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RESEARCH ARTICLE

Preparation, characterization and *in vitro* evaluation of ε-polylysine-loaded polymer blend microparticles for potential pancreatic cancer therapy

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ABSTRACT

Peptide active ingredients show great promise regarding the treatment of various health-endangering diseases. It is reported that L-lysine inhibits the proliferation of several tumour lines *in vitro* and *in vivo*. However, proteins and peptide drugs possess certain disadvantages such as *in vivo* instability and short biological half-life. On the grounds that drug delivery systems can overcome a wide spectrum of bioactive compounds issues, a biopolymeric blend-based microparticulated system capable of delivering ε-polylysine (PLL) was developed. PLL-loaded poly((L)Lactic acid)/poly(D,L-Lactide)-co-poly(ethylene glycol)-based microparticles (PLL-PB-MPs) were prepared and fully characterised exhibiting a narrow size distribution ($1.2 \pm 0.12\,\mu m$), high loading efficiency (81%) and improved thermal stability (T_d from 250 °C to 291 °C). The cytotoxicity and antiproliferative effect of PLL-PB-MPs in pancreatic adenocarcinoma cell lines BxPC3 and MIA PaCa-2 were confirmed. Due to their physicochemical and biopharmaceutical properties, PB-MPs constitute a promising carrier to deliver bioactive peptides.

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Biopolymers; ε-polylysine; microparticles; polylactic acid

Introduction

Cancer was responsible of an estimated 8.2 million deaths in 2012, being one of the leading causes of morbidity and mortality worldwide (World Health Organization, 2014). With 338,000 new cases diagnosed in 2012, pancreatic cancer is the twelfth most common type of cancer globally. Considering the fact that the disease is usually asymptomatic during early stages, this kind of cancer is commonly diagnosed at an advanced stage. Therefore, pancreatic cancer remains an extremely lethal disease, with a median survival of only 6 months and a dismal 5-year overall survival of less than 5%. It is the seventh most common cause of cancer deaths worldwide (Ferlay et al., 2012; Ferlay et al., 2015), and no systemic therapy is effective against pancreatic cancer.

Peptide active ingredients play a key role in new pharmaceutical developments intended to treat several health-endangering diseases, such as cancer, diabetes, infectious conditions, and autoimmune disorders (Wu et al., 2014; Yeh et al., 2015). In particular, ε-polylysine (PLL) has been largely employed for different purposes in the field of experimental medicine, including its use as nanoparticles, hydrogels, liposomes, drug carrier for small molecules, gene carriers, interferon inducers, lipase inhibitors, coating material, among others (Shukla et al., 2012). For instance, how high-molecular weight DNA undergoes a cooperative structural transition which results in different kind of compact configurations in the presence of PLL has been reported decades ago (Laemmli, 1975).

More importantly, L-lysine inhibits the growth of several transplantable animal tumours, reducing tumour cell proliferation both *in vitro* and *in vivo* without toxic side effects (Szende et al., 2002). In addition to directly inhibiting cell proliferation, PLL also enhances the production of immunoglobulin-M and interferon-β without stimulating cell proliferation (Yamamoto et al., 2001). As a consequence, the

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immunostimulatory activity of PLL also contributes to restrain tumour growth (Szende et al., 2002).

Despite the fact that proteins and peptides, such as PLL, are valuable active pharmaceutical ingredients, these have certain intrinsic properties that limit their pharmaceutical use, namely *in vivo* instability and short biological half-life. Even though overcoming these limitations represents a considerable challenge (Leo et al., 2006), the development of a peptide carrier system capable of improving the performance of bioactive peptides is warranted.

It is important to highlight that significant progress has been made in the development of new pharmaceutical technology platforms based on different types of systems (Ali et al., 2005, 2011, 2015). Particularly, polymeric microparticles (MPs) are micron-sized entities which can be made from a wide variety of natural and synthetic polymers. Due to their ability to improve the efficiency of current therapeutic treatments, MPs are being extensively studied and used as drug carriers in the field of biomaterials, medicine and pharmaceutical sciences (Mantripragada and Jayasuriya, 2016; Obayemi et al., 2016).

There are many properties of biopolymeric MPs that can overcome the problems associated with peptide therapeutics, such as particle size, size distribution, porosity, drug-loading percentage, and entrapment efficiency, which are the most relevant characteristics defining the unique properties of a system (Joye and McClements, 2014). Regarding versatility in the administration route, MPs can be delivered orally and/or by pulmonary inhalation to reach systemic circulation, and also locally via injection or implantation (Chew et al., 2017). In addition, MPs can load fragile hydrophilic agents, such as peptides and proteins, using electrohydrodynamics (EHD) (Moghe and Gupta, 2008), a simple and inexpensive technique that allows one-step encapsulation.

The final goal of drug delivery systems is that it must be stable in the bloodstream long enough to allow recognition and localisation of its specific target (Owens and Peppas, 2006; Ali, 2011; Saleem et al., 2013). The human body recognises hydrophobic particles as foreign, and consequently the reticuloendothelial system (RES) removes them from the bloodstream in a few minutes. This complex biological barrier represents one of the most important challenges to be overcome by particulated carrier systems (Kumari et al., 2010). In consequence, to improve blood circulation half-life of polymeric drug carriers, several camouflaging strategies have been developed. The most explored and successful strategy to avoid RES recognition is to interfere with opsonin binding, which relies on the surface treatment of particles with poly(ethylene glycol) (PEG). It is well known that hydrophilic polymers, especially PEG, can be attached to the surface of MPs providing them steric stabilisation and "stealth" properties. The PEG surface functionalization represents one of the most promising approaches to confer stability to particulated systems, showing the lowest occurrence of harmful *in vivo* effects. Hence, when designing biopolymeric particles to perform carrier systems, PEG-based block copolymers are within the most frequently chosen matrix alternatives (Otsuka et al., 2003). Moreover, poly(lactic acid) (PLA) blends involving PEG have been greatly explored and used for biomedical applications (Saini et al., 2016).

Considering the advantages of MPs as drug carriers and the potential of PLL as a therapeutic agent for cancer therapy, the purpose of this work was to prepare biopolymeric MPs able to successfully deliver this peptide active ingredient and to explore the *in vitro* therapeutic effect of PLL-loaded polymer blend MPs (PLL-PB-MPs) over the viability of two different pancreatic cancer cell lines.

Methods

Materials

Poly((L)Lactic acid) acid endcap (PLA, Mn: 25,000–35,000 Da) was purchased from Akina Inc. and Poly(D,L-Lactide)/poly(ethylene glycol) with carboxylic acid end, PLA-*b*-PEG-COOH (PEG-b-PLA, Mw: 12,600 Da), was purchased from Advanced Polymer Materials Inc. Poly-lysine labelled with fluorescein (PLL-FITC) sequence: FITC-C6-Lys10was purchased from American Peptide Company. The 2,2,2-trifluoroethanol (TFE), 3-(4,5-dimethyl-thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich® (St. Louis, MO) and the foetal bovine serum (FBS) from Gibco Invitrogen® Corporation (New York, NY).

Roswell Park Memorial Institute medium (RPMI 1640) and Dulbecco's modified Eagle's medium (DMEM) were supplied by Gibco® Laboratories. Human pancreatic carcinoma cell lines BxPC3 (CRL1687) and MIA PaCa2 (CRMCRL1420) were from ATCC® (Manassas, VA).

All other reagents were of analytical grade, and used as received without further purification. All experiments were carried out with ultra-purified water (Milli-Q, Millipore®, Bedford, MA).

Preparation of PLA/PEG-b-PLA MPs

PLL-loaded (1% w/w) MPs were prepared using a proprietary electrohydrodynamic technology (Bio-Target Inc., Chicago, IL; LNK Chemsolutions LLC, Lincoln, NE).

Briefly, for all MPs, organic solutions containing all the necessary components were processed using this technology, resulting in a dry collection of the specified particles. A polymer/copolymer blend (PB) using two different ratios were prepared: PLA/PEG-*b*-PLA blend ratio of 50–50% and 75–25%. PLL-loaded PB MPs (PLL-PB-MPs) and unloaded particles (PB-MPs) were prepared.

Characterization of PLA/PEG-b-PLA MPs

Morphological characterisation of MPs

Particle diameter and morphology were assessed by scanning electron microscopy (SEM). The images were taken with Quanta 200 FEG Environmental Scanning Electron Microscope using an accelerating voltage of 15–20 kV and working distance of approximately 10 mm. A Cressington 108 Auto Sputter Coater was used for sample coating with metallic gold for 30 s before SEM characterisation to enhance the contrast of non-conducting samples. Statistical analyses of particle diameter measurements from SEM images were performed on sets of at least 150 counts within each specimen.

Confocal microscopy

MPs carrying the PLL-FITC conjugate were collected in a microscope glass slide, and analysed using a confocal microscope (Olympus IX 81).

Thermal analysis

Thermogravimetric analysis (TGA) was performed in a TA Auto-MTGA Q500 Hi-Res. Three mg of free PLL, PB-MPs and PLL-PB-MPs samples were hermetically sealed in aluminium pans. The dynamic scans were taken in N_2 atmosphere (flow rate of 50 ml/min) at the heating rate of 10 °C/min. The TGA curves were obtained in a temperature range of 25–650 °C.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra (Perkin Elmer Spectrum 100.) were measured from potassium bromide discs containing the sample (KBr, spectroscopy grade, Merck®). The discs were dried under vacuum for at least 2 h before recording the spectra. The samples were measured from 4000 to 400 cm^{-1} with a resolution of 8.0 cm^{-1} and 40 scans.

Loading efficiency (LE)

In order to determine the amount of PLL loaded within the polymeric matrix, the absorbance of different amounts of PLL-FITC in TFE solutions were measured at 436 nm and a calibration curve was calculated, obtaining a $R^2 = 0.996$ which indicates a strong linear correlation between absorbance and PLL concentration. To determine the LE, 7.85 mg of PLL-PB-MPs with a blend ratio 75–25% were dissolved in 0.3 ml of TFE and the absorbance of the solution obtained was measured. According to the amount initially incorporated of PLL, 1% w/w of PLL-loaded MPs, the LE was calculated according to Equation (1),

$$\text{LE } \% = \frac{A_m \times C_i}{A_i} \times 100 \quad (1)$$

where LE% is the percentage of loading efficiency of PLL into MPs, A_m is the absorbance of the sample measured, C_i is the initial concentration of PLL determined theoretically according to 1% w/w of PLL-loaded MPs and A_i is the absorbance corresponding to the solution at C_i of PLL.

Cell viability assays

Human pancreatic carcinomaBxPC3 and MIA PaCa2 cell lines were used to perform cell viability assays according to ISO standards (ISO, 2009). The BxPC3 was cultured in RPMI and MIA PaCa2 in DMEM medium, both supplemented with FBS to a final concentration of 10%, pH 7.2. Cells were seeded at a confluence of 6×10^3cells/well in 96-well adherent plates in 100 µL of complete medium and incubated for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. Next, the cells were treated with 100 µL of complete medium containing free PLL and PLL-PB-MPs at a concentration gradient of 25, 50 and 100 µg/mL as final concentration of PLL for 24 and 72 h. PB-MPs were also tested using the same concentration equivalents as previously explained. Cells alone without treatment were used as negative controls. After treatment, the mitochondrial metabolic activity of cells was quantitatively determined by incubation with 5 mg/mL MTT dissolved in complete medium for 1h at 37 °C in order to allow MTT to reduce to formazan (Ciapetti et al., 1993). Formazan crystals were then resuspended in DMSO and the reduction of MTT to formazan was measured colorimetrically at 570 nm using a EON plate reader (Biotek®). Absorbance readings of treated samples were used to calculate cell viability relative to the absorbance readings of the negative control samples, which were considered to be 100% viable. All conditions were done in quadruplicate for both cell lines.

Statistical analysis

Statistical significance to compare different experimental conditions by comparisons of mean values (assessed by a two-tailed Student *t*-test) and an analysis of variance (one-way ANOVA) with the Bonferroni's *post hoc* test were performed, using GraphPad software. A p value $< .05$ or less was considered significant.

Results

Morphological characterization of MPs

Figure 1 shows the PLL-PB-MPs SEM images obtained for the two different PB ratios. The morphology of both types of particles was characterised by a non-spherical, but highly uniform raisin-like structure. These results indicate that the selected fabrication method allows control over the formation of MPs, their dimensions, and their homogeneous properties. Biopolymeric MPs diameter was also assessed by SEM through statistical image analysis and the values obtained are presented in Table 1.

75%/25% PLA/PLA-b-PEG blend ratio MPs showed a non-spherical but highly uniform morphology with a mean diameter of $1.2 \pm 0.12\,\mu m$ confirming the narrow size distribution observed in SEM micrographs. On the other hand, 50%/50% PLA/PLA-b-PEG blend ratio MPs presented several issues regarding particle aggregation and formation. These results are attributed to the hydrophobic and hydrophilic character of the components constituting the polymer blend.

Confocal microscopy

To evaluate PLL loading on PB-MPs, we used FITC-labelled PLL and evaluated PLL-PB-MPs by confocal fluorescence microscopy. As observed in Figure 2, FITC-labelled PLL not only co-localized with MPs (merged images), but also showed a homogeneous distribution along the PB matrix.

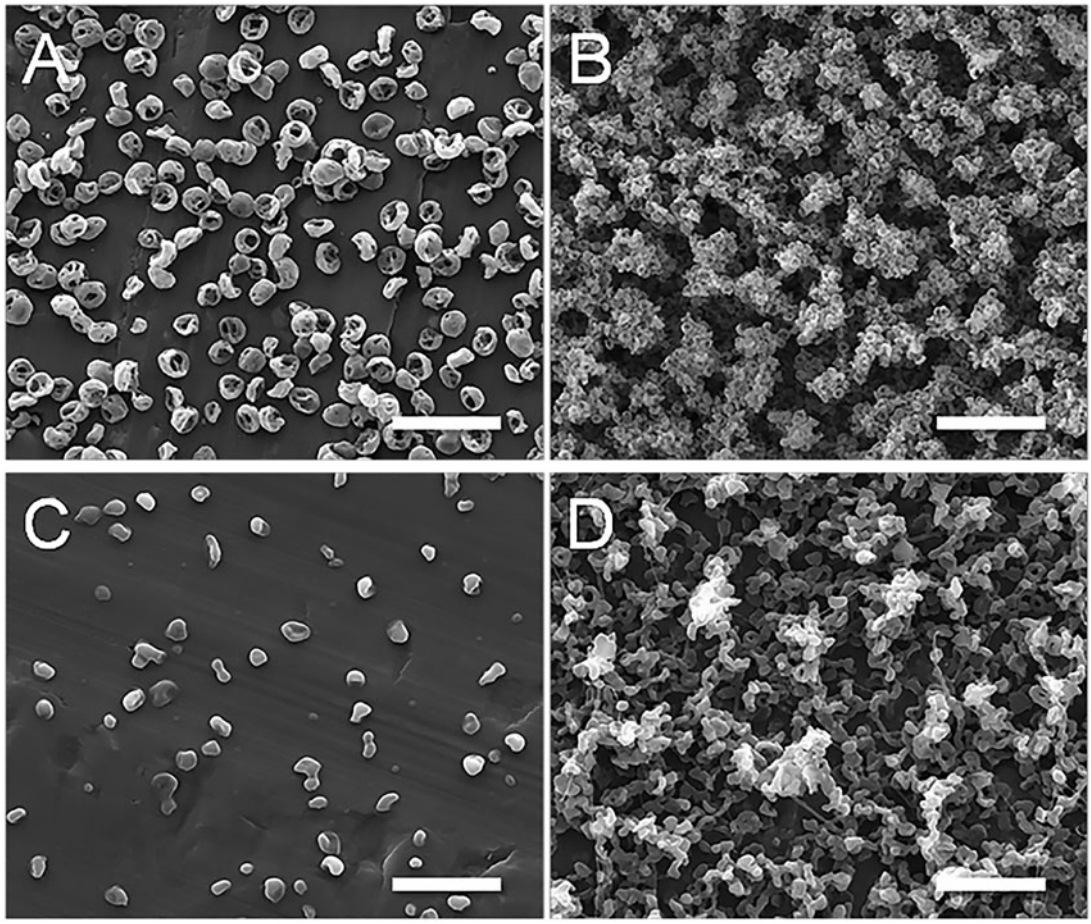

Figure 1. (A) Dried PLL-FITC loaded PLA/PEG-*b*-PLA blend ratio of 75–25% microparticles (Scale bar: 5 μm). (B) Dried PLL-FITC loaded PLA/PEG-*b*-PLA blend ratio of 75–25% microparticles collected for 24 h (Scale bar: 10 μm). (C) Dried PLL-FITC loaded PLA/PEG-*b*-PLA blend ratio of 50–50% microparticles (Scale bar: 5 μm). (D) Dried PLL-FITC loaded PLA/PEG-*b*-PLA blend ratio of 50–50% microparticles collected for 24 h (Scale bar: 10 μm).

Table 1. Data summary corresponding to microparticulated studied systems indicating polymer blend, polymer blend ratio (PB ratio) expressed as %PLA/% PLA-b-PEG, used PLL-FITC percent by weight relative to the polymer blend (PLL-FITC %w/w), size distribution values extracted by SEM ($n = 150$), quantified PLL-FITC average loading efficiency ($n = 3$) and degradation temperature extracted from obtained thermograms (t_d).

System ID	Polymer blend	PB ratio %	PLL-FITC % w/w	Size (μm)	LE (%)	T_d (°C)
PLL-PB-MPs	PLA/PLA-b-PEG	75/25	1	1.2 ± 0.12	81	291
PLL-PB-MPs	PLA/PLA-b-PEG	50/50	1	0.9 ± 0.25	68	–
PB-MPs	PLA/PLA-b-PEG	75/25	–	0.95 ± 0.19	–	275
Free PLL	–	–	–	–	–	250

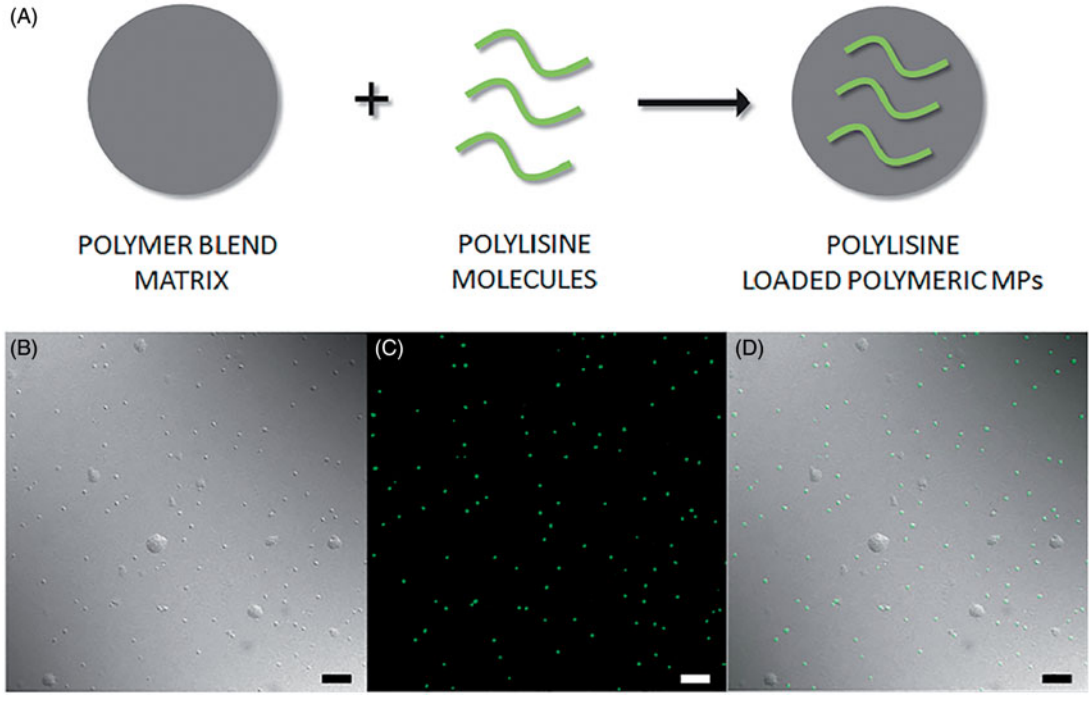

Figure 2. (A) Scheme of obtained PLL-FITC loaded polymer blend microparticles (PLL-PB-MPs), (B) Images obtained by confocal microscopy of PLL-PB-MPs, (C) PLL-FITC fluorescence of PLL-PB-MPs and (D) Merged of B and C showing co-location as a qualitative indication of successful PLL accommodation within the polymer matrix. Scale bar = 10 μm.

Thermogravimetric analysis (TGA)

The thermogravimetric analyses to free PLL, PB-MPs and PLL-PB-MPs were carried out from room temperature to 650 °C. As shown in Figure 3(A), free PLL started to degrade at a lower temperature when compared to PLL-loaded MPs. The unloaded PB-MPs presented a degradation temperature higher than free PLL, but lower than PLL-PB-MPs. The degradation temperature (T_d) values for each one is presented in Table 1.

Fourier transform infrared spectroscopy (FTIR)

Figure 3(B) shows the FTIR spectra for PLL-loaded PB-MPs and unloaded PB-MPs. The FTIR spectrum exhibits bands associated with PLA, PEG and PLL: Group of C-H stretching bands centred at 2955 cm^{-1} of the methyl groups in the PLA and the methylene groups of the PEG, C–O–C peaks of the PLA and PEG from 1096 cm^{-1} to 1186 cm^{-1}. CH_2 scissoring band of the PEG and the CH_3 asymmetric deformation band of PLA appear also in the range 1388 and 1459 cm^{-1}. C=O at 1762 cm^{-1} is associated with the carbonyl ester groups of the PLA. The band centered in 3480 cm^{-1} is associated with O–H stretching possibly overlapped with the reported PLL band around 3300 cm^{-1} that corresponds to the proton mode band of the peptide group that appears with its isotopic counterpart near 2400 cm^{-1}, in this case at 2370 cm^{-1} (Rozenberg and Shoham, 2007; Wang et al., 2013).

Loading efficiency (LE)

The LE of PLL found for PB using a blend ratio of 75%/25% was 81%, meanwhile this value was lower (68%) for PB when a blend ratio of 50%/50% was chosen, as can be observed in Table 1. These results

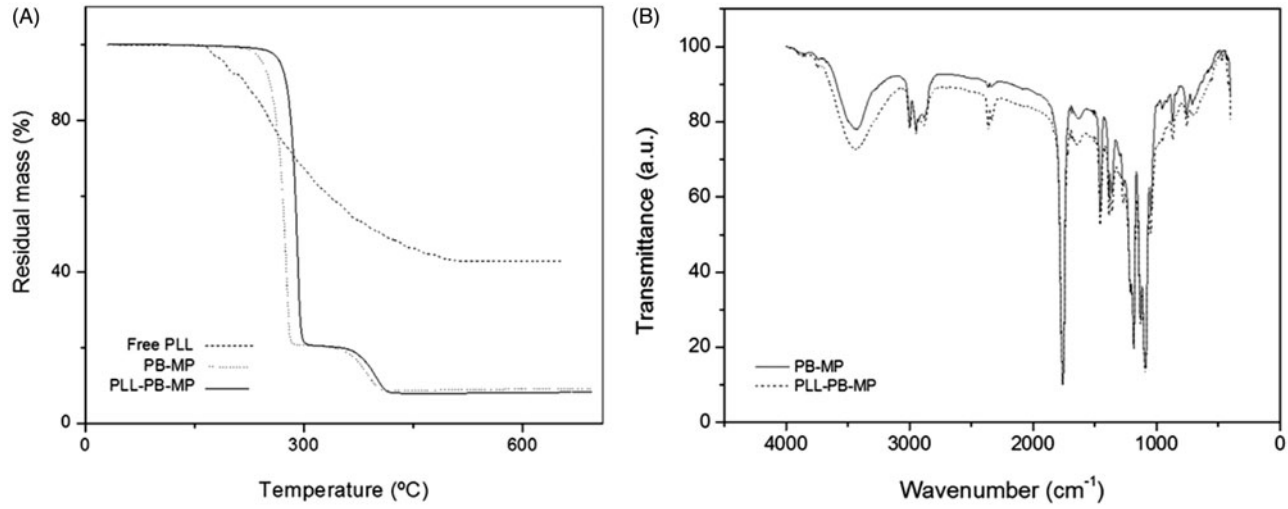

Figure 3. (A) TGA curves of free PLL, unloaded polymer blend microparticles (PB-MPs) and PLL-FITC loaded polymer blend microparticles (PLL-PB-MPs) representing the thermal stability behaviour of the microparticulated systems. (B) FT-IR spectra of PLL-PB-MPs and unloaded PB-MPs presenting the following characteristics bands: C-H stretching bands at 2955 cm^{-1}, C-O-C from 1096 cm^{-1} to 1186 cm^{-1}. CH_2 scissoring from 1388 and 1459 cm^{-1}, C=O at 1762 cm^{-1}, O–H stretching at 3480 cm^{-1}, peptide proton mode band 3300 cm^{-1} and is isotopic counterpart at 2370 cm^{-1}.

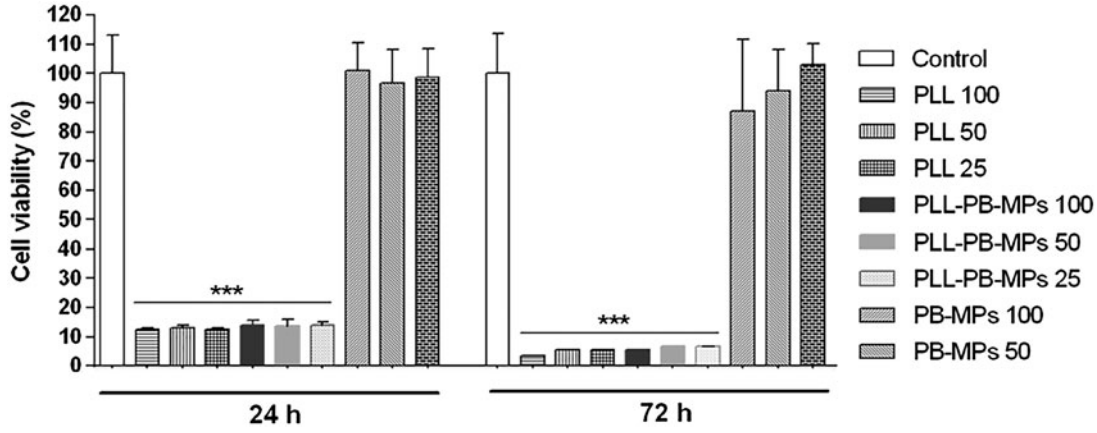

Figure 4. Cytotoxicity evaluation of BxPc3 pancreatic cells treated with free PLL-FITC, PLL-FITC loaded polymer blend microparticles (PLL-PB-MPs) and unloaded polymer blend microparticles (PB-MPs) employing the MTT assay. Asterisks indicate a significant difference between the indicated groups relative to the control at each time (***$p < .001$). All groups under line present the same significant differences relative to the control.

are supported by the anionic nature of PLA chains and the cationic nature of PLL.

In vitro *cell viability analysis*

MTT-based viability assays were performed for free PLL, PLL-PB-MPs and PB-MPs in human BxPc3 and MIA PaCa2 pancreatic adenocarcinoma cell lines. These cell lines were selected because these were sensitive to free PLL (Figures 4 and 5). Therefore, we used these cell lines to determine if the effect of PLL would be preserved after loading into MPs. As can be seen in Figures 4 and 5, treatment with PLL-PB-MPs or free PLL for 24 and 72 h led to a considerable reduction in cell viability when compared to the control group ($p < .001$). Furthermore, in BxPC3 cells (Figure 4), the cytotoxicity and antiproliferative effect observed at 24 and 72 h, respectively, with PLL-PB-MPs was similar to the effect observed for free PLL at the same concentration of peptide, indicating that a high cellular uptake of PLL was achieved using PLL-PB-MPs.

On the other hand, in MIA PaC-2 cells (Figure 5), even though the cytotoxic effect observed at 24 h for free PLL and PLL-PB-MPs at the same concentration of peptide was similar, at 72 h PLL-PB-MPs at 25 and 50 μg/mL showed enhanced cytotoxicity and

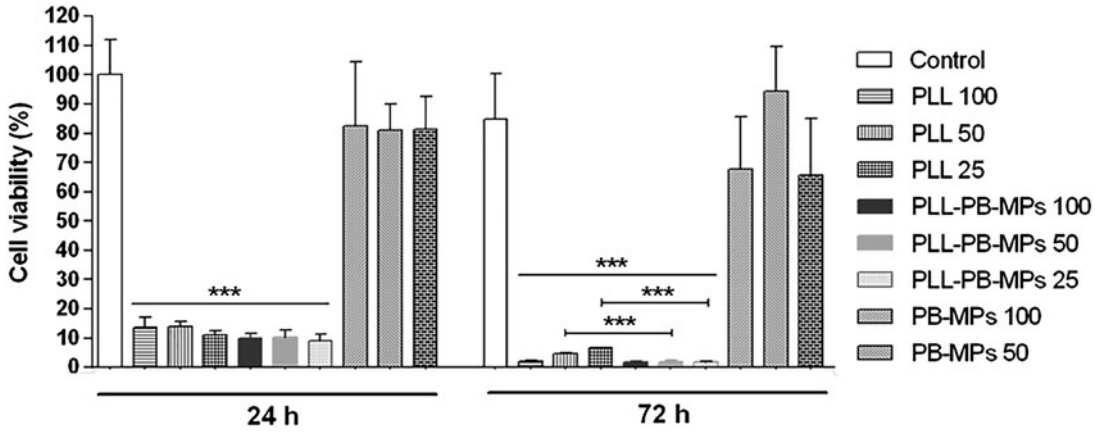

Figure 5. Cytotoxicity evaluation of MIA PaCa2 cells treated with free PLL-FITC, PLL-FITC loaded polymer blend microparticles (PLL-PB-MPs) and unloaded polymer blend microparticles (PB-MPs) employing the MTT assay. Asterisks indicate a significant difference between the indicated groups relative to the control at each time (***$p < .001$). All groups under line present the same significant differences relative to the control. At 72 h, horizontal small bars represent statistical comparisons made between free PLL and PLL-PB-MPs groups.

antiproliferative effect when compared with the same concentrations of free PLL ($p < .001$). At 100 μg/mL of PLL, no differences were observed between free PLL and PLL-loaded MPs, as both treatments led to a complete loss of viability. Additionally, both cells treated with unloaded PB-MPs showed no loss of cell viability, even at higher concentration (Figures 4 and 5), indicating that PB-MPs are not cytotoxic *in vitro* and suggesting that PB-MPs might constitute a promising carrier to deliver bioactive peptides.

Discussion

This work dealt with the obtention, characterisation and *in vitro* evaluation against human pancreatic cancer cell lines of PLL loaded PLA/PEG-*b*-PLA blend based MPs.

It was possible to successfully obtain PLL-PB-MPs with adequate physicochemical characteristics considering its potential biomedical application. According to SEM micrographs shown in Figure 1, MPs obtained using a PLA/PLA-b-PEG blend ratio of 75%/25% presented a non-spherical but highly uniform "raisin-like" morphology (shrunken spheres) which, based on our experience, is typically indicative of rapid solvent evaporation during the elestrospraying process. Moreover, this system presented a mean diameter of 1.2 ± 0.12 μm exhibiting a narrow size distribution. In comparison, MPs fabricated using a PLA/PLA-b-PEG blend ratio of 50%/50% exhibited considerable issues regarding particle aggregation and homogeneous particle formation. These differences can be explained considering the hydrophobic and hydrophilic character of the components constituting the PB. When a higher proportion of hydrophilic PEG chains are present, the proper formation of MPs is limited due to the low affinity of PEG in organic media and the intrinsic characteristics of the proprietary technology processing used to obtain the particles (Moghe and Gupta, 2008). In accordance with this, size, size distribution and LE% were improved when using a higher proportion of PLA compared to that from PLA-*b*-PEG (Table 1). The results obtained indicate that, using a really low percentage of peptide (1%) relative to the PB, PLL was very efficiently encapsulated confirming the capability of biopolymers to carry proteins and peptides even when these hydrophilic molecules present several challenges. The outstanding LE% reported reaches 81% for the PLL-PB-MPs (blend ratio of 75%/25%), whereas the same parameter fell to 68% when taking into account PLA/PLA-*b*-PEG blend ratio of 50%/50%. This result was expected considering the reported polyanionic nature of PLA chains (Shan et al., 2009) and the polycationic nature of PLL (Kim et al., 2005). In light of the evident electrostatic interaction between PLL and PLA within the polymer blend, it is noticeable how a higher proportion of PLA will be translated into increased entrapment efficiency. As can be observed in Figure 2, confocal microscopy was a useful tool to qualitatively confirm the presence of PLL within the PLL-PB-MPs through FITC labeling since it was possible to carry out a co-localisation of MPs and PLL.

These results indicate that the selected fabrication method allows control of the formation of MPs, their dimensions, and their homogeneous properties. Therefore, PLL-PB-MPs with a blend ratio of 75%/25% were selected to perform further characterisation studies and *in vitro* cytotoxicity assays.

The TGA is an important analysis used to investigate loss weight and thermal decomposition of polymeric materials. The thermal stability of free PLL, PLL-loaded and unloaded MPs was studied (Figure 3(A)). Free PLL exhibited a weight loss around 100 °C corresponding to water loss and a T_d of 250 °C. The PB-MPs presented a T_d of 275 °C which is in agreement with the reported values for this kind of biopolymer. Meanwhile, PLL-PB-MPs possess a T_d of 291 °C demonstrating how the PLL accommodation within the biopolymeric matrix results in increased thermal stability.

The spectra obtained for PLL-PB-MPs showed soft shift in characteristic peaks of each component which confirm interactions between polymer and peptide (Figure 3(B)). Furthermore, the band centered in 3480 cm^{-1} presented an increased intensity in PLL-PB-MPs compared to unloaded PB-MP, that could be explained with the contribution of the reported proton mode band of the PLL, which is confirmed by the peak at 2370 cm^{-1} assigned as its isotopic counterpart and identified in this study as the only band that appears in the FTIR spectrum after PLL loading (Rozenberg and Shoham, 2007; Wang et al., 2013).

To study the efficacy of the developed MPs, *in vitro* cell viabilities were evaluated in human pancreatic adenocarcinoma (BxPC3 and MIA PaCa2) cell lines (Figures 4 and 5). Regardless of the assayed time, unloaded PB-MPs were not cytotoxic, probably due to the remarkable biocompatibility of the selected polymers used in the preparation of the MPs. It is known that PLA degrades to produce lactic acid, which is considered a well-tolerated non-toxic material (Rasal et al., 2010). Besides, several *in vitro* and *in vivo* studies demonstrated that PLA in general is well tolerated and does not induce a significant immune response (Athanasiou et al., 1996; de Tayrac et al., 2008).

Free PLL and PLL-PB-MPs exhibited similar cytotoxic and antiproliferative effect at the same concentrations of peptide, with both pancreatic cancer cell lines, exhibiting a significant decrease in cell viability when treated with PLL and PLL-BP-MPs ($p < .001$). This indicates that, even when entrapped in the MPs, PLL was able to inhibit mitochondrial activity, which could be associated with cell death (Tait and Green, 2010). These results are in agreement with previous reported work (Szende et al., 2002). The ability of PLL to inhibit the growth of cells is likely related to its capacity to increase the efflux of small molecules from the cell, which are required for DNA and protein synthesis (Arnold et al., 1979).

The cell viability decrease over time observed for both cell lines treated with free PLL or PLL-PB-MPs could be attributed to the internalisation of PLL into cells. Interestingly, PLL-PB-MPs seem to display an increased potency compared with free PLL against MIA PaCa2 pancreatic cells, as PLL-PB-MPs were slightly more cytotoxic than free PLL for these cells at 72 h (Figure 5). This suggests that these cells might have an enhanced ability to uptake PLL-PB-MPs when compared to free PLL. Moreover, these results indicate that the entrapment of PLL within the biopolymeric matrix of MPs either preserve or increase the therapeutic effect of the peptide against pancreatic adenocarcinoma cell lines.

In addition, the polymeric matrix employed has the safety required for human use, because their component PLA and PEG are generally recognised as safe (GRAS) by the United State Food and Drug Administration (Jamshidian et al., 2010; USFDA, 2011).

Finally, PLL-PB-MPs are capable to display preserved and even improved *in vitro* therapeutic efficacy for potential pancreatic cancer treatment than the one observed using free PLL. Even though *in vivo* studies were not performed, these results are auspicious, not only since both PB and PLL are considered safe substances, but also because they validate the use of PB-MPs for bioactive peptides delivery.

Conclusion

PLL-PB-MPs were successfully prepared and characterised. Adequate physicochemical properties to address biomedical applications were observed. Furthermore, PLL-PB-MPs could display a potent antiproliferative effect when tested against two different human pancreatic adenocarcinoma cell lines achieving a promising dose-response ratio. On the light of the obtained results, the developed PLL-PB-MPs represent a novel system with improved properties to potentially treat pancreatic cancer due to their capability to enhance the PLL antitumoral activity.

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Disclosure statement

The authors do not have any declarations of interest to report.

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