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PLGA/PEG-derivative polymeric matrix for drug delivery system applications: Characterization and cell viability studies

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Abstract

The incorporation of additives such as polyoxyethylated oleic acid glycerides (PEG-derivative) can modify the release of drugs from microparticles. PEG-derivative decreases the release rate of drugs that are dissolved in PLGA matrices but if un-dissolved the initial release rate slightly increases. To clarify this behaviour the influence of adding PEG-derivative in the preparation of microspheres was investigated by scanning electron microscopy, differential scanning calorimetry, gel permeation chromatography, nuclear magnetic resonance and infrared spectroscopy. Cytotoxicity of this resulting PLGA/PEG-derivative matrix was evaluated in cell lines (fibroblasts) which are more reproducible but less specific and in primary cell cultures (splenocytes and human leucocytes) which have the advantage of their specificity.

Scanning electron microscopy revealed that PLGA/PEG-derivative microspheres exhibited small surface concavities with a highly porous polymeric matrix. The incorporation of PEG-derivative caused a slight reduction in the T_g values of PLGA. In vitro degradation studies showed that PEG-derivative remains within the microspheres as long as the matrix does. This PLGA/PEG-derivative matrix was well tolerated exhibiting cell viabilities similar to PLGA microspheres and can be used to modulate the release of drugs from microparticulate systems destined for parenteral administration.

Keywords: PLGA/PEG-derivative; Microspheres; Cell viability

1. Introduction

Aliphatic polyesters with hydrolysable backbones are promising candidates to control the release of drugs entrapped into microparticulate systems. Among them, one of the most commonly employed systems is microparticles fabricated from poly(D,L-lactic-co-glycolic acid) (PLGA) (Lecaroz et al., 2006; Martínez-Sancho et al., 2006; Herrero-Vanrell et al., 2000; Bao et al., 2006; Wischke and Borchert, 2006). These copolymers are attractive for this application due to many favourable characteristics such as good biocompatibility, their ability to degrade into natural metabolites and their safety profile for human use (Jain, 2000; Gander et al., 2001). However, such systems will be inadequate if they release drugs too rapidly. To control drug release different approaches may be undertaken: polymer blending or the use of different additives (Ruan and Feng, 2003; Choi et al., 2001; Zheng et al., 2006).

For instance, di- or triblock copolymers that contain at least two different groups in their structure (with both, hydrophobic and hydrophilic properties) have lately gained increasing attention for controlled drug delivery (Mi et al., 2003). Nowadays PLGA-PEG and PLA-PEG-PLA copolymers are being used exhibiting adequate characteristics but the synthesis reaction needed for their production increases their cost (Mallardé et al., 2003; Ruan and Feng, 2003; Venkatraman et al., 2005). On the other hand, it is well known that the incorporation of hydrophilic and lipophilic additives can modify the release rate of drugs from microparticulate systems (Chung et al., 2006; Srinivasan et al., 2005; Mallard et al., 2000; Martínez-Sancho et al., 2003; Sansdrap and Moës, 1998). They are usually added to control the initial release from these systems however, it has not been demonstrated if they remain within the system

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for a long time. Among additives, we have previously used a PEG-derivative (polyoxyethylated oleic glyceride), which is frequently employed in the preparation of microemulsions and lately in controlled release systems. This additive has proven to decrease the release rate of indomethacin and ibuprofen from PLGA microspheres destined to intraarticular administration (Fernández-Carballido et al., 2004; Puebla et al., 2005), but when aciclovir was encapsulated for intraocular administration the opposite release behaviour was obtained (Martínez-Sancho et al., 2003), however, the mechanism involved should be clarified.

Biocompatibility of PLGA is a well-known fact but there is a lack of information regarding the behaviour of additives incorporated in the preparation of microparticles.

For this, the objective of the present work was to study the effect that the inclusion of an additive (PEG-derivative) has on the final characteristics of the polymeric matrix of PLGA microspheres and to determine if the additive remains as long as the polymeric matrix exists. In addition, cell viability studies of this PLGA/PEG-derivative matrix have been carried out. To fulfil these objectives PLGA microspheres were prepared with and without the incorporation of PEG-derivative by means of the solvent evaporation technique from an O/W emulsion. The influence of the additive was investigated in vitro by several analytical techniques. Another important aspect of this work is that cytotoxicity is evaluated not only in cell lines (fibroblasts) but in primary cell cultures as well (from animal and human origins).

2. Materials and methods

2.1. Materials

PLGA 50:50 (Resomer® RG 503, Mw = 34,000 Da), was purchased from Boehringer Ingelheim (Ingelheim, Germany), polyoxyethylated oleic acid glyceride (Labrafil® M 1944 CS) (PEG-derivative) was supplied by Gatttefossé (Saint-Priest, France). Methylene chloride (CH_2Cl_2) (Merck, Darmstadt, Germany) and polyvinyl alcohol (PVA) (Mw = 49,000 Da) were supplied by Sigma–Aldrich Chemical (Madrid, Spain). Other solvents and reagents were of analytical grade. Distilled and deionized water (Millipore Corporation, USA) was used in the preparation of solutions and buffers.

2.2. Preparation of microspheres

Microspheres were prepared with and without PEG-derivative by the solvent evaporation technique from an O/W emulsion (Fernández-Carballido et al., 2004). Briefly, the organic phase was prepared by dissolving PLGA (200 mg) in 1 ml CH_2Cl_2 using a vortex mixer (IKA, Labortechnick, Germany). The aqueous phase consisted of 5 ml of PVA solution (1%). Emulsion formation took place in a Polytron homogenizer (Kinematica, Lucerne, Switzerland) operated at a speed setting of 2000 rpm for 1 min. Upon formation of the emulsion, 6 ml of distilled water was added and stirring continued at the same speed setting for 2 min. Finally, the immature microspheres were suspended in 250 ml of distilled water and stirred gently and continuously using a magnetic stirrer for 4 h in order to allow for complete evaporation of the organic solvent. The microspheres were then washed and separated by vacuum filtration using a 5-μm filter and finally dried under vacuum at 25 °C for 48 h. Microspheres containing PEG-derivative (PLGA/PEG-derivative microspheres) were prepared by adding 20 μl of this additive to the internal phase of the emulsion.

2.3. Characterization of microspheres

Morphological characterization: the morphology of the microspheres prepared with and without PEG-derivative was investigated by scanning electron microscopy (SEM) (Jeol, JSM-6400, Japan). Samples were coated with a thin layer of colloidal gold applied in a cathodic vacuum evaporator before observation by SEM at 20 kV. In order to observe their internal morphology, microspheres were cross-sectioned and analyzed.

Particle size: particle size analysis was performed by laser diffraction in a Galai model Cis-1 computerized inspection system (Galai Production Ltd., Israel). Size range used was 0.5–150 μm. Samples of microspheres were suspended in distilled water and sonicated for 1 min to prevent clumping. Mean microsphere diameters were expressed as volume-diameter and then, size distribution of the microspheres was graphically represented using log-linear volume distribution curves.

2.4. Differential scanning calorimetry (DSC)

The physical state of the microspheres components was characterized by thermal analysis in a Mettler 820 DSC analyser (Mettler Toledo, Switzerland). Samples (10 mg) were sealed into aluminium pans and heated in an inert atmosphere of nitrogen (40 ml/min). An empty aluminium pan was used as reference. Samples were scanned from 25 to 180 °C with heating rate of 10 °C/min. Calibration of the system was performed using indium standards. Data obtained was processed on the system software and glass transition temperatures (T_g) identified.

2.5. Gel permeation chromatography (GPC)

Samples were dissolved in tetrahydrofuran (THF) (10 mg/ml). After filtration (PTFE filter, pore size 0.45 μm, Tracer, Spain), 20 μl of the solution was injected. Two columns HR 4E (7.8 mm × 300 mm, Waters Associates, MA, USA) and Ultrastygel 103 Å (Waters Associates) were consecutively connected to augment sensitivity of the procedure. All measurements were performed at a flow rate of 1 ml/min at 35 °C with a Waters 1525 binary HPLC pump (Waters Associates). The refractive indexes were measured using a 2414 refractive index detector (Waters Associates). Molecular weights were calculated by the system calibration software using narrow polystyrene reference materials of known molecular weights: 114,000, 43,700, 18,600, 9650, 6520 and 2950 Da (Waters Corporation, GmbH, Germany). Evaluation was performed according to a cubic universal calibration curve (Waters, MA, USA). Molecular weights are expressed as weight-average

molecular weight (Mw) and number-average molecular weight (Mn). GPC analysis was performed on PLGA (Mw and Mn) and PEG-derivative (AUC). Moreover GPC measurements (Mw, Mn and AUC) were carried out with PLGA/PEG-derivative microspheres at time zero and at different stages of the in vitro degradation assays.

2.6. Infrared absorption spectrophotometry (IR)

IR spectra were recorded on Fourier transform infrared (FT-IR) spectra with a Nicolet Nexus spectrophotometer (MA, USA) within the range 4000–400 cm^{-1}. The FT-IR spectra were obtained at room temperature in KBr pellets for PLGA, PEG-derivative, PLGA microspheres and PLGA/PEG-derivative microspheres. The weight ratio of KBr to powder was about 100.

2.7. Nuclear magnetic resonance (NMR)

^{13}C NMR spectra analysis was carried out in a Bruker Avance 400 MHz (Bruker Española S.A., Madrid, Spain). ^{13}C solid-state NMR spectra were obtained with the cross-polarization/magic angle spinning (CP/MAS) technique. Magic angle spinning was carried out with 4-mm double bearing rotors of ZrO_2 and spinning rate of 12,000 Hz at probe temperature of 295 K. The proton 90° pulse length was 4.7 μs with contact and delay times of 2 ms and 4 s, respectively. A total of 10 K transients were accumulated at a time domain size of 2 K data point with an acquisition time of 40 ms.

2.8. In vitro degradation studies

The degradation behaviour of the polymeric matrix was evaluated by means of the molecular weight reduction with time (33 days) upon in vitro incubation in isotonic phosphate buffer saline (PBS) at pH 7.4. Twenty mg of PLGA/PEG-derivative microspheres was added to PBS (3 ml) and placed in a water shaker bath (NE-5, Clifton, UK) at 37 °C with constant agitation (50 strokes/min). At fixed time intervals all sample volume was withdrawn and the particles were desiccated. Thereafter, the solid residues obtained were dissolved in THF, filtered through 0.45 μm filters (Teknokroma, Spain) and injected in the gel permeation chromatographic (GPC) system (Waters).

2.9. Cell viability studies in fibroblasts

The toxicity of PEG-derivative, PLGA, PLGA microspheres and PLGA/PEG-derivative microspheres was estimated using the MTT colorimetric assay (3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide). All samples were previously sterilized by gamma-irradiation with a sterilizing dose of 25 kGy from a Co-60 source (Aragogamma, Barcelona, Spain). NIH-3T3 fibroblast cells were seeded onto well plates. Fibroblasts (reference cells) and fibroblasts with PEG-derivative, PLGA, PLGA microspheres and PLGA/PEG-derivative microspheres were suspended in a complete RPMI-1640 medium (Sigma–Aldrich Chemical, USA) and incubated at 37° in 5% CO_2 95% air-humidified atmosphere for 48 h. Different concentrations of PEG-derivative (0.1–1 mg/ml), PLGA (0.8–2.5 mg/ml), PLGA microspheres (0.8–2.5 mg/ml), PLGA/PEG-derivative microspheres (0.8–2.5 mg/ml) and control fibroblasts were tested under the same experimental conditions. To account for cell viability the absorbance was measured at an optical density of 570/630 nm. Untreated cells were used as control (100% viability) and cells without addition of MTT were used for calibration of the system to zero absorbance. Relative cell viability (%) was calculated by means of $[abs]_{test}/[abs]_{control} \times 100$. All assays were performed in triplicate.

2.10. Cell viability studies in splenocytes

Male Wistar rats (250–300 g) were housed under standard environmental conditions at temperatures of 22–24 °C, under a 12:12 h light: dark cycle, with access to food and water ad libitum. Animals were sacrificed and the splenic cells were obtained using differential centrifugation. Splenocytes (reference cells) and splenocytes with microspheres were suspended in a complete medium RPMI-1640 (Sigma–Aldrich Chemical, USA) and incubated at 37° in 5% CO_2 95% air-humidified atmosphere for 48 h. Different concentrations of PEG-derivative, PLGA microspheres and PLGA/PEG-derivative microspheres (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml) were tested under the same experimental conditions. After incubation, the samples were placed in 96 wells. Afterwards, 100 μl sulforhodamine (0.4% in 1% acetic acid) was added to each well and the plates were incubated for 15 min at room temperature. The unbound dye was removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was then solubilized with 100 μl Tris buffer (100 mM) and the absorbance measured in a microplate reader (Bio-Rad, Hercules, CA) at a wavelength of 492 nm (Houghton et al., 2007).

2.11. Cell viability studies in human leucocytes

Blood samples were drawn from healthy human subjects and cells isolated by centrifugation. Leucocytes with microspheres were suspended in a complete medium RPMI-1640 (Sigma–Aldrich Chemical, USA) and incubated at 37° in 5% CO_2 95% air-humidified atmosphere for 48 h. Different concentrations of PLGA, PEG and microspheres with- and without PEG-derivative (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml) were tested. After incubation, the samples were placed in 96 wells. Afterwards, sulforhodamine (0.4% in 1% acetic acid) was added to each well and the plates were incubated for 15 min at room temperature. The unbound dye was removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was then solubilized with Tris buffer and the absorbance measured in a microplate reader (Bio-Rad) at a wavelength of 492 nm (Houghton et al., 2007).

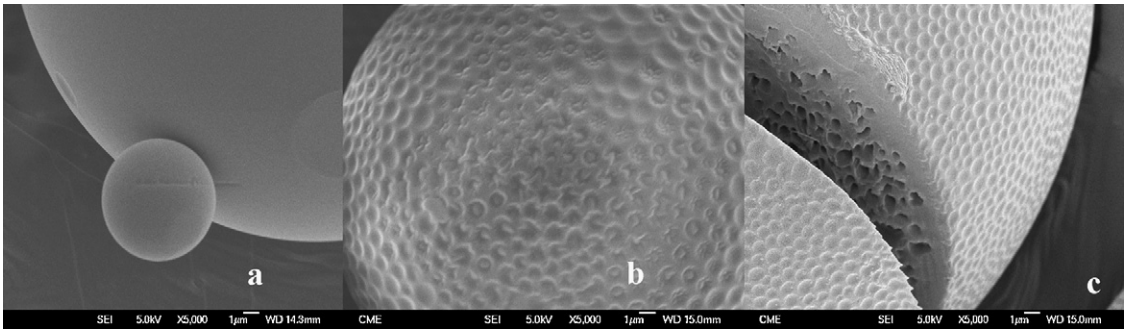

Fig. 1. SEM of PLGA microspheres (a), PLGA/PEG-derivative microspheres (b) and trasversal cross-section of PLGA/PEG-derivative microspheres (c).

2.12. Statistical analysis

Values are expressed as mean ± S.D. The differences obtained with respect to control samples were analyzed by means of ANOVA. Significant differences were analyzed using the Tukey–Kramer's test. The level of significance was set at $p < 0.05$.

3. Results and discussion

Particle size is an important factor that can influence the biopharmaceutical properties of microparticles (Siepmann et al., 2004; Berkland et al., 2002). Mean particle sizes obtained were 39.1 ± 4.3 and 38.4 ± 6.0 μm for PLGA microspheres and PLGA/PEG-derivative microspheres, therefore, particle size was not significantly influenced ($p < 0.05$) by the incorporation of the additive. Morphologically, SEM revealed that PLGA microspheres were spherical with no signs of aggregation whereas PLGA/PEG-derivative microspheres exhibited homogeneous shapes with small concavities on their surfaces (Fig. 1). Other authors have pointed out that irregular surfaces appeared in microspheres due to the plastificant effect of other PEGs different from the one used in the present study (Blanco-Prieto et al., 2000; Choi et al., 2001). In our case, concavities of homogeneous sizes were regularly distributed throughout the surface of the microspheres. Analysis of transversal cross-sections of the microparticles revealed a highly porous polymeric matrix that can be attributed to a reduction of the rate at which solvent evaporation occurs during formation of the microparticles.

PLGA, PLGA microspheres and PLGA/PEG-derivative microspheres were also analyzed by differential scanning calorimetry (DSC) (Fig. 2). PLGA microspheres exhibited the same transition temperature as PLGA (T_g = 45.4 °C) (Alléman et al., 1998). However, the incorporation of PEG-derivative caused a slight reduction in the T_g values of the polymer (43.8 °C). The plasticizing effect of this additive is based on the reduction of the attractive forces among the polymer chains. A decrease in the attractive forces leads to an increase in the mobility of the macrochains that could affect (diminishing) the rate at which solvent evaporation occurs during microencapsulation. In this case remnants of the organic solvent could have an influence on the cytotoxicity of the polymeric matrix.

Gel permeation chromatography was used to determine if the additive remains within the PLGA matrix throughout its degradation process. The results obtained for PLGA and PLGA/PEG-derivative microspheres were very close (35,834 ± 2135 and 35,023 ± 2901 Da, respectively) and similar to label value (34,000 Da, GPC), demonstrating that the microencapsulation technique has no influence on polymer molecular weight. Analysis of PEG-derivative showed only one peak at 802 ± 32 Da whereas PLGA/PEG-derivative microspheres showed two peaks at 35,023 ± 2915 and 823 ± 41 Da, which correspond to PLGA and PEG-derivative, respectively.

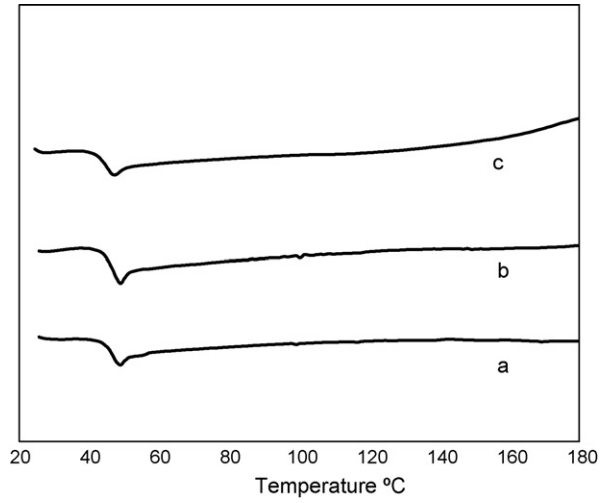

Fig. 2. DSC thermograms of PLGA (a), PLGA microspheres (b) and PLGA/PEG-microspheres (c).

Table 1 summarizes the evolution of mean molecular weight (Mw) and mean molecular number (Mn) of PLGA/PEG-derivative microspheres during their in vitro degradation from time zero to 33 days. Also included in the table are the AUC

Table 1
GPC results obtained for PLGA/PEG-derivative microspheres

Time (days)	PLGA (Mw) (Da)	PLGA (Mn) (Da)	PEG-derivative (AUC) (mV time)
0	35,023	25,835	64,369
5	32,828	23,070	18,508
14	19,406	11,969	13,933
17	14,588	9658	10,003
20	10,966	7690	7869
33	7533	6487	5234

values obtained for PEG-derivative since no significant modifications of Mw occurred during the release tests due to hydrolytic scission. The decreases observed in AUCs of PEG-derivative could be explained by the amphiphillic character of this compound which slowly dissolves as the polymeric matrix degrades. We have previously seen a more rapid dissolution of PEG-derivative alone when compared to the PLGA/PEG-derivative matrix. Therefore, the fact that PEG-derivative remains for a long time makes it an adequate candidate to control the release of drugs from microparticulate systems. This behaviour could explain the release characteristics of indomethacin and ibuprofen-like drugs from microspheres prepared with PEG-derivative (Fernández-Carballido et al., 2004; Puebla et al., 2005), and it could also explain the release behaviour of drugs such as aciclovir that are incorporated un-dissolved in the polymeric matrix (Martínez-Sancho et al., 2003). In this case initial release of aciclovir from the microspheres occurred more rapidly probably due to an increase of the amount of drug which is being dissolved in the matrix whereas in a second stage, the release rate decreased when compared to that of microparticles prepared without PEG-derivative, and exhibited the same release behaviour of indomethacin and ibuprofen-like drugs that are incorporated dissolved in the polymeric matrix.

With respect to Mw and Mn values shown in Table 1 for PLGA, both values decreased very rapidly in the first stages of the in vitro degradation assay. This fact has been already explained by random chain scission as the mechanism for hydrolytic copolymer degradation (Hausburger et al., 1995). As degradation continues, decreases of both Mw and Mn are less marked since shorter chains are present.

The fact that PEG-derivative remains as long as the matrix does makes it necessary to determine if an interaction between PGLA and PEG-derivative occurs. For this, nuclear magnetic resonance and infrared absorption spectra were obtained.

Nuclear magnetic resonance (NMR) is a non-destructive technique used to solve problems related to elucidation of structures. ^{13}C NMR analysis of PLGA (a) and PLGA microspheres (b) revealed the presence of three sets of peaks (Fig. 3): the first one corresponds to carboxylic and carbonyl bonds (168.95 ppm), the second one (71.10–63.48 ppm) corresponds to CH bonds in lactic acid and CH_2 in glycolic acid and the third one corresponds to methylen groups of the D,L-lactic acid repeated units (18.20 ppm). The ^{13}C NMR of PLGA and PLGA microspheres are similar which again confirms that the technique used did not modify the structural characteristics of the polymer (Fig. 3a and b). On the contrary, ^{13}C NMR analysis of PLGA/PEG-derivative microspheres (Fig. 3c) revealed a marked increase in the intensity of the first peak (171.63–169.11 ppm), with a significant inversion in the intensity of the second set of peaks that is even noticeable at the lower proportion at which PEG-derivative was used (<10% with respect to PLGA).

The infrared absorption spectra obtained for PLGA, PEG-derivative and microspheres prepared with and without PEG-derivative are shown in Fig. 4. FT-IR spectrum of PLGA is

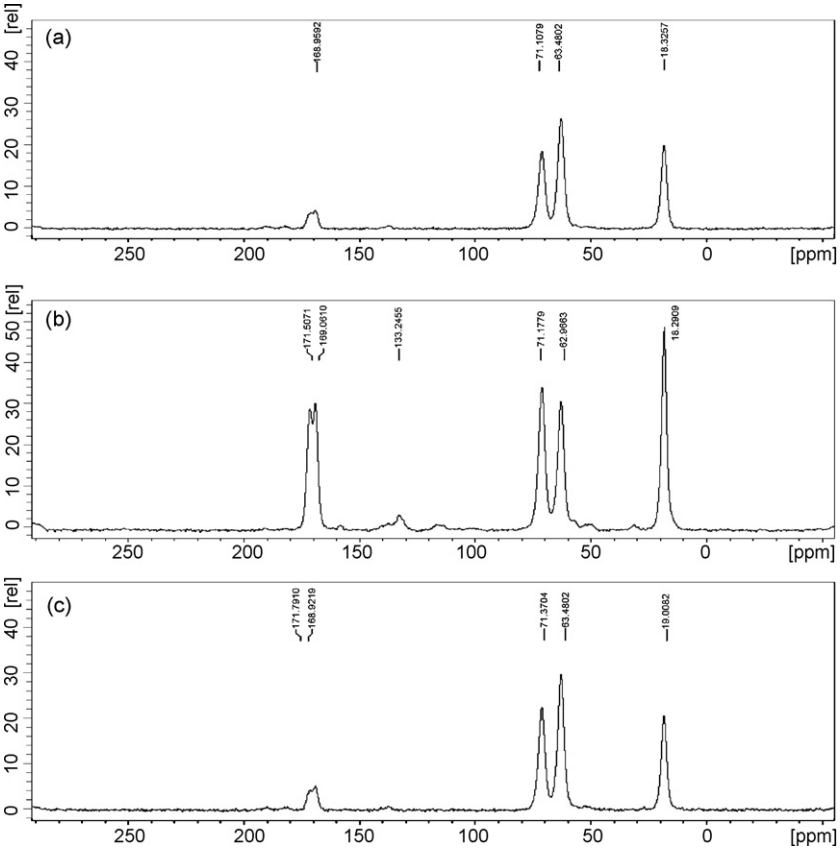

Fig. 3. NMR patterns of PLGA (a), PLGA microspheres (b) and PLGA/PEG-derivative microspheres (c).

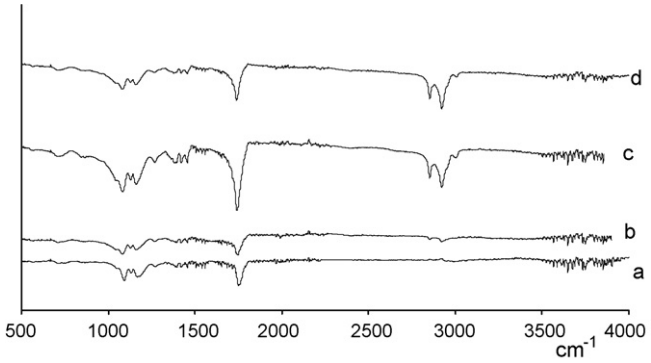

Fig. 4. IR spectra of PLGA (a), PLGA microspheres (b), PEG-derivative (c) and PLGA/PEG-derivative microspheres (d).

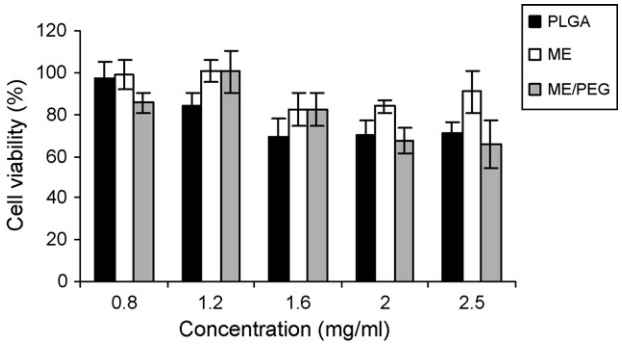

Fig. 5. Cell viabilities (%) obtained in fibroblasts after testing different concentrations of PLGA, PLGA microspheres (ME) and PLGA/PEG-derivative microspheres (ME/PEG).

consistent with the expected structure of the copolymer showing a very weak band in the region of 2860–2940 cm^{-1} that is due to C-H stretch of CH_2 and CH_3. A strong band at 1760 cm^{-1} is assigned to C=O stretch and absorption at 1100–1200 cm^{-1} is due to C=O stretch. The IR spectrum corresponding to PLGA microspheres prepared without PEG-derivative did not show any changes with respect to that of PLGA alone thereby indicating, as previously said, that the microencapsulation process did not modify the polymer characteristics. When PEG-derivative was analyzed by IR, similar peaks to those of PLGA were obtained but with a strong band appearing at 2750–3010 cm^{-1} due to C–H stretch of CH=CH. IR spectrum of microspheres prepared with PEG-derivative was similar to that of PEG, which confirms that it is incorporated within the polymeric matrix.

The results obtained by NMR an IR analyses do not demonstrate the presence of an interaction between both compounds.

Biocompatibility of PLGA is a well-established fact but there is no information regarding cell viability of multiparticulate systems prepared with PEG-derivative. The paucity of data that demonstrate that the PLGA/PEG-derivative matrix is non cytotoxic when used in parenteral medications led us to carry out cytotoxicity tests in cell lines and primary cell cultures. Cell lines are more reproducible but less specific whereas primary cell cultures have the advantage of their specificity. Among the advantages of using cell lines are that they do not produce contact inhibition being able to grow in an endless manner. On the other hand, primary cell cultures exhibit greater variability in their results thereby more closely resembling real situations (Mannerströma et al., 2006).

Two major techniques were used to assess cell growth: the first one uses 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The MTT method was firstly developed and introduced in 1986 (Houghton et al., 2007), in which the formation of colour relies on the activity of the mitochondria so, if the function of these is inhibited by variations in cellular levels of NADH, glucose and other factors, variable results are obtained and a similar result may be given as if the cells were not alive or not proliferating.

The second major technique for testing cytotoxicity is the more preferred sulphorhodamine B (SRB) assay. The SRB assay is sensitive, simple, reproducible and more rapid than the formazan-based assays and gives better linearity, a good signal-to-noise ratio and has a stable end-point that does not require a time-sensitive measurement, as do the MTT assays (Houghton et al., 2007).

Cell viability results obtained in fibroblasts are depicted in Fig. 5. The viabilities values obtained for PEG-derivative ranged from 120.8 to 133.2% (data not shown) with an increase in cell viability as PEG-derivative concentration increased. Values obtained for PLGA ranged from 68.8 to 96.8%, from 82.8 to 100.8% for PLGA microspheres and from 65.6 to 100.5% for PLGA/PEG-derivative microspheres. It is well known that PLGA polymers are composed of nontoxic monomers (lactic and glycolic acid units) with excellent biocompatibility (Wang et al., 2000), and according to our results PLGA/PEG-derivative microspheres showed non-statistically significant differences with both PLGA and PLGA microspheres. Data corresponding to PEG-derivative showed cell viabilities higher than 100% at all concentrations assayed. These results could be attributed to the fact that this additive is a polyethilenglycol derivative and other studies have proven that a PEG solution resulted in significantly greater viability than other organ preservation solutions when assayed in kidney tubular cells (Moutabarrik et al., 1998). The mechanism by which PEG improved cell preservation is not clear. PEG interacts with the cell membrane probably by association with its phospholipids, and this interaction may stabilize the membrane. When incorporated in the preparation of microspheres this effect is negligible since PEG may not be able to interact and stabilize cell membranes.

Cell viability results obtained in splenocytes are shown in Fig. 6. The viabilities values obtained for PEG ranged from 68.8

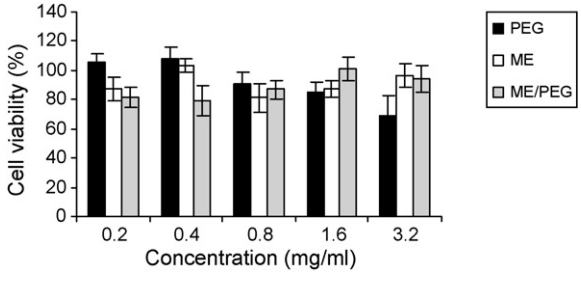

Fig. 6. Cell viabilities (%) obtained in splenocytes after testing different concentrations of PEG, PLGA microspheres (ME) and PLGA/PEG-derivative microspheres (ME/PEG).

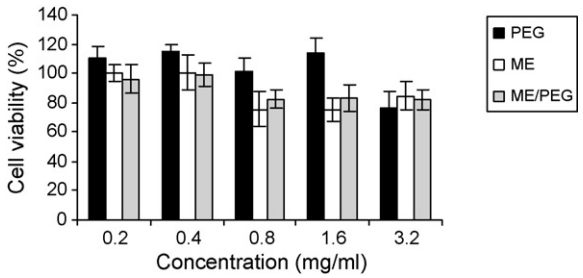

Fig. 7. Cell viabilities (%) obtained in leucocytes after testing different concentrations of PEG, PLGA microspheres (ME) and PLGA/PEG-derivative microspheres (ME/PEG).

to 105.7%. The highest PEG concentrations tested in this assay showed a decrease in cell viability since these high concentrations could difficult cell growth which is limited by contact in primary cell cultures. PLGA microspheres exhibited cell viabilities of 81.2–103.1% and 79.2–100.6% results were obtained for PLGA/PEG-derivative microspheres with no significant differences found between both types of microspheres.

Cell viability results obtained with leucocytes are shown in Fig. 7. As occurred with splenocytes, viabilities higher than 100% were obtained for PEG-derivative with only one value lower than 100% which was obtained at a concentration of 3.2 mg/ml (highest concentration tested). PLGA microspheres showed cell viabilities ranging from 75.3 to 100.3% and from 81.9 to 99.4% for PLGA/PEG-derivative microspheres. In both cases no direct correlation was obtained between the amount of microspheres assayed and the viability response. Moreover, significant differences were not found between both types of microspheres.

These results show that cytotoxicity was similar for both primary cell cultures (splenocytes and leucocytes) and when compared to cell lines similar good viability results were obtained with independence of the technique employed.

4. Conclusions

In conclusion it can be stated that the PLGA/PEG-derivative matrix is easy to obtain, exhibits cell viabilities similar to PLGA microspheres and can be used to modulate the release of agents from drug delivery systems destined to parenteral administration.

Acknowledgments

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