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Potential applications of PLGA film-implants in modulating in vitro drugs release

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Abstract

In this work we evaluate poly(lactic/glycolic) acid (PLGA) film-implants as potential biodegradable devices for controlled release of two different drugs: 5-Fluorouridine (5-FUR), a conventional low molecular weight water-soluble compound and SPf66 malaria vaccine, a therapeutic synthetic polypeptide. Three types of devices were prepared by solvent-casting techniques alone or combined with compression method: simple monolithic discs (SMD), multilayer discs with a central monolithic layer (MLDM), and multilayer discs with a central drug-reservoir (MLDR). For the highly water-soluble drug, 5-FUR, in vitro release from SMD showed an initial burst (24% in 2 h) followed by prolonged release over 20 days. In contrast, from a MLDM (two drug-free PLGA discs were added to the SMD) showed an initial lag-time of 12 days followed by a very fast second release phase. Finally, when the load of this system was increased from 3 to 9%, an extended release over 20 days with a low burst effect was obtained. For SPf66, the central reservoir containing the synthetic polypeptide MLDR reduces the possibility of degradation due to peptide contact with polymer solution. When four layers were added, 10 days sustained-release was obtained without any burst effect. With six layers a moderate pulse was obtained, 18–22 days from the beginning of the release. The results show the suitability of the proposed devices to control release and avoid the burst effect with highly water-soluble drugs; as well as modulate in vitro peptide release.

Keywords: PLGA film-implants; Simple monolithic discs; Multilayer discs; 5-Fluorouridine; Synthetic polypeptide SPf66

1. Introduction

Various controlled release systems have been prepared using biodegradable polymers as carriers for delivering pharmaceutical agents of a wide variety of types including, antibiotics (Webber et al., 1998), anticancer drugs, steroids (McCarron et al., 2000; Zhou et al., 1998), peptides (Rothen-Weinhold et al., 1999a), proteins (Singh et al., 2001) and many other therapeutic agents. However, the development of controlled release technology currently remains a stimulating challenge for a large number of research groups world-wide.

The physicochemical properties of both polymer and drug are important factors in the design of controlled release delivery systems. The toxicity,

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biocompatibility, and immunogenicity of polymer devices are also critical due to their direct interface with the biological environment, into which they are injected, implanted or inserted (Jeong and Kim, 1986). Among biodegradable polymers, polylactic acid and poly(lactic/glycolic) acid (PLGA) have been the most commonly used in sustained-release drug delivery, as they degrade by simple hydrolysis of the ester bonds into natural metabolites, glycolic and lactic acid. These are removed from the body by normal metabolic pathways and therefore do not require surgical removal after completion of drug release. These polymers are therefore biocompatible, biodegradable and considered safe (Jain et al., 1998; Kunou et al., 2000).

PLGAs may be prepared with any monomer composition, the properties of these copolymers can thus be controlled by adjusting their molecular weight (Mw) and the molar ratio of lactic to glycolic acids; an important factor in system design. In drug delivery systems, lactic acid is more frequently chosen as the predominant specie because it is more hydrophobic. Furthermore, lactic acid is optically active and the option of using of optically pure or racemic monomer in the preparation of PLGA offers an additional measure of system design control (Jain et al., 1998; Miyajima et al., 1998).

Polymer devices can be classified into two main classes: monolithic and reservoir devices. In the monolithic systems the drug is dissolved or dispersed in an inert matrix and in the reservoir systems the therapeutic agent forms a core surrounded by an inert polymer diffusional barrier. Combinations of both devices can also be prepared. Drugs formulated in these systems are released either by diffusion through the polymer barrier, by its erosion, or by a combination of both mechanisms (Jain et al., 1998). Drug release is affected by factors such as polymer Mw, copolymer ratio, polymer crystallinity, preparation method, and the physicochemical properties of the incorporated drug. In fact, polymer-drug interactions can be critical in controlling drug release characteristics (Miyajima et al., 1998; Sung et al. 1998; Rothen-Weinhold et al., 1999b; Vogelhuger et al., 2001). On the other hand, the drug release kinetics from bulk-eroding PLGA matrices is complex because the polymer phase properties change continuously during degradation, resulting in drastic changes in drug diffusivity and permeability (Kunou et al., 2000).

All these reasons make precise drug profiles difficult to predict and so the design and development of a biodegradable drug delivery system is carried out with the purpose to obtain a drug release kinetics aimed at optimum therapeutic effect.

The aim of this study was to evaluate PLGA film-implants as potential biodegradable devices for controlled release of several types of drugs. The film-implants were selected for their versatility of design and easy manufacture. Since most of the drugs are small molecules with Mw < 500 excepting polypeptides and proteins, the pharmaceutical agents selected were: 5-Fluorouridine (5-FUR) (a low Mw antimetabolite) (Zhou et al., 1998), and SPf66 malaria vaccine (synthetic polypeptide) (Valero et al., 1993).

2. Materials and methods

2.1. Materials

5-FUR was purchased from Sigma®; SPf66 malaria vaccine (batch 15–7) was synthesised at the Instituto de Inmunología, Hospital San Juan de Dios, Bogotá, Colombia. PLGA (Mw: 47000; LA/GA 63/37) was synthesised in our laboratory by ring-opening polymerisation of initial monomers D,L-lactide (Aldrich) and glycolide (Boheringer Ingelheim) following the method described by Gilding and Reed (1979)All other reagents were analytical grade.

2.2. Characterisation of copolymer

The weight-average Mw was determined by gel permeation chromatography (GPC, Waters), relative to polystyrene standards (Tokyo Soda Ltd) with Mws 2800–700 000. Tetrahydrofuran was used as the mobile phase at a flow rate of 0.9 ml/min. The copolymer composition and the relative proportions of lactic-glycolic acid bond (GA–GA)

were assessed by ^{1}H-RMN and ^{13}C-RMN, respectively using a Bruker AMX-400 spectrometer (Dorta et al., 1993).

2.3. Preparation of formulations

Three types of biodegradable circular film-implants (discs) composed of one or more PLGA-layers were prepared by a solvent-casting technique alone or combined with compression methods: simple monolithic discs (SMD), multilayer discs with a central monolithic layer (MLDM), and multilayer discs with a central drug-reservoir (MLDR).

2.3.1. Preparation of drug-free film-implants (PLGA-layers)

Drug-free PLGA-layers were manufactured using a solvent-casting technique. Briefly, PLGA solution (25–30%, w/v) in dichloromethane (DCM), was prepared. This solution was then poured onto an aluminium surface (4.7 cm in diameter) and the DCM allowed to evaporate slowly at between 2–8 °C for 48 h. The films were then vacuum-dried in a dessicator at room temperature for 12 h to remove the residual solvent. These drug-free films were then used in preparing the different formulations.

2.3.2. Simple monolithic discs

5-FUR PLGA-film was prepared as described above, but in this case the 5-FUR (60 mg) was dispersed into the polymer solution (30%, w/v) and mixed well prior to casting. The resulting simple monolithic film (4.7-cm diameter) was cut into small discs of 3-mm diameter and each was weighed. The final weight and thickness of the discs thus obtained was 1.79 ± 0.38 mg ($n = 10$) and 0.148 ± 0.028 mm ($n = 10$) respectively.

Fig. 1 shows the schematic representation of the preparation method of both drug-free film-implants and SMD.

2.3.3. Multilayer discs with a central monolithic layer

Two film sandwiches (4.7 cm in diameter) were manufactured containing 5-FUR, one 60 mg and the other 180 mg. Each sandwich was formed of three layers prepared as previously described: the two external drug-free layers and a third central monolithic drug-PLGA-layer. The sandwich films were prepared by compressing the above three layers at a pressure of 0.5 metric ton applied for 3 min at room temperature using a manual hydraulic press (Perkin Elmer). The devices produced were cut into discs of 3 mm in diameter. Fig. 2 shows the schematic representation of the preparation method of MLDM.

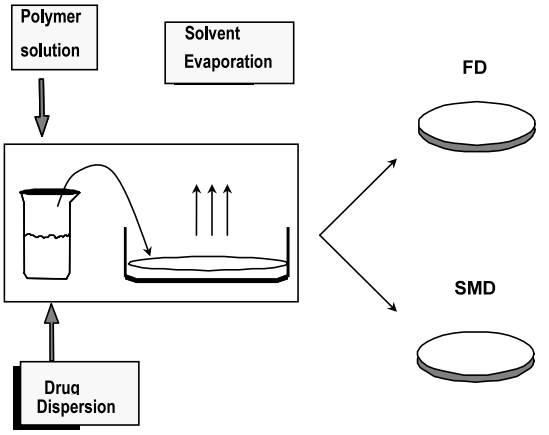

Fig. 1. Schematic representation of the film-implant preparation method: drug-free discs and SMD.

The final weight and thickness of the resulting discs were 6.11 ± 0.60 mg and 0.490 ± 0.07 mm for 3.03% MLDM, and 6.53 ± 0.66 mg and 0.571 ± 0.062 mm for 9.09% MLDM respectively.

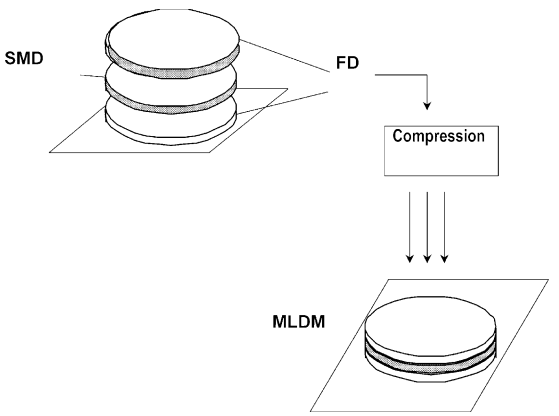

Fig. 2. Schematic representation of the preparation method for MLDM.

2.3.4. Multilayer discs with central drug-reservoir

Two reservoir devices were prepared containing SPf66 with four and six layers. First, an aqueous solution of SPf66 synthetic polypeptide and hydroxypropyl β-cyclodextrin (HPβ-CD), therapeutic agent and cryoprotectant excipient respectively, was prepared. The SPf66/HPβ-CD weight ratio in the solution was 5/95. A certain amount (0.5 ml) of this solution was cast over a drug-free PLGA-film prepared as previously described. This SPf66 polymer-film and several drug-free PLGA films were freeze-dried in a lyophilizer (Labconco Lyphlock® 6) following a freezing step of 1 h to −40 °C, a primary drying step with a shelf temperature of −40 °C (for up 16 h); and finally, a secondary drying step while raising the temperature to 15 °C, maintaining this for 1 h. After, a lyophilised drug-free PLGA-film was then poured onto polymer loaded film to form a sandwich. This system was compressed at a pressure of 0.5 metric ton for 15 s at room temperature using a hydraulic manual press (Perkin Elmer®). Smaller discs of 1.5 mm diameter were cut from this system.

Three groups of discs 1.5 mm in diameter were individually encased by two (one on top and one on the bottom) or four drug-free PLGA-films (two on top and two on the bottom). The resulting multilayer systems, one with a total of four layers and another with six layers, were compressed at a pressure of 1 metric ton for 1 min. The devices obtained were cut into small discs 4 mm in diameter. Fig. 3 shows the schematic representation of the preparation method of MLDR.The final weight and thickness of the resulting discs were 7.64 ± 1.14 mg and 0.398 ± 0.109 mm respectively for $n = 4$ and 10.28 ± 1.51 mg and 0.431 ± 0.09 mm for $n = 6$.

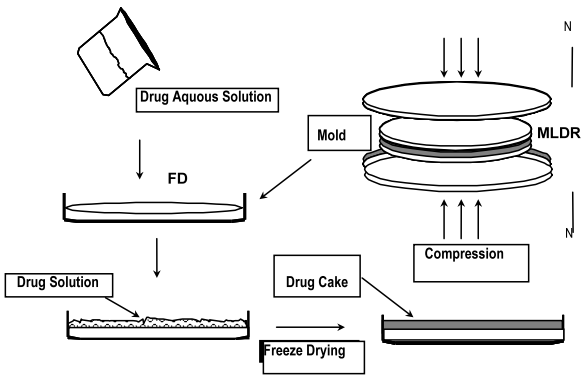

Fig. 3. Schematic representation of the preparation method for MLDR.

2.4. Drug content determination

The drug content in the film-implants and the amount of released drug were determined by high performance liquid chromatography (HPLC) using a waters apparatus (Waters, Milford, MA) consisting of a pump, model 600 E multisolvent delivery system, 700 Wisp sample processor, and a programmable multiwavelength detector. deionized water used to prepare the mobile phase was purified in a Millipore Milli-Q system; all other chemicals and reagents were HPLC grade. All solvents were filtered with 0.45 μm pore size filters (Millipore). The mobile phase was filtered and degassed.

2.4.1. 5-Fluorouridine

Each disc was dissolved completely in DCM, and the drug, which is insoluble, was then extracted from polymer solution with distilled water. The aqueous phase was analysed by reverse-phase chromatography (RP-HPLC). A RP C-18 column (Resolve® 8 × 100 mm^2) was used as stationary phase. The mobile phase was a mix (96/4) of 50 mM ammonium dihydrogen phosphate (adjusted to pH 3.5 with phosphoric acid) and acetonitrile, and at a flow rate of 1.7 ml/min at room temperature. UV detection at 268 nm was used.

2.4.2. SPf66

Each disc was dissolved completely in tetrahydrofuran and the dispersion centrifuged at 3000 rpm for 15 min, and the supernatant was removed. This precipitate was dissolved in mobile phase and the SPf66 concentration measured by size exclusion chromatography (SEC-HPLC). A Protein Pack 125 column (370 × 7.8 mm^2 I.D.; Waters) was used as stationary phase. The mobile phase was an acetonitrile/water mix (30/70) with 0.05% trifluoracetic acid at a flow rate of 1.0 ml/min at

room temperature. UV detection at 214 nm was used.

Both methods were conveniently validated (Dorta et al., 1997; Santoveña et al., 2002).

2.5. In vitro release studies

2.5.1. 5-Fluorouridine

The in vitro experiments were carried out in triplicate, by placing the preweighed film-implants (discs) in individual silanated vials containing 3 ml of a 0.066 M isotonic phosphate buffer saline at pH 7.4 in a bath kept at 37 °C. The release medium was periodically withdrawn and replaced by equal volumes of fresh buffer taken into account in the calculations of the cumulative amount of released drug, which was analysed by HPLC as previously described.

2.5.2. SPf66

The in vitro experiments were carried out in triplicate. The preweighed film-implants (discs) were placed in individual silanated vials that contained 0.5 ml of a Glycin I (Sörensen) buffer at pH 2.0 (Geigy, 1965) in a bath kept at 37 °C. At appropriate time intervals the vials were withdrawn (three vials at a time) and the implant in each vial separated from the release medium. The residual content of SPf66 in each implant was determined by HPLC as described above. This method allows to detect possible variations due to peptide degradation or any other process with respect to an initial or predetermined value (Santoveña et al., 2002). The cumulative amount of released polypeptide was calculated from the difference between the dose and the residual content per implant at each time.

2.6. Data analysis

The model parameters were obtained by fitting linear least-square regression of the order-zero linear equations treating the results by analysis of variance (ANOVA) of the linear regression (significance level $P < 0.05$), and by fitting non-linear least-square regression of the exponential equations using Microsoft Excel Solver function (Billo, 1997).

3. Results and discussion

The objective of this work was to evaluate the potential of biodegradable PLGA film-implants for controlled release of two kinds of drugs. First, film-implants containing 5-FUR were prepared by dispersion into PLGA solution before to casting. In the former case, an SMD was obtained. 5-FUR was selected as the model drug due to being a low Mw water-soluble compound (Mw 262.2 g/mol).

The drug release from biodegradable monolithic systems may combine diffusion and dissolution of both polymer and drug. For this reason, the release profiles often present several phases depending on which process predominates. Generally, the drug delivery from monolithic PLGA systems shows either a triphasic release pattern, characterised by a secondary phase of lower release preceded and followed by higher release phases, or a biphasic release composed of an initial burst and a subsequent sustained-release phase (Park et al., 1998). Drug release kinetics from bulk-eroding PLGA matrices is complex because the polymer phase properties change continuously during degradation, resulting in drastic changes of drug diffusivity and permeability (Kunou et al., 2000). Most of the authors fit the experimental data separately by stages. The kinetics most used are zero-order, first-order and the square root of time (Higuchi). The last predicts that the predominant release mechanism is diffusion controlled, but very few authors determine the diffusion coefficients, therefore these fits are empirical.

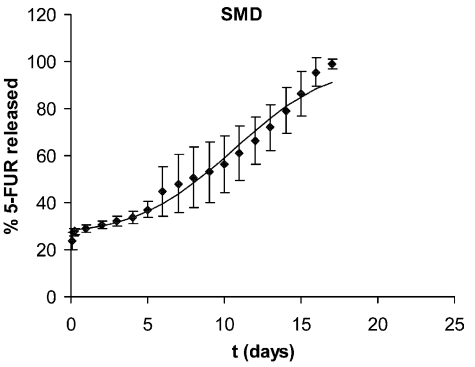

Fig. 4. Plot of percentage 5-FUR released from SMD versus time (mean ± S.D.).

Fig. 4 shows the cumulative release of 5-FUR from SMD. The experimental data were fitted to the empirical model (Dorta et al., 2002):

$$X = X_{01} + \frac{X_{02}}{1 + e^{-k_2(t-t_0)}} \quad (1)$$

where X is the percentage of drug released from the implant at time t, X_{01} and X_{02} are the drug released (%) initially and during the second stage of release, and k_2 the release rate constant. This model allows calculate the amounts of released drug for every stage of release, so as the specific rate release constants of the release process.

The release showed a profile characterised by an initial burst (24% of the drug dose released within 2 h) followed by a second stage of gradual delivery over 20 days with a constant rate of $k_2 = 0.314 \pm 0.047$ days^{-1}, although a slight increase in release rate was observed after 11 days ($t_0 = 10.64 \pm 0.891$ days). The high initial release or burst effect is a frequent phenomenon associated with drug delivery from monolithic polymer controlled release systems. Despite the fast release of drug in a burst stage being useful in certain drug administration strategies, the negative effects brought about by the burst can be pharmacologically dangerous and economically inefficient (Huang and Brazel, 2001). In addition, in the particular case of highly water-soluble compounds, such as 5-FUR, this difficulty is increased and the in vitro profiles from this type of biodegradable system have frequently presented a large initial burst and incomplete release (Benoit et al., 1997; Kader and Jalil 1998).

The MLDM were designed to improve the release properties and two formulations were prepared. Since the burst effect is generally due to the dissolution or diffusion of drug particles deposited on or near the film surface, two drug-free PLGA discs were added above and below the 5-FUR-containing SMD. Therefore, the resulting loading of this system was three times lower. As can be seen in Fig. 5, the release pattern shows an initial lag-time (6% of the drug dose released within 12 days) with a very slow rate delivery ($k_1 = 1.1 \times 10^{-3} \pm 1.5 \times 10^{-4}$ mg/day; $r = 0.883$), immediately followed by a very fast second phase of release ($k_2 = 0.03 \pm 0.004$ mg/day; $r = 0.968$). In

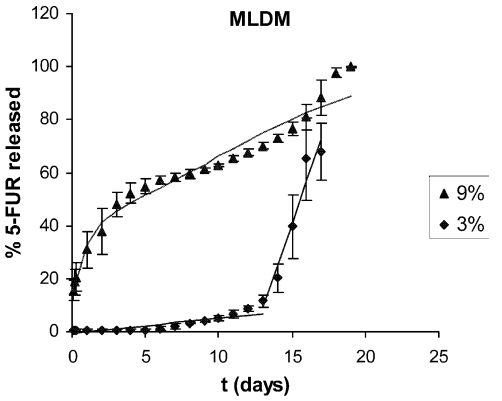

Fig. 5. Plot of percentage 5-FUR released from MLDM versus time. Dots are experimental data (mean ± S.D.) and solid line are predictions from model equations.

this case the experimental data fitted to zero-order kinetics which indicates that 3% MLDM behaviour was close to that of a reservoir device. However, a continuous prolonged release during the active phase of disease is the ideal release profile for most drugs, such as 5-FUR, from a controlled biodegradable system.

Therefore, since in general the initial amount of drug released increases proportionally with the drug load (Kader and Jalil 1998; Zhou et al., 1998), the load in the system was increased from 3 to 9% to avoid the initial lag-time obtained from the above formulation. A continuous delivery from the beginning of the assay and sustained-

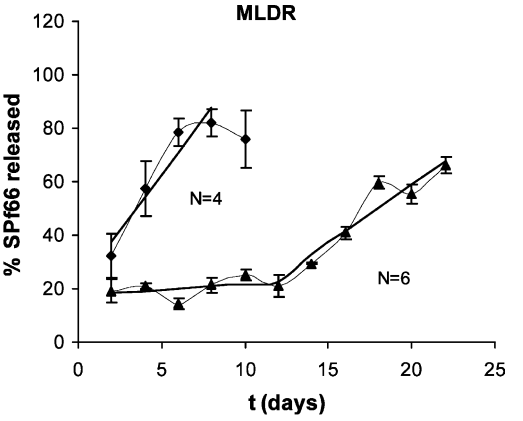

Fig. 6. Plot of percentage SPf66 released from MLDR versus time. Dots are experimental data (mean ± S.D.) and solid line are predictions from zero-order linear equation.

release over 20 days was obtained from 9% MLDM (Fig. 5). As described above, the experimental data fitting to the empirical model, but in this case the release was governed by two constants (Eq. (2)):

$$X = X_{01}(1 - e^{-k_1 t}) + \frac{X_{02}}{1 + e^{-k_2(t-t_0)}} \quad (2)$$

where k_1 is the rate constant during the first stage of release and all the others parameters as in Eq. (1).

In addition, the release profile from this formulation was very similar to that obtained from SDM (Fig. 4), where however the burst effect was reduced by 8%. Therefore, the most extended release for 5-FUR (without burst effect) was obtained from 9% MLDM.

Conversely, many therapeutic peptides and proteins need to be administered in a pulsed pattern, rather than continuously. Indeed, the 5-FUR pulse obtained from 3% MLDM would be desirable for therapeutic peptides. It is even easier to achieve for peptides because of their higher Mw and lower aqueous solubility. Thus, the following step was to evaluate this kind of system for releasing the synthetic polypeptide SPf66 malaria vaccine. Peptides and proteins differ from conventional small-molecule drugs in several aspects, but one of the most important is the complexity of protein structure. Due to the close correlation of protein efficacy with three-dimensional molecular structure, it is essential to maintain structural integrity through all formulation steps until the drug is released from the dosage form at the delivery site. Otherwise the activity of the protein or peptide is lost (Johnson, 2000). In addition, the need to use organic solvents, indispensable in the method of preparing PLGA systems, mainly microspheres and implants, is another drawback because of the poor stability of protein drugs (Chen et al., 1997; Crotts and Park, 1997; Putney and Burke, 1998).

For these reasons and to avoid the preparation process damaging the polypeptide, and thus reducing its biological activity or rendering the polypeptide immunogenic, we modified the multilayer system to a central reservoir of peptide drug surrounded on both sides by several drug-free PLGA-layers. The SPf66 stayed unaltered as demonstrated in content tests.

The first MLDR prepared consisted of a central reservoir of SPf66 surrounded by 4 drug-free PLGA-layers (Section 2.1). In this case a constant release ($k = 2.2 \times 10^{-3} \pm 5 \times 10^{-4}$ mg/day; $r = 0.954$) over 10 days was achieved without any initial burst (Fig. 6). However, when six drug-free PLGA-layers were added (Section 2.1) a moderate pulse was obtained, 14–22 days from the beginning of the release (Fig. 6). Thus, a 20.4% of the dose was released during the first 2 days, release then levelled off over the following 10 days ($k = 0$; $P = 0.384$), increasing from this moment with zero-order release rate constant $k = 7.3 \times 10^{-4} \pm 1.7 \times 10^{-4}$ mg/day ($r = 0.907$). For SPf66 malaria vaccine this profile is desirable, as mentioned above.

These results show the suitability of the proposed film-implants as potential biodegradable delivery system for controlled release of various drug types. The versatility of their design facilitates obtaining: (a) different release patterns, ranging from continuous to pulsed release both for highly water-soluble low Mw substances as 5-FUR and for the synthetic polypeptide SPf66; (b) implants without damaging protein structure; (c) devices with reduced size for easier administration.

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