, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay, the Kenacid Blue R-binding method, and by the cell proliferation test on polymer films; the hemocompatibility was tested by the contact activation both of the coagulation cascade (intrinsic pathway), by the plasma prekallikrein activation test, and of the thrombocytes, by measuring the release of platelet factor 4 and β-thromboglobulin. The experimental results show that such a polymerization process permits high-molecular mass block copolymers with relatively good tensile and mechanical properties to be obtained. Their cyto- and hemo-compatibility makes them suitable for employment as biomaterials.

1. Introduction

Biodegradable polymers are widely used in such biomedical applications as drug delivery systems, surgical sutures, bone fracture healing devices and partially resorbable vascular grafts. A new class of biodegradable materials, the poly(ester–ether–ester) block copolymers, have been recently proposed for biomedical applications, since their properties appear very promising [1, 2]. Such types of copolymers are generally synthesized by means of inorganic or organometallic catalysts, which can leave toxic impurities in the biomaterials: consequently, non-catalysed synthetic procedures are generally preferable.

In this paper, we report the synthesis and characterization of tri-block poly(ε-caprolactone)-poly(oxyethylene)-poly(ε-caprolactone) copolymers obtained by reacting poly(ethylene glycol)s (PEG) with ε-caprolactone (CL) in bulk under vacuum at 185 °C, without added catalysts. The copolymer formation occurs according to a ring-opening mechanism, where the active hydrogen atoms of the preformed PEG induce a selective acyl-oxygen cleavage of the lactone ring [3–5]. Starting from PEG prepolymers with different molecular mass and varying the initial CL/PEG molar ratio, copolymers with different molecular mass, composition and average length of the blocks can be obtained.

To investigate the biocompatibility of the copolymers synthesized, both their cytotoxicity and their hemocompatibility were tested.

The lack of cytotoxic effects is one of the most important properties to assess the biocompatibility of new materials identified as candidates for the construction of artificial prostheses. To estimate the cytotoxic characteristics of the new materials, two levels of test were performed:

1. Level **I** cytotoxicity tests, e.g. the Neutral Red uptake (NR) assay; the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay; and the Kenacid Blue R-binding (KB) method, early in the synthesis program.

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2. Level II cytocompatibility tests, e.g. the cell proliferation test on polymer films.

The hemocompatibility was tested by the contact activation both of the coagulation cascade (intrinsic pathway) and of the thrombocytes. The start of the intrinsic pathway was tested by measuring the activation of plasma prekallikrein (PKK) to kallikrein (KK) [6, 7]. The thrombocyte activation was tested by evaluating platelet factor 4 (PF4) and β-thromboglobulin (βTG), two platelet-specific proteins secreted by the α-granules during the release reactions induced by the contact between blood and materials [8, 9].

2. Materials and methods

2.1. Materials

ε-Caprolactone (CL): the commercial product (Janssen Chimica) was purified by vacuum distillation over CaH_2. The fraction collected at 96–98 °C (5 mm Hg) was used in all the polymerization experiments.

Poly(ethylene glycol)s (PEG): commercial products (Fluka) with molecular mass of 9200 (PEG 9200), 20 000 (PEG 20 000) and 35 000 (PEG 35 000) were extracted using boiling ethyl ether and used without further purification.

2.2. Polymerization procedure

The reaction mixtures were prepared by introducing under a nitrogen atmosphere a known volume of CL monomer into a Pyrex phial containing a pre-weighed amount of PEG. The phial was connected to a vacuum line, evacuated, sealed off and placed in a thermostat at 185 °C. After the quantitative conversion of the CL–PEG mixture, the copolymer was recovered in the form of a solid, showing different degrees of friability depending on the CL to PEG ratio.

2.3. Characterization tests

Gel permeation chromatography (GPC): polymer GPC analyses were carried out using a Millipore-Waters apparatus with tetrahydrofuran (THF) as an eluant and Ultrastyragel columns with different pore size. Chromatographic diagrams of the different polymerization products showed that all the materials were free from undesired CL homopolymer (PCL) and unreacted PEG.

Vapour pressure osmometry (VPO): number-average molecular masses ($\overline{M}_n$) were measured in 1,2-dichloroethane solution at 30 °C, using a Wescan 233 isopiestic osmometer. Sucrose octaacetate was used as the calibration standard. The copolymers tested were those with $\overline{M}_n$ values lower than 25 000 Da, which is the instrumental limit of the osmometer used.

Proton magnetic resonance spectroscopy (^{1}H NMR): spectra of polymers in $CDCl_3$ solution were recorded on a Bruker instrument with a relaxation delay of 0.9 s. The scan numbers were in most cases 32 and the sample concentrations were in the range 20–30 mg cm^{-3}. Chemical shifts were referred to tetramethylsilane, assuming the $CHCl_3$ signal as resonating at 7.26 ppm. The temperature of the probe was 25 °C.

Infrared spectroscopy (IR): the IR analysis of the copolymers, as solid films obtained by melting the samples on KBr discs, was carried out with a Perkin-Elmer 283-B instrument.

Differential scanning calorimetry (DSC): the measurements were carried out on a Perkin-Elmer DSC-4 instrument equipped with an Intracooler-I apparatus. Samples were cooled from the melt at a 0.1 °C min^{-1} rate.

X-ray diffractometry (RX): X-ray diffraction diagrams of powder samples of different copolymers were obtained with a Siemens 810 apparatus using CuK_α radiation.

Stress–strain testing: the mechanical characterization of the copolymers was performed on an Instron 4302 instrument, with a 5 mm min^{-1} crosshead speed. Both the "yield-stress" (σ_y) and the "elongation at break" (ε_r) of film and fibre samples were measured.

2.4. Cytotoxicity and cytocompatibility tests

The copolymers C23, C24, C25 and C26 underwent NR, MTT and KB assays. Modified FRAME protocols [10] were used. Two series of 100 mg samples were prepared: 5 ml of phosphate buffered saline (PBS) was added to one set of samples which were then sterilized by autoclave (121 °C, 1 h). The second set was first sterilized by ethylene oxide, then added with 5 ml of PBS and left at 37 °C for 5 days. Extracts were filtered by 0.2 μm cellulose acetate filters (Nalgene) and used for the assays. 3T3 cells, taken from a mouse fibroblast cell line, were seeded onto 96-well plates at two different densities: 1.2×10^4 cells/cm^2 for the 24 h and 6×10^3 cells/cm^2 for the 72 h exposure period assay.

24 h after seeding (a.s.), 100 μl/well of the extracts were added to the wells, then the plates were incubated for 24 h or 72 h at 37 °C in a CO_2 atmosphere. No extracts were added to negative control (NC) wells, while 2,4-dinitrophenol (70 μg ml^{-1}) was added to the positive control wells. After the incubation period, the procedures were different for the 3 assays.

NR assay: the medium was replaced with 150 μl NR medium (50 μg ml^{-1}) per well. The plates were incubated for 3 h at 37 °C, the NR medium was removed, the cells were rinsed twice with PBS and 150 μl of destain solution (1% glacial acetic acid + 50% ethanol + 49% distilled water) per well were added. The plates were shaken for 10 min and the absorbance was read at 540 nm against a reference well which contained no cells.

MTT assay: the medium was replaced with 10 μl MTT solution per well. After 4 h incubation the solution was removed, 100 μl DMSO were added and after 5 min of slow agitation the absorbance was read at 550 nm.

KB assay: the medium was replaced with 150 μl KB dye per well. After a 20 min slow agitation, the dye was aspirated and the wells washed twice with a washing solution. The desorbing solution was then added and

the plates shaken for 20 min. The absorbance was read at 570 nm.

Cell adhesion and proliferation test: Films were obtained from copolymers C23 and C25 by evaporating 2% (W/W) solutions in $CHCl_3$, on the bottom of glass Petri dishes (area 20 cm^2), sterilized with ethylene oxide and precoated with fibronectine (5 $\mu g\,ml^{-1}$). Human umbilical vein endothelial cells (HUVEC) were seeded on the polymer films in duplicate, at the density of 3×10^5 cells/ml. HUVEC were trypsinized at 40 h, 65 h and 88 h a.s. Cell numbers were estimated by total protein determination (Biorad Protein Assay).

2.5. Hemocompatibility tests

The activation of PKK to KK, caused by the contact of plasma with a foreign material, was determined by the proteolytic reaction between KK and the chromogenic substrate H–D–Pro–Phe–Arg–pNa (S-2302 Kabi Diagnostica). A pool of citrated plasma from eight healthy donors was dispensed in samples of 0.5 ml, and then frozen at − 20 °C. Before carrying out the activation test, the plasma was thawed at 37 °C and diluted 1:10 with 0.05M TRIS-HCl buffer (pH = 7.8). Volumes of 0.50 ml of diluted plasma were put in plane-bottom borosilicate glass tubes of 0.50 cm inner radius, both uncoated and internally coated with the materials to be tested, and incubated at 37 °C under a constant stirring speed (1100 rpm) for 3 min; 0.2 ml of activated plasma were added to 0.2 ml of 2 mM solution of S-2302 and 0.6 ml of the same buffer in a spectrophotometric cell thermostated at 37 °C. *p*-Nitroaniline release from S-2302 was followed spectrophotometrically at 405 nm (molar absorptivity 9950); each initial rate was obtained by extrapolating to zero conversion the logarithms of the instantaneous rates, calculated by a "tailor made" computer program. Kallikrein-like activity (KLA) values (in units per litre) were obtained from the initial rates by the "initial velocity" method [7, 11].

To carry out the thrombocyte activation test, human blood was collected into sodium citrate anticoagulant (1:9). Just after the venipuncture (no blood-flow stopping), 2 ml of blood were put into Petri dishes (6 cm inner diameter) with 14.8 $cm\,ml^{-1}$ contact surface, then thermostated at 37 °C and gently stirred on a tilting table. After a 15 min contact time, aliquots of the activated blood were collected, treated with 2-chloroadenosine and procaine-HCl (1:5) to arrest any further platelet activation and finally centrifuged (3000 rpm at 0 °C for 30 min) to separate plasma from cells. The so obtained plasma was then diluted with a phosphate buffer containing albumin and Tween 20, in order to measure the amounts of PF4 and βTG by the Enzyme Linked Immuno-Sorbent Assay (ELISA).

The ELISA test is based on the "sandwich" principle. In the first incubation step, an antibody specific for the antigen (PF4 or βTG) is coated onto a plastic support. In the second incubation step, the first immunologic reaction, the antigen in the sample is bound by this antibody. Since antigen has several antigenic determinants, a second immunologic reaction peroxidase (POD)-labelled antibodies occurs, with the formation of "sandwich" antigen-POD complexes in a quantity proportional to the antigen content in the sample. The unbound POD conjugate is removed in the following washing step (separation of bound and free antigens). The wall-bound POD activity is determined photometrically at 492 nm after addition of H_2O_2 and chromogen *o*-phenylenediamine. The results are obtained from a calibration curve that must be set up for each series using standard PF4 and βTG samples. A commercial kit (Boehringer, Mannheim) was used to carry out the test, following the standard procedure suggested by the manufacturer.

3. Results and discussion

The experimental data concerning the feed composition together with the molar compositions and the molecular weights of the copolymers obtained are collected in Table I.

Three series of copolymers were obtained by using PEGs with $\overline{M}_n$ values of 9200, 20 000 and 35 000, respectively. All the polymerizations were carried out at 185 °C, since in preliminary experiments it was found that at this temperature a quantitative conversion to copolymer can be obtained in a suitable time period varying from 48 to 72 h, depending on feed composition. Higher temperatures were not used since it was found that above 200 °C an intermolecular dehydration of the hydroxyl end groups of the copolymer takes place, with an undesired formation of double bonds. Copolymerization progress was checked in most cases by GPC chromatography,

TABLE I Experimental results concerning block copolymers obtained by reacting CL with PEGs with different molecular masses in bulk at 185 °C

Exp. number	Feed			Copolymer composition (mol %)		$10^{-4}\overline{M}_n$
	(mole ratio) CL/PEG	(wt %)				
		CL	PEG	CL	OE	
		with PEG 9200				
C13	37.0	31.2	68.8	15	85	1.34
C17	98.1	54.8	45.2	32	68	2.04
C12	161.2	64.8	35.2	42	58	2.76
C15	418.0	83.8	16.2	67	33	5.69
C14	740.0	90.1	9.9	78	22	9.37
		with PEG 20000				
C19	80.0	31.5	68.5	15	85	2.91
C20	328.0	65.1	34.9	42	58	5.75
C21	906.2	83.8	16.2	67	33	12.35
C16	1613.4	90.2	9.8	79	21	20.40
		with PEG 35000				
C24	143	31.7	68.3	14	86	4.99
C26	531	64.0	36.0	35	65	8.32
C28	759	72.2	27.8	48	52	11.75
C25	1475	82.8	17.2	62	38	18.73
C27	1493	85.0	15.0	66	34	20.37
C23	2878	90.2	9.8	70	30	23.68

which permits one to follow the progressive consumption of CL monomer and of unreacted PEG with the appearance of a new elution peak due to the formation of a PCL–POE–PCL copolymer.

Experimental GPC diagrams also showed that all the copolymers obtained have $\overline{M}_w/\overline{M}_n$ ratios ranging from 1.15 to 1.20. Molar compositions of the copolymers reported in Table I were determined from ^{1}H NMR spectra carried out at 100% conversion and are expressed as the molar percentage (mol %) of ε-caprolactone (CL) and oxyethylene (OE) units in the copolymer. From the ^{1}H NMR spectra $\overline{M}_n$ values of the different copolymers were also obtained and compared, if lower than 25 000 Da, with the corresponding values determined by VPO. In all cases the differences between respective values were within 2–4%.

The ether–ester nature of the copolymers has been proved by IR and ^{1}H NMR spectroscopies, which demonstrated also the presence of two terminal hydroxyl groups per macromolecule. All the copolymers obtained exhibit cristallinity, even if they show a quite different behaviour depending on the different molar compositions. The crystallization pattern of different copolymers has been investigated by RX and DSC measurements.

The calorimetric traces (Fig. 1) indicate that the PCL blocks crystallize in all the cases, independently of the copolymer composition, while the central POE blocks crystallize only in the copolymers richer in oxyethylene units (OE units ⩾ 58 mol %, as shown in Table I). These results show that the crystallizability of POE segments is significantly affected by the links of its ends to PCL blocks of various lengthes. In contrast, there is a tendency of PCL blocks in the copolymer to crystallize over the whole range of composition examined.

The calorimetric results are confirmed by the X-ray analysis carried out on powder samples, which indicate the typical reflections of the orthorhombic structure of PCL blocks (2θ = 21.2, 21.8 and 23.5) in all the copolymers obtained, and the POE reflections (2θ = 18.9 and 23.0) only in copolymers with OE units ⩾ 58 mol %. Fig. 2 shows typical RX diagrams for pure homopolymer samples and for two copolymers with different compositions.

Fibre samples were obtained from the melt of the copolymers with higher molecular masses. These fibres exhibit the typical behaviour of ductile polymers, i.e. after reaching a load maximum (yield stress: $\sigma_y \cong 9$ MPa) as strain increases, flow of the sample takes place at a slightly increasing stress and ultimately the specimen fails at an elongation $\varepsilon_r \cong 700\%$ (elongation at break), as shown in Fig. 3 for the sample C16 in Table I.

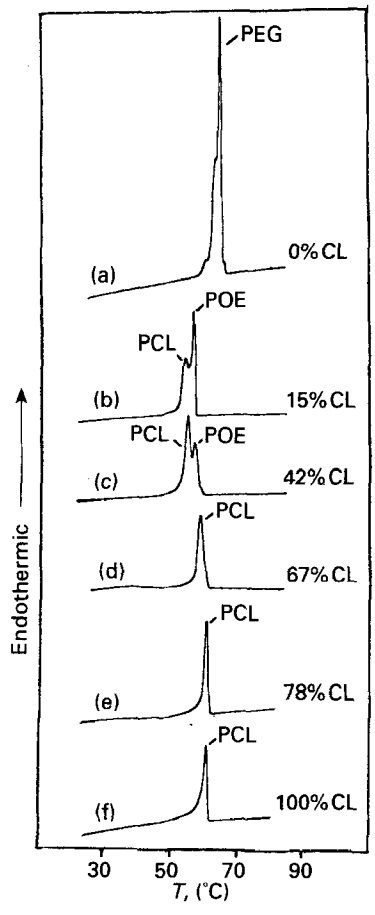

Figure 1 DSC thermograms of PEG (a), PCL (f) homopolymers and PCL–POE–PCL copolymers with different molar compositions; (b) sample C13; (c) sample C12; (d) sample C15; (e) sample C14 in Table I.

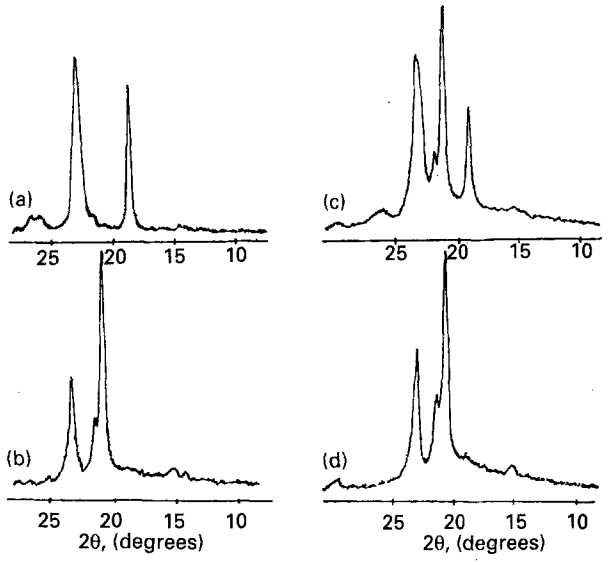

Figure 2 Powder X-ray diffraction diagrams of PEG (a), PCL (b) homopolymers and typical PCL–POE–PCL copolymers with different molar compositions; (c) sample C12; (d) sample C14 in Table I.

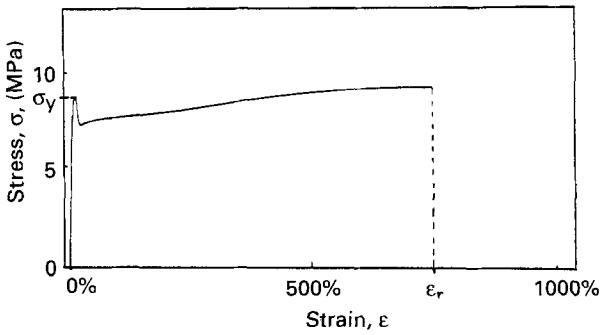

Figure 3 Typical stress–strain behaviour for a fibre sample obtained from the copolymer C16 in Table I.

Preliminary cytotoxicity experiments showed that the sterilizing agent (autoclave or ethylene oxide) did not significantly (one-factor ANOVA, $p > 0.05$) affect the results (data not shown). We therefore adopted as a routinary procedure sterilization based on ethylene oxide.

NR assay: Neutral Red uptake was significantly affected by the copolymer nature (2-factor ANOVA, $p < 0.05$) and by the cell exposure duration to copolymer extracts ($p < 0.01$). After a 24 h exposure period (Fig. 4), copolymer C25 gave significantly different results (F-test, $p < 0.05$) from those of the negative control, while after 72 h both copolymers C25 and C26 behaved worse than the negative control ($p < 0.05$).

MTT assay: the results were dependent both on copolymer nature (2-factor ANOVA, $p = 0.01$) and exposure time ($p = 0.0001$). The separate comparison of the copolymers at 24 and 72 h with the negative controls (Fig. 5) showed no significant differences (F-test, $p > 0.05$). On the contrary, copolymer C24 extract showed a positive influence on mithocondrial functionality compared with the negative control (F-test, $p < 0.05$).

KB assay: total protein content (Fig. 6) was not affected by either copolymer type (2-factor ANOVA, $p > 0.05$) or exposure time ($p > 0.05$). Even the comparison of each copolymer with the negative control

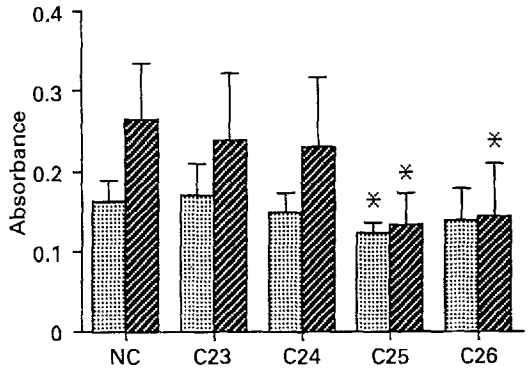

Figure 4 NR assay: viability of 3T3 cells is measured as a function of Neutral Red uptake into the lysosomes and expressed as absorbance at 540 nm. Means of three experiments ($n = 12$, mean ± S.E.; *significant at the 95% level). ▨ NR 24 h; ▧ NR 72 h.

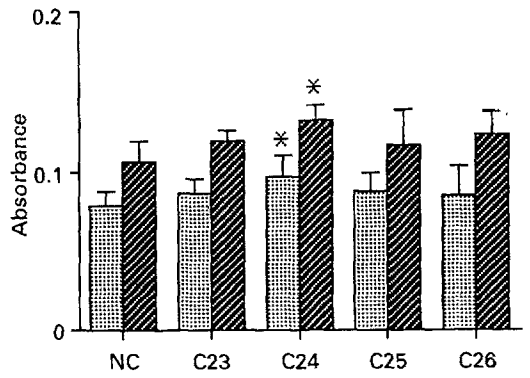

Figure 5 MTT assay: viability of 3T3 cells is measured as a function of mitochondrial integrity and expressed as absorbance at 550 nm. Means of two experiments ($n = 10$, mean ± S.E.; *significant at the 95% level). ▨ MTT 24 h; ▨ 72 h.

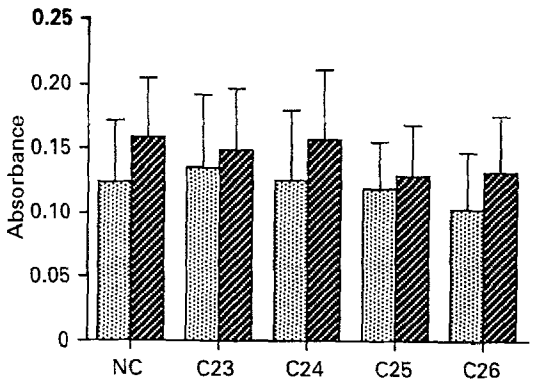

Figure 6 KB assay: total protein content of the 3T3 cultures exposed to the different copolymer extracts and expressed as absorbance at 570 nm. Means of two experiments ($n = 10$, mean ± S.E.). ▨ KB 24 h; ▧ KB 72 h.

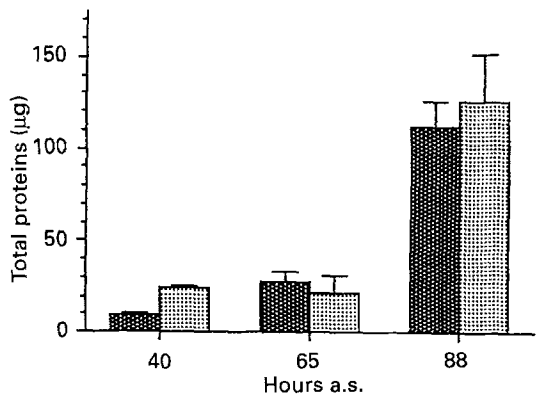

Figure 7 Cytotocompatibility test: HUVEC were seeded on C23 and C25 copolymer films and their growth, expressed as total cell protein content, tested at fixed time intervals. Means of two experiments ($n = 4$, mean ± S.E.) ▩ CL 23; ▨ CL 25.

both at 24 and 72 h exposure did not show any significant difference (F-test, $p > 0.05$).

Cell adhesion and proliferation test: the results show (Fig. 7) that HUVEC growth curves on both copolymers C23 and C25 follow normal patterns of growth with a lag phase followed by an exponential phase. The 2-factor ANOVA, in which the significant variable is total protein content, and the two factors simultaneously tested are time and copolymer nature, reveals that there is no significant difference between copolymers C23 and C25, while time significantly ($p < 0.001$) affects the results.

The rationale of our cytotoxicity tests is that cells in culture proliferate at a known optimal rate, which would be reduced by chemicals that affect one or more essential functions, such as mitochondrial activity, DNA synthesis, maintenance of membrane integrity or protein synthesis. The cytotoxic effects of chemicals upon culture cells can therefore be measured by the change in total cell protein content arising from the cell proliferation inhibition. Growth inhibition degree, compared with the negative control, provides a toxicity indication.

Neutral Red is preferentially taken up into cell lysosomes. Any chemicals having a localized effect upon the lysosomes will, therefore, affect cell viability and cell number. This factor makes this method useful

to detect those chemicals that selectively affect the lysosomes, especially when it is used in conjunction with other tests capable of determining cell number (e.g. KB assay).

In the MTT assay the tetrazolium salt is taken up into the cells and reduced in a mitochondria-dependent reaction to yield a formazan product. The product accumulates within the cells, because it cannot pass through the plasma membrane. On solubilization of the cells, the product is liberated and can readily be detected and quantified by a simple colorimetric method. The ability of the cells to reduce MTT provides an indication of mitochondrial integrity and activity that, in turn, may be interpreted as a measure of viability.

Our results suggest that none of the copolymer extracts under study affects cell proliferation as shown by the total protein measures at both 24 and 72 h (Fig. 6), even if copolymers C25 and C26 seem to exert some damaging effects on lysosomal function (Fig. 4). The mitochondrial activity of the cells seems also not to suffer from the exposure to copolymer extracts (Fig. 5). In contrast, copolymer C24 seems to exert a beneficial effect on cell viability, as revealed by the MTT assay (Fig. 5).

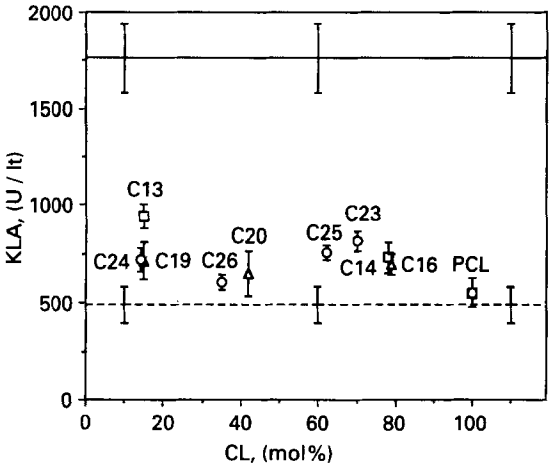

Figure 8 KLA versus molar percentage of CL in the copolymers for the following materials: borosilicate glass (——); silicone (- - -); PCL (100 mol % CL); the following PCL–POE–PCL copolymers: C13 (15 mol % CL) and C14 (78 mol % CL), from PEG 9200 (□); C19 (15 mol % CL), C20 (42 mol % CL) and C16 (79 mol % CL), from PEG 20 000 (△); C24 (14 mol % CL), C26 (35 mol % CL), C25 (62 mol % CL) and C23 (70 mol % CL), from PEG 35 000 (○). Plasma contact time 3 min at 37 °C.

The cytocompatibility (including cytotoxicity) test (Fig. 7) on the two copolymer films C23 and C25, which show mechanical properties suitable to support cell seeding, suggests that HUVEC proliferation occurs equally well on both substrates. Therefore, the relative lysosomal impairment by C25 extract, suggested by the NR assay (Fig. 4), is not confirmed by the HUVEC adhesion and growth on the same copolymer.

To check the hemocompatibility of the new materials, nine of the copolymers listed in Table I were tested for the contact activation tests both of PKK and of the thrombocytes. Fig. 8 shows the KLA induced in the plasma by these copolymers, compared with that induced by PCL (100 mol % CL), borosilicate glass (as a high-activation reference) and silicone (as a low-activation reference).

Each point is the mean value of five experiments. The activation induced by the copolymers is, as expected, nearer to that induced by silicone than to that induced by glass.

The different copolymers tested can be collected into three different groups depending on their composition expressed as CL to OE units molar ratio (Table II).

A first group comprehends the copolymers with a ratio lower than 0.5, the second one those with a ratio between 0.5 and 1.0, the third one those with a ratio higher than 1.0. A statistical treatment, based on student *t*-test, has been carried out to check the presence of significant differences between the KLA values of the different groups.

Both the differences of the first versus the second group, and of the second versus the third one appear highly significant ($p < 0.01$), while the difference between the first and third group does not appear significant ($p > 0.05$).

Our results show that the second group contains the most hemocompatible copolymers, even if the difference in PKK activation between the members of the same group and the low-activation reference silicone is significant.

TABLE II Student *t*-test carried out on the KLA values shown in Fig. 8. Plasma contact time: 3 min at 37 °C

Material	CL/OE	group	Significance (f)			
			versus I	versus II	versus III	versus silicone
C13	0.17					
C19	0.18	I	—	0.0008	0.116	0.00005
C24	0.16					
C20	0.72	II	0.0008	—	0.0001	0.0061
C26	0.53					
C14	3.54					
C16	3.55					
C25	1.68	III	0.116	0.0001	—	0.0001
C23	2.23					
PCL	—	—	0.00045	0.0545	0.0001	0.1353

TABLE III Activation of the thrombocytes by PCL–PEG–PCL block copolymers after 15 min contact time at 37 °C

Exp. number	Material	$10^{-4}\,\bar{M}_n$	βTG (ng ml^{-1})	PF4 (ng ml^{-1})	βTG/PF4
1	none	—	104	45	2.31
2	C27	20.37	131	77	1.70
3	C23	23.68	119	71	1.67
4	silicone	—	143	113	1.27
5	C16	20.40	196	155	1.26
6	C20	5.75	208	171	1.22
7	C24	4.99	196	167	1.17
8	glass	—	190	178	1.07
9	C28	11.75	131	143	0.92
10	C26	8.32	161	184	0.87
11	C25	18.73	161	184	0.87

Concerning the PCL homopolymer, it shows a good hemocompatibility (no significant difference versus silicone), but it is a brittle material with very poor mechanical and tensile properties, so that it cannot be used to make biomedical devices.

Table III shows the activation of the thrombocytes measured by the release of βTG and PF4 for the reference materials and eight of the copolymers listed in Table I, together with the βTG and PF4 values in the plasma from blood never put in contact with any material. It is known [8] that the ratio of βTG to PF4 plasma levels ranges between 2.3 and 3.9; an increase of both levels and a decrease of their ratio after contact with a foreign material suggests an *in vitro* release due to platelet activation by the material.

Concerning the data shown in Table III, Exp. 1 shows that an activation occurs before the incubation of blood with the materials, since βTG and PF4 ratio is at the lower limit of the values normally found and their levels are markedly higher [8]. All the materials tested (Exp. 2-11) give values indicating a platelet activation, since the βTG and PF4 levels increase and their ratio decreases. It is noteworthy, however, that two copolymers, C27 ($\bar{M}_n = 20.37 \times 10^4$ Da) and C23 ($\bar{M}_n = 23.68 \times 10^4$ Da) exhibit a quite low thrombocyte activation, which is even lower than that induced by silicone.

4. Conclusions

High-molecular-mass block copolymers have been obtained from ε-caprolactone and poly(ethylene glycol) through a simple synthetic procedure, which avoids the use of potentially toxic initiators.

The ring-opening mechanism permits the synthesis of copolymers with different structures and molecular masses, by varying PEG chain length and CL to PEG molar ratio in the feed.

Relatively good tensile and mechanical properties have been observed for film and fibre samples obtained from materials with molecular masses higher than 18×10^4 Da.

The results of the cytotoxicity tests show that all the copolymers examined do not induce relevant cell damage. In particular, the copolymers C23, C24 and C25 give results that are not significantly different from the negative controls. Furthermore C23 and C25 constitute an excellent support for human endothelial cell adhesion and growth, as shown in Fig. 7.

Concerning the hemocompatibility, the results obtained show that all the copolymers tested induce a PKK activation relatively low and near to that of silicone (Fig. 8). In contrast, the thrombocyte activation tests show results whose interpretation is less straightforward (Table III). In particular, C27 and C23 copolymers seem to induce the lowest platelet activation.

In conclusion, the characterization tests show that the biocompatibility properties of the different copolymers are quite good. Their physico-mechanical properties are in some cases less satisfactory; nevertheless, we believe that they can be improved in order to obtain materials suitable for the production of biomedical devices.

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