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Paclitaxel delivery system based on poly(lactide-co-glycolide) microparticles and chitosan thermo-sensitive gel for mammary adenocarcinoma treatment

Juan I. Pesoa[a,†], María J. Rico[b,c,†], Viviana R. Rozados[b,c], O. Graciela Scharovsky[b,c], Julio A. Luna[a] and Luciano N. Mengatto[a]

[a]Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), Universidad Nacional del Litoral-CONICET, Santa Fe, Argentina, [b]Instituto de Genética Experimental, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Rosario, Argentina and [c]Consejo Nacional de Investigaciones Científicas y Técnicas, Rosario, Argentina

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chitosan; hydrogel; mammary cancer; microparticle; paclitaxel; poly(lactide-co-glycolide)

Correspondence
Luciano Mengatto, Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), Universidad Nacional del Litoral-CONICET, Colectora Ruta Nac 168, Santa Fe, Argentina.
E-mail: lmengatto@santafe-conicet.gov.ar

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[†]These authors contributed equally to this work.

Abstract

Objectives To evaluate the combination of more than one release system in the same formulation as a useful strategy to achieve paclitaxel delivery in a more sustained and controlled manner.

Methods The present study deals with the preparation of poly(lactide-co-glycolide) microparticles loaded with paclitaxel and included in a chitosan thermo-sensitive gelling solution. The microparticles were characterized by their size, shape and drug loading. The formulation was characterized by scanning electron microscopy, in vitro release experiments and was evaluated in mice bearing mammary adenocarcinoma.

Key findings The formation of paclitaxel crystals in a pharmaceutical formulation reduces its efficacy. In this work, the use of microparticles avoided this phenomenon. Combining more than one delivery system allowed delivering paclitaxel in a more sustained and controlled manner leading to a long-term effect in the site of action. The formulation showed an inhibition in tumour volume of 63.0% in comparison with the control group.

Conclusions One intratumour injection of gelling solution containing the microparticles was at least as efficacious as four intraperitoneal injections of a commercial formulation. In addition, the delivery system was nontoxic, and the treated mice presented the highest percentage of tumour regression and median survival time.

Introduction

Hydrophilic gels that are usually called hydrogels are networks of polymer chains. In this three-dimensional structure, the polymer unions can involve covalent bonds, association bonds such as van der Waals interactions or hydrogen bonds, or physical cross-links, among others. Therefore, according to the interactions type, hydrogels can be named permanent or chemical in the case of covalent bonds and reversible or physical when other interactions such as ionic, hydrogen or hydrophobic bonds are involved.[1,2] Both categories have been investigated as injectable sustained release drug delivery systems that form a gel depot *in situ*. Due to the hydrophilic functional groups present in the polymeric backbone, hydrogels exhibit the ability to retain a significant fraction of water within its structure and to swell without dissolving. According to the mechanism of depot formation, these systems can be classified into two categories: platforms based on *in situ* phase separation and platforms based on *in situ* cross-linking.[3] In the latter, *in situ* gels are formed by photopolymerization, chemical cross-linking or physical cross-linking. In the former, phase separation and gel formation can be induced by changing the solubility of the polymer through changes in pH, temperature or by elimination of solvent.[3] Amongst this kind of hydrogels, those which react to temperature are the most studied. These hydrogels are characterized by the ability to undergo reversible volume-phase transitions in response to subtle changes in their surrounding temperature. The transition has been attributed to a critical balance

of hydrophilic and hydrophobic moieties within the polymer network.[4] Therefore, a thermo-sensitive hydrogel can be applied as an injectable solution and undergo a transition to gel due to a change in temperature. Chitosan (CHT), a cationic amino polysaccharide, combined with β-glycerophosphate disodium (GP) is an outstanding *in situ* gel-forming system, which was first reported by Chenite *et al.*[5] This system is available at a physiologically acceptable pH and is liquid at room temperature. When the temperature is raised to 37 °C, the physiological temperature, it transforms into a gel. CHT and its derivatives have been used to get water-soluble *in situ* gel-forming systems.[6–9] One of the most promising applications for CHT thermosensitive hydrogel is the intratumour delivery of anticancer drugs.[10–13] In these systems, paclitaxel (PTX) is the anticancer drug more frequently studied. PTX is a diterpenoid pseudoalkaloid that promotes the polymerization of tubulin, forming extraordinarily stable and dysfunctional microtubules. This stabilization causes eventually the death of the cell by interrupting the normal tubule dynamics required for cell division.[14]

Continuous delivery over a period of several months may be achieved by entrapping in a CHT hydrogel matrix, drug-loaded carriers which behave themselves as long-term sustained release systems, such as cyclodextrin complexes[9,11] or micro- and nanoparticles.[15] In this approach, biodegradable polymeric microparticles represent a suitable system to provide controlled drug release. A popular polymer used in the preparation of microparticles is poly(lactide-co-glycolide) (PLGA). The versatility of PLGA microparticles allows adjustments in characteristics such as size, drug load and degradability to be made by tuning of the design parameters. The desired release rate and residence can thus be achieved. For example, it has been reported that the delivery of PTX from PLGA microparticles resulted in slower drug clearance and longer residence time, in comparison with the PTX in the Cremophor formulation.[16]

The present study reports the preparation and characterization of a paclitaxel delivery system based on poly(lactide-co-glycolide) microparticles and chitosan thermosensitive gel. In addition, it is shown that the system can be administered in an intratumoral manner in mammary adenocarcinoma-bearing mice.

Materials and Methods

Materials

Chitosan was purchased from China Easter Group (China). GP was kindly provided by Surfactan S.A. (Argentina). PLGA Resomer RG 502H, 8650 Da was manufactured by Boehringer Ingelheim Pharma KG (Ingelheim am Rhein, Germany). PTX was purchased from Laboratorios Filaxis (Martinez, Buenos Aires, Argentina). Polyvinyl alcohol (PVA) was purchased from SERQUIN (San Martín, Buenos Aires, Argentina). Acetic acid and methylene chloride were PA grade (Cicarelli, San Lorenzo, Santa Fe, Argentina). Solvents used for PTX quantification were HPLC grade (Merck, Darmstadt, Germany).

Quantification of PTX

PTX concentration was determined by a HPLC system (Prominence Series 20A, Shimadzu, Kyoto, Kansai, Japan, with UV detection by diode array). The chromatographic system and conditions of analysis were as follows: C18 column (HICROM Ultrasphere®, Hichrom Ltd., Theale, Berkshire, UK, 250 × 4.6 mm), mobile phase water/methanol/acetonitrile (25 : 11 : 64, v/v), flow rate 1 ml/min, oven temperature 30 °C, wavelength 227 nm and injection volume 20 µl. Standard solutions were prepared in the release medium. The calibration curve was fitted to a straight line using linear regression analysis. The evaluation of performance of the method showed that the model can explain ~99.97% (R^2) of the variation in the response variable. The corresponding correlation coefficient (R) was 0.9999 and indicated a large relationship between the variables. The P-value of the model indicated a significant relationship between peak area and PTX concentration ($P < 0.05$).

PLGA microparticles

Microparticles preparation

Microparticles (MP) containing PTX (MPloaded) were prepared using an emulsion and solvent evaporation method adapted from Tsai *et al.*[16] and Boimvaser *et al.*[17] PTX and PLGA were dissolved in methylene chloride. This solution was added dropwise into a PVA aqueous solution (2% w/v) to obtain the O/W emulsion. During the addition, the aqueous phase was stirred at 4000 rpm using an Ultra-Turrax T25D homogenizer (IKA, Staufen, Germany). After 5 min of homogenization, 70 ml of diluted PVA aqueous solution was added to the emulsion and stirred during 30 min. A rotary evaporation system was used for the removal of methylene chloride. The MP were collected by centrifugation, washed with distilled water and lyophilized. In addition, the same procedure was used for the preparation of MP without drug (MP blank).

Microparticles characterization

For the study of MP size distribution, suspensions of the particles were prepared in distilled water and observed in an optical microscope (Leica DM2500M, Leica

Microsystems, Wetzlar, Germany) coupled with a DFC 290HD camera. The particles size distribution (mean ± standard error of the mean) was determined by observing at least 50 MP and analysing the images with the ImageJ software, National Institutes of Health (NIH), Bethesda, Maryland, USA.

The morphology of MP was studied by scanning electron microscopy (SEM). Samples of lyophilized MP were put over an aluminium stub and were then sputter coated with gold using soft conditions under argon atmosphere (SPI Supplies, 12157-AX) and examined using an acceleration voltage of 20 kV, in a JEOL JSM-35C equipped with the image acquisition program JEOL SemAfore.

MP were dissolved in methylene chloride, and then, the samples were conditioned and analysed by HPLC. Entrapment efficiency (*EE* %), drug loading (*DL* %) and yield (*Y* %) were calculated as follow:

$$EE(\%) = \frac{\text{mass of entrapped drug}}{\text{mass of drug used in preparation}} \times 100$$

$$DL(\%) = \frac{\text{mass of entrapped drug}}{\text{mass of particles}} \times 100$$

$$Y(\%) = \frac{\text{mass of microparticles produced}}{\text{mass of drug and polymer used in preparation}} \times 100$$

Infrared spectroscopy experiments (IR) were performed to find out drug-polymer interactions in the solid state. Nearly, 2 mg of sample was blended with potassium bromide and compressed to obtain discs. The IR spectrums were recorded on a FTIR-8001 PC Shimadzu spectrophotometer in the frequency range of 4000–400 cm^{-1}.

Differential scanning calorimetry (DSC) was carried out using a calorimeter (Mettler-Toledo 821; Mettler Instrument AG, Greifensee-Zurich, Switzerland). Aluminium pans containing approximately 2 mg of sample were heated up from 10 to 240 °C at a rate of 10 °C/min.

Chitosan gel with PLGA microparticles

System preparation

Chitosan was dissolved in a 0.15 M acetic acid solution to obtain a concentration of 2% w/v. GP solution was prepared with water. Different amounts of MPloaded were suspended in the polymer solution to obtain gels with different PTX concentration. CHT and GP solutions were mixed (3 : 1, CHT:GP) at room temperature using a syringe.[18] In addition, to prepare a gel only with PTX, the drug was suspended in the polymer solution and then mixed with GP solution.

The structural morphologies of gels were studied by SEM. Gels were freeze-fractured in liquid nitrogen, put over an aluminium stub and subsequently lyophilized. All prepared samples were then arranged and observed as performed for MP.

In-vitro experiments

After CHT and GP solutions were mixed, the syringe containing the mix was put into a thermal bath at 37 °C to allow the transition to gel. Subsequently, pieces of gel of approximately 0.3 g were carefully cut with a scalpel, weighed and immersed into bottles with 100 ml of release medium. Phosphate-buffered saline (PBS, pH 7.4) containing SDS (0.3% w/v) was used as release medium. SDS was included to increase the solubility of PTX. The bottles were maintained at 37 °C with a stirring speed of 100 rpm (orbital shaker). Aliquots were withdrawn at predetermined time intervals and assayed by HPLC. An equal volume of fresh medium was added to maintain a constant volume. Sampling time was selected in order to keep sink condition. In addition to the release experiments from gel containing dispersed drug or MPloaded, experiments with MPloaded alone were also performed. Release experiments were repeated three times.

In vivo experiments

The M234-p mouse mammary tumour, established at the Institute of Experimental Genetics (School of Medical Sciences, National University of Rosario), is a type B moderately differentiated mammary adenocarcinoma[19] that shows a mixed pattern and develops lung metastasis. This tumour appeared spontaneously in a BALB/c female mouse, and it is maintained *in vivo* by serial subcutaneous passages in syngeneic mice.[20]

Six- to eight-week-old BALB/c female mice (25–30 g) were used. Animals were provided with commercial chow and water *ad libitum* and were maintained in a 12-h light/dark cycle. All the experiments were carried out during the first half of the light cycle. The study and all protocols were approved by the Bioethics and Biosafety Committees of the School of Medical Sciences, National University of Rosario (Resolution 4972/2013). The animals were treated in accordance with the guidelines issued by the Canadian Council on Animal Care.[21] Two trials were carried out separately: trial 1 (T1) for the evaluation of antitumor effect and

toxicity and trial 2 (T2) for the evaluation of survival percentage. In both trials, mice were implanted subcutaneously with ~1 mm^3 M-234p tumour fragments in their right flanks. When the tumours reached a volume of 80 mm^3, the animals were distributed in four groups (n = 6–8) and treated as follows: *Control group* received intraperitoneally (IP) 0.2 ml/day of saline solution during 4 days (treatment days 0–3); *PTX group* received IP 10 mg/kg per day of PTX (Paklitaxfil®, Fresenius-Kabi, Buenos Aires, Argentina) during 4 days (treatment days 0–3); *Gel group* received intratumour (IT) 50 µl of gelling solution; and *Gel/MP group* received in an IT manner 50 µl of gelling solution with MPloaded. Tumour sizes were measured with a vernier calliper, and tumour volume was calculated as follows: $v = 0.4\ (ab^2)$, where v = volume (mm^3), a = largest diameter (mm) and b = smallest diameter.[20,22] Toxicity was evaluated by the evolution of the weight of the animals during treatment. T1 was finished after 11 days of treatment when the first mouse reached the maximum tumour volume ethically allowed, and all the animals were sacrificed. In T2, animals of all groups were maintained alive until each one reached the maximum tumour volume ethically allowed, at which point it was sacrificed. Animals that did not show tumour were sacrificed at day 120 when the trial was concluded.

The results were subjected to a multiple comparison test: ANOVA or Kruskal–Wallis. If the null hypothesis was rejected, pairwise comparison was done to determine where the difference lied. Results were considered statistically significant if $P < 0.05$.

Results and Discussion

PLGA microparticles

Figure 1 shows pictures obtained by optical microscopy. MP size distribution study indicated that MP blank and MPloaded showed comparable diameter (Figure 1a,b). The diameter was 7.48 ± 0.19 µm and 6.38 ± 0.13 µm for MP blank and MPloaded, respectively. Pictures of MP obtained with polarized light determined that visible PTX crystals were not present (Figure 1c). Figure 2 shows pictures obtained by SEM. MP were spherical in shape with a smooth exterior surface without visible paclitaxel crystals. The morphology of MP blank (Figure 2a) and MPloaded (Figure 2b) was similar. In different batches of MP preparation, it was observed that *EE* and *Y* were generally high and were 87.2% (77–94%) and 87.7% (85–89%), respectively. The range of *DL* variation was between 7.8% and 9.4% across the different MP preparation batches. These results agree with those reported by Tsai *et al.*[16] and Li *et al.*[23]

Figure 3 shows IR spectra of PTX, PLGA, MP blank, MPloaded and a physical mixture of PLGA/PTX. This mixture was prepared taking into consideration the percentage

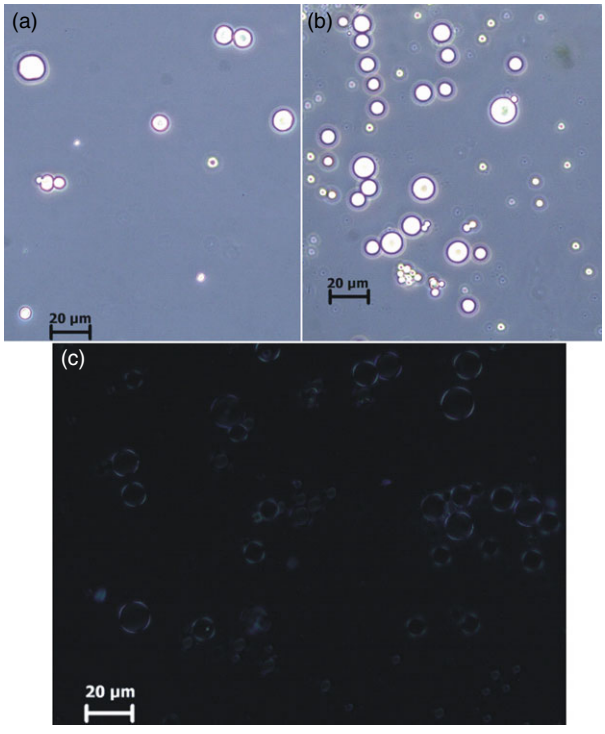

Figure 1 Pictures obtained from optical microscopy. (a) MP blank, (b) MPloaded and (c) MPloaded observed with polarized light.

of drug loading. The spectrum of PTX (Figure 3a) showed the presence of -OH from the alcohol group at 3437 cm^{-1} and –CH bonds at 2800–3000 cm^{-1}. In addition, in the carbonyl stretching region, contributions from ester/ketonic C=O (1715–1730 cm^{-1}) and amide C=O (1647 cm^{-1}) were observed. In the region from 900 to 675 cm^{-1}, a characteristic peak due to aromatic CH bond was seen at 709 cm^{-1}.[24] The spectra of PLGA and MP blank (Figure 3b) were similar, as expected. The $–CH_3$,$–CH_2$ and –CH stretching vibrations were observed at 2900–3000 cm^{-1}; the strong band at 1759 cm^{-1} and the bands at 1250–1500 were attributed to stretch of the C=O group and $–CH_3$ deformation, respectively. In addition, C–O ester stretching (1000–1250 cm^{-1}) and –CH bending (500–750 cm^{-1}) were observed.[25] The IR spectra of the physical mixture and MPloaded showed differences compared to that of MP blank (Figure 3c). The regions where the differences were important are marked between dashed lines. In these regions, the bands at 3400, 1647 and 709 cm^{-1} showed more intensity in the mixture as compared to MPloaded. As previously mentioned, these bands correspond to group characteristic of PTX. This suggests that in the MPloaded most of the drug was entrapped in the particle.

Differential scanning calorimetry thermograms (Figure 4) were obtained to investigate the physical state of the

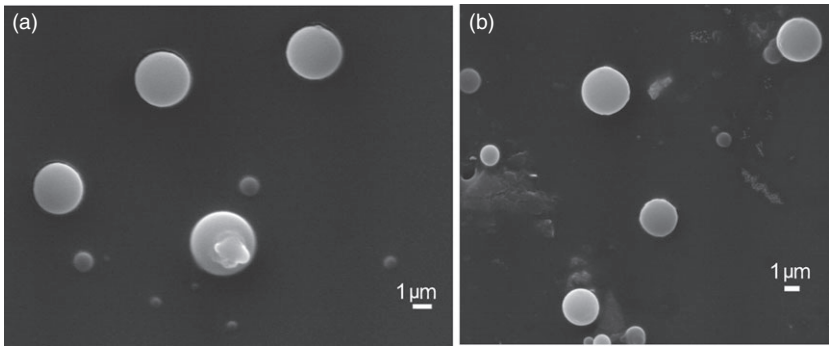

Figure 2 Pictures obtained from SEM. (a) MP blank and (b) MPloaded.

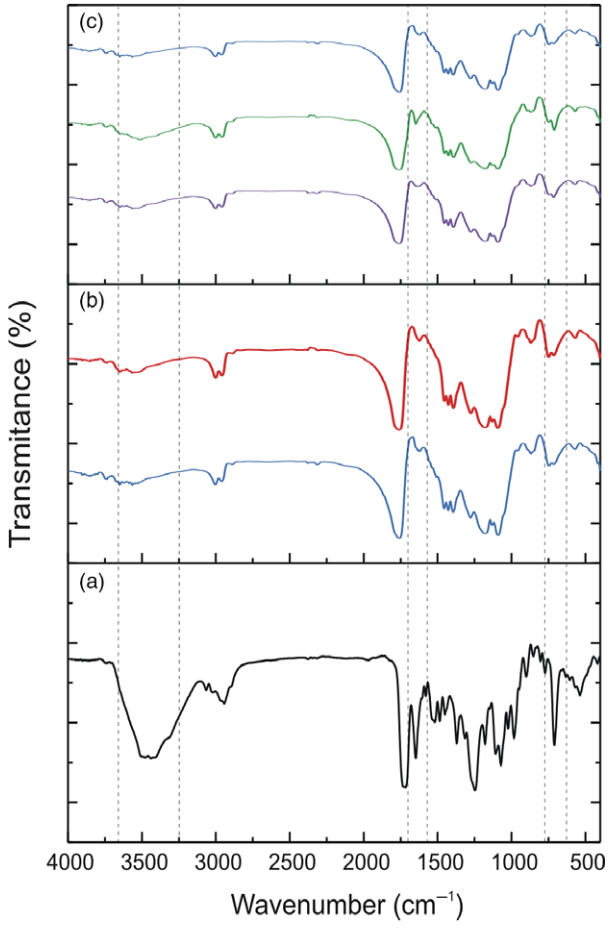

Figure 3 FTIR spectra of (a) PTX; (b) PLGA (red) and MP blank (blue); (c) MP blank (blue), MPloaded (purple) and physical mixture of PLGA/PTX (green).

drug in the MP. In the case of PTX, the results agree with the finding of Liggins *et al.*[26] A small change in baseline around 50–60 °C due to the removal of residual water and a single melting endotherm at 220 °C, before degradation, were observed. MP blank and MPloaded showed

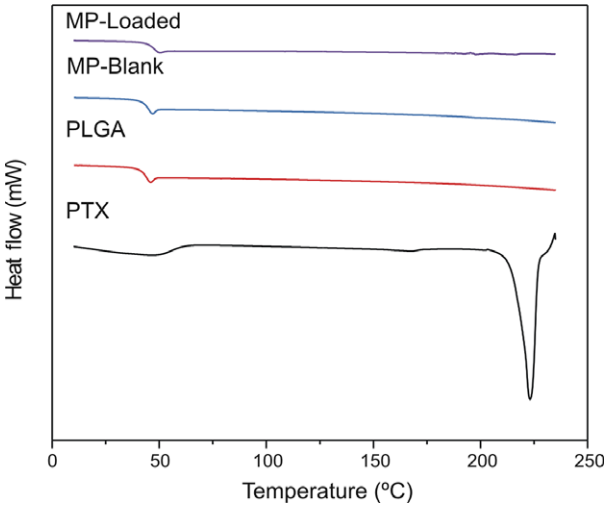

Figure 4 Differential scanning calorimetry thermograms of PTX (black), PLGA (red), MP blank (blue) and MPloaded (purple).

comparable glass transition temperatures, which were similar to that of PLGA (48–51 °C), suggesting no drug–polymer interactions in the particle.[16] Moreover, the melting peak of PTX was not present in the calorimetric curve of MPloaded; therefore, the drug was not in a crystalline form. Then, PTX was in an amorphous form or homogeneously dispersed in the polymer matrix.

Chitosan gel with PLGA microparticles

Chitosan solutions not only present the ability to undergo sol-gel transition in response to temperature change, but also the polymer itself possesses antitumor activity.[22,27] Nevertheless, in this kind of hydrophilic systems, the direct incorporation of the hydrophobic PTX leads to the formation of drug crystals which are very stable in aqueous environments. Castro *et al.*[28] prepared different hydrogels with PTX concentrations similar, or even lower, to those reported in the literature and observed PTX crystals. In

addition, these authors proposed that the crystallization of the drug can decrease its therapeutic effect. Supporting this theory, Chun *et al.*[29] reported that the hydrogel with the lowest concentration of PTX was more effective in tumour inhibition than the hydrogel with the highest concentration when tested in experiments with rats. Although the authors mentioned that this result was not clear, one explanation could be that the phenomenon of PTX crystallization, which is enhanced at higher concentrations, reduced the efficacy of the drug.[28] On the other hand, micro- and nanocrystals have been used as carriers for PTX.[30,31] Nevertheless, nanocrystals may require some additives to ensure stabilization.[32] In addition, PTX crystals can be formed by homogeneous and heterogeneous nucleation, displaying different morphologies and sizes and affecting the dissolution and release of the drug.[33] In general, the smallest nanocrystals exhibit similar *in vivo* behaviour to a drug solution, while the larger are rather phagocytosed by the reticuloendothelial system (RES) and preserved as drug depots. And, further, charge on crystal surface affects cell internalization and cytotoxicity.[34] To avoid the complications of PTX crystallization, polymer particles loaded with the drug can be prepared and included in systems such as the temperature-sensitive hydrogels.[15] As CHT, PLGA also displays antitumor activity.[35] According to our knowledge, this work is the first time that PLGA MP loaded with PTX were included in CHT thermo-sensitive hydrogels. This kind of system allowed preparing hydrogels with different PTX concentration by varying the amount of MP. While the MP contributes to avoid PTX crystallization, the hydrogels help to improve the effectiveness of MP by immobilizing them in the treatment site. The transition of CHT solutions from liquid to gel state was not affected by the presence of MP, since the gelling time (approximately 150 s) was not modified in comparison with CHT solution without MP. In addition, the gel with MP presented a good macroscopic consistency and near neutral pH (6.95). Figure 5 shows pictures obtained from SEM of gel (a) and gel with MP (b). The gel matrix displayed an interconnected porous structure. MP were located in the cavities of the pores or even embedded in the polymer structure (Figure 5b). Also, no crystals of the drug were observed.

In-vitro experiments

A direct loading of the active ingredient into the solutions prior to gel formation represents an easy way to add agents. However, the release of the loaded molecules may not be well regulated. For long-term controlled applications, another release system loaded with the drug may be incorporated into the hydrogel. Different amounts of MP of different characteristics (drug load, size, polymer) may provide a wider range of possibilities for control. In this work, three amounts of MPloaded were suspended in the polymer solution to obtain gels with different PTX concentration (1.30; 2.00 and 2.70 mg_{ptx}/g_{gel}). After the gel was obtained, pieces of 0.3 g were immersed into bottles containing the release medium.

Figure 6a shows the cumulative release of PTX (mg_{ptx}/g_{gel}) from gels as a function of time. For each of the PTX concentration, a three phase pattern of release was observed. The release profile over 192 h presented an initial burst phase over about 1 h, followed by a slower release phase (insert Figure 6a). The initial release rate and the cumulative release during the whole experiment were statistically higher ($P < 0.05$) for the higher drug loading. The rate of PTX release underwent a slight increase since hour 5, until to the total drug content was delivered, due to a combination of diffusion and degradation of the MP. Figure 6b shows the cumulative release of PTX (%) from MPloaded alone and gels containing dispersed drug or MPloaded as a function of time. Gels containing dispersed drug or MPloaded with a final PTX concentration of 1.30 mg_{ptx}/g_{gel} and sufficient amount of MPloaded (*DL* 7.8–9.4%) were used; therefore, the total amount of drug was approximately the same (0.4 mg) in the three tested systems. MPloaded alone presented a phase of rapid release followed by a slower release phase up to 88 h when the total drug content was delivered. In the insert of Figure 6b, it can

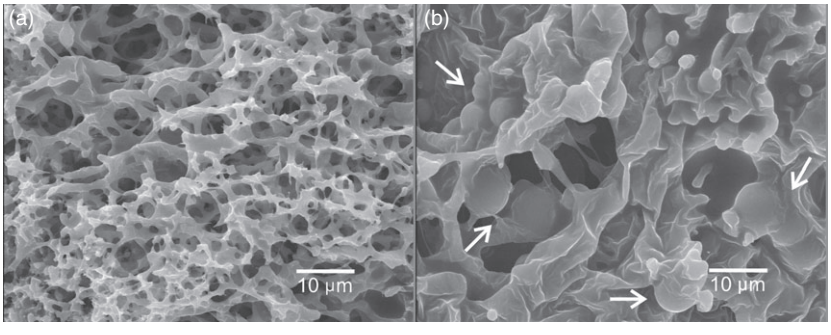

Figure 5 Pictures obtained from SEM. (a) Gel and (b) gel with MP (arrows indicate MP).

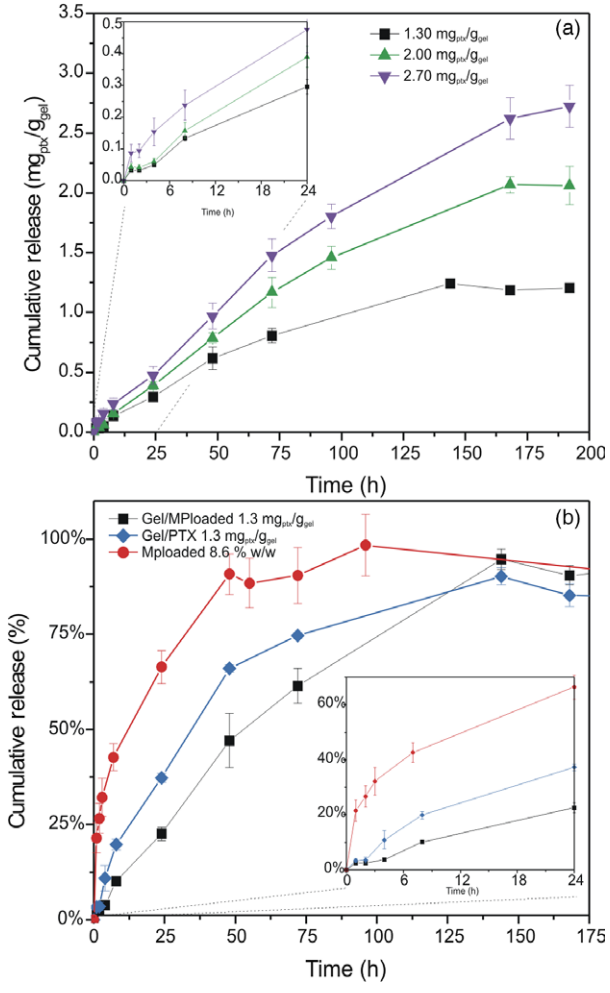

Figure 6 (a) Cumulative release of PTX (mg$_{ptx}$/g$_{gel}$) from gels with different amounts of MPloaded. (b) Cumulative release of PTX (%) from gels containing dispersed drug or MPloaded and MPloaded alone.

be observed that the gel contributed to control the release since the initial release rate was lower than the rate of the MPloaded alone. In addition, the entrapment of the drug into the MP and their inclusion in the gel rendered the deliver more controlled. The cumulative release during the whole experiment was lower for the gel with MPloaded due to a combination of diffusion from the MP to the gel and then from the gel to the medium. Also, degradation of the MP and the gel could contribute to the release.

Taking into consideration that the gel containing MPloaded at the lowest PTX concentration (1.30 mg$_{ptx}$/g$_{gel}$) was the system with the best behaviour during in-vitro experiments, it was selected for *in vivo* experiments. Supporting this selection, the results reported by Chun *et al.*[29] showed that the lowest concentration of PTX is more effective *in vivo* at tumour inhibition than the highest concentration.

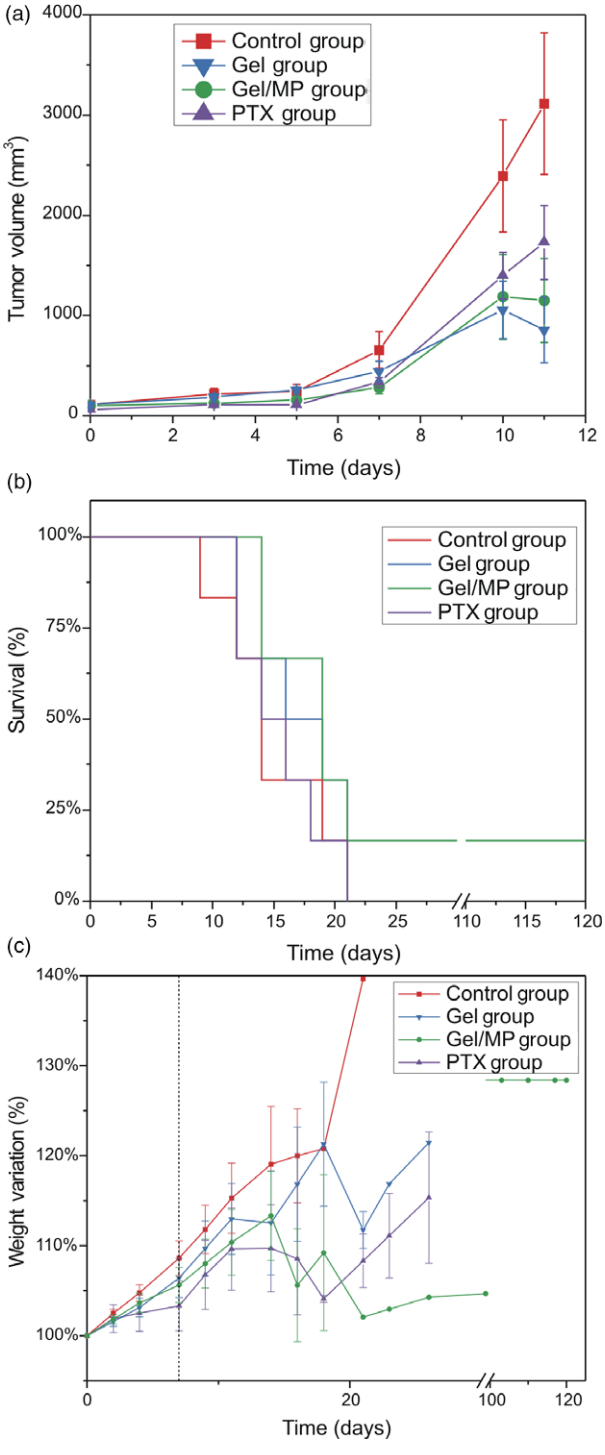

Figure 7 (a) Tumour volume in T1 experiment, (b) survival percentage in T2 experiment and (c) evolution of the mice body weight during T2 experiment.

In vivo experiments

Localized delivery of therapeutic agents has emerged as a way of reducing systemic toxicity, and it is a popular area of interest for systems such as CHT hydrogels. In this work, the IT delivery of PTX was tested with the M-234p BALB/c tumour model. When the tumours reached a volume of ~80 mm^3, generally 7–10 days after tumour cells implantation, the animals were distributed into four groups and treated. T1 was conducted to evaluate the antitumor effect and toxicity of the system.

Figure 7a shows that after 7 days of treatment, tumour volume in the *Control group* was higher than in the other groups, and the difference was statistically significant ($P < 0.01$). At the end of the trial, the *Gel/MP* and *PTX groups* showed an inhibition in tumour volume of 63.0% and 44.0%, respectively, in comparison with the C*ontrol group*. In addition, it is noteworthy that one IT injection of *Gel/MP* was at least as efficacious as four IP injections of Paklitaxfil®. The injection of gelling solution without PTX also impacted on tumour growth. This result agrees with the findings of Ruel-Gariépy *et al.*[22] and can be explained on the base of the antitumor activity of CHT itself.[27] T2 was carried out to evaluate the effect of the treatment on survival. It has to be noted that one animal belonging to the *Gel/MP group* survived until the end of the experiment (day 120). In this animal, the tumour was neither visible nor palpable. During necropsy, neither primary tumour nor macroscopic alterations in lungs, liver, spleen and pancreas were observed. The mean survival time of the animals in the *Control* and *PTX groups* was 15 days, followed by *Gel group* (17.5 days). The higher survival was shown by the *Gel/MP group* (19 days; Figure 7b), a difference though not statistically but biologically significant.

The evolution of the mice body weight was used as an indicator of general health status during treatment. There were no weight losses; instead, an increase in the weight of all the animals was observed (Figure 7c). In addition, other characteristics controlled throughout the experiment, such as motor activity, fur quality, food intake, response to stimuli and breathing, remained without alterations.

Based on the results on tumour volume and regression and survival percentage, the IT injection of gelling solution with MPloaded exhibited the best antitumor action and this fact could be explained by three reasons. First, the PTX concentration remained high in the tumour because the treatment was localized (IT), leading to low dose requirements. Second, the MP and the gel may have allowed PTX delivery in a more sustained and controlled manner allowing a long-term effect on the tumour cells. Third, a synergistic effect may occur between PTX, PLGA and CHT.

A key requirement of *in situ* gelling systems for local delivery and more specifically, IT delivery, is the injectability from a prefilled syringe. These solutions can easily flow during administration, but once injected rapidly form gel networks. Nevertheless, leakage from the site of injection must be controlled.[3] The researchers who carried out the *in vivo* experiments showed here, expressed conformity regarding injectabilty and prevented leakage by leaving the needle inside the tumour for 10 s after injection.

Conclusions

Intratumour drug delivery is a notable tool to obtain high treatment efficacy and low toxicity and side effects. The aim of the present paper was the preparation of a PTX delivery system based on PLGA microparticles and CHT thermo-sensitive gel for breast cancer treatment. This platform, combining more than one delivery system, allowed delivering the drug in a more sustained and controlled manner leading to a long-term effect in the site of action. The use of microparticles avoided the formation of drug crystals. One intratumour injection of gelling solution containing the microparticles was at least as efficacious as four intraperitoneal injections of a commercial formulation. The delivery system was nontoxic, presented the highest median time of survival, and showed one complete regression. These interesting results are the foundation for further studies that will allow obtaining improved therapeutic results.

Declarations

Conflict of interest

None.

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