Biodegradable hollow fibres containing drug-loaded nanoparticles as controlled release systems

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Abstract: A ‘multiple’ delivery system was studied, consisting of hollow microfibres containing drug-loaded nanoparticles. Both fibres and nanoparticles are made of biodegradable polymers, so that the system does not need any surgical operation to be removed. The main advantage of the system is that it allows the contemporaneous release of different kinds of drugs. Copolymers of poly(lactic acid) and ε-caprolactone were used for the preparation of the fibres through both wet and dry–wet spinning procedures. Two types of nanoparticles, gelatin and poly(DL-lactide-co-glycolide) nanoparticles, were prepared by simple water-in-oil and oil-in-water emulsions, respectively. Drugs such as dexamethasone and methotrexate were used to load the particles. The technique employed for the preparation of the nanoparticles filled fibres was described and the drug release characteristics of this system were investigated and compared with those of the free nanoparticles.

INTRODUCTION

During the last two decades, significant advances have been made in the controlled delivery of therapeutic agents. An important goal in controlled drug release is ‘drug targeting’, ie delivery of a drug to specific cells and organs in the body. The main advantage of drug targeting is the delivery of an active agent only to a particular cell population or to a restricted site. In this way the therapeutic effect of the drug is increased and therefore a lower dose is required, the exposure of other cells is minimized and the toxic side-effects are reduced.

The main disadvantage of drug targeting, however, is that very often the release apparatus has to be implanted in situ (and eventually removed) by a surgical procedure. An interesting solution for this drug targeting problem is represented by the use of nanoparticles.1–4 These are polymeric particles less than 1 μm in size, used as drug carriers both because of their small dimension and high surface-to-volume ratio, which favours the release of loaded drugs. Nanoparticles are generally administered by intravenous, intramuscular or subcutaneous injection or by peroral or ocular administration.

However, if the small size of the particles represents an advantage for the reasons mentioned above, it also makes it difficult to control the position of the particles inside the human body after administration. Therefore, it is not always possible to have a specific therapeutic effect restricted to the target site. It could be hypothesized that the use of a matrix, able to provide a mechanical immobilization for the particles without affecting the release of the drug could provide a solution to this problem. In this respect, a release device was designed, based on the entrapment of drug-loaded nanoparticles into a poly(vinyl alcohol)-based hydrogel matrix.5,6

In the present work a ‘multiple’ delivery system has been developed. This system is based on a biodegradable hollow microfibre filled with drug-loaded biodegradable nanoparticles. The biodegradation of both fibre and particles would guarantee the complete release of the loaded drug. The system should allow the release of various drugs loaded into the nanoparticles, directly and exclusively to the affected body site. Of course the use of this system does not avoid the need of a surgical operation to be implanted. However, the interest in developing this system is related to two main reasons: the first is that the system, being completely biodegradable, does not require a removal operation; the second is that it is a ‘multiple’ system able to release different agents to the same target site either simultaneously or in sequence, depending on the specific requirements.

A schematic representation of the device is shown in Fig 1(a). The fibre has a thin wall and the inner cavity is filled with nanoparticles loaded with specific drugs. In the first phase after the implant, before biodegra-
dation of either fibre or particles starts, the drugs are released only by diffusion. The drugs first diffuse out of the particles and then pass through the fibre wall (Fig 1(b)). This is a two-step diffusion process with apparently slow kinetics. However, the drug release is favoured by the high surface-to-volume ratio of the particles, the small thickness of the fibre wall (that can be easily maintained below 20 μm) and the high concentration of the drug. Subsequently, when the fibre starts to decompose through biodegradation, the particles pass through the fibre wall and head towards the target site (Fig 1(c)). Obviously the particles also start to degrade, so the kinetics of the drug release will depend on both diffusion and material degradation.

The materials to be used for the production of the fibres should possess some specific properties, such as:

- spinning ability, which is the most important characteristic for the production of fibres
- elasticity, that is fundamental for applications in which a variation of the shape and/or of the dimension of the device is required during its running
- biocompatibility and, for some applications, also haemocompatibility
- biodegradation kinetics: it must be possible to calibrate the lifetime of the device and therefore the duration of drug release depending on the desired effects
- capacity to undergo sterilization.

Poly(DL-lactide-co-(ε-caprolactone) (PLA-
PCL) was chosen, among the many biodegradable polymers investigated for the preparation of the fibres, because it possesses most of the above properties. It is a well-known copolymer which combines the high elasticity of poly(caprolactone) (PCL) with the mechanical properties and high degradation kinetics of poly(lactic acid) (PLA).7,8

Fibres were produced by both dry and wet spinning and filled with one of two different kinds of nanoparticles:

(1) poly(DL-lactide-co-glycolide) (PLGA) nanoparticles loaded with dexamethasone (DXM), were produced by a solvent evaporation procedure based on a single oil-in-water (O/W) emulsion;7
(2) gelatin nanoparticles loaded with methotrexate (MTX) were produced by a solvent evaporation procedure based on a single water-in-oil (W/O) emulsion.9

The morphological characteristics of both nanoparticles and fibres were investigated by optical and scanning electron microscopy. The in vitro release of the drugs from nanoparticles entrapped into the fibres was evaluated and compared with that from free nanoparticles.

MATERIALS AND METHODS
Materials
Poly(DL-lactide-co-glycolide 50/50) (PLGA) with an
average molecular weight $M_w$ 40000–75000 g mol$^{-1}$, dexamethasone, gelatin (type B, from bovine skin), poly(methyl methacrylate) (PMMA) ($M_w$ 120000 g mol$^{-1}$), methotrexate and glutaraldehyde (GTA) (25% and 8% aqueous solutions) were supplied by Sigma Aldrich.

Poly(vinyl alcohol) (PVA) with $M_w$ 15000 g mol$^{-1}$ was supplied by Fluka Chemika.

Dichloromethane, chloroform, toluene, acetone and Tween 80 were supplied by Carlo Erba Reagenti, Italy. All of the above chemicals were used as received.

Poly (ε-lactic acid)-co-poly(ε-caprolactone) (PLA–PCL) was synthesized according to a procedure described in literature. In brief, the polymerization was conducted in bulk, at 140°C, under a nitrogen atmosphere using stannous octoate as a catalyst and glycerol as initiator. Five different copolymers were synthesized with different composition and viscosimetric molecular weights as reported in Table 1. After the synthesis, the materials were accurately dried under vacuum at 30°C for 1 day and then stored in a desiccator chamber.

### Preparation of PLGA nanoparticles loaded with dexamethasone

PLGA nanoparticles were prepared by an O/W emulsification process described in detail in a previous work. Briefly, DXM was dissolved in acetone, then PLGA and dichloromethane were added to obtain the ‘oil phase’. This solution was added dropwise to an aqueous PVA solution, while mixing by a high-speed homogenizer (Miccra-D8, Falc Instruments). Mixing was continued for a total of 10 min. After the addition of Tween 80 as a surfactant, solvent evaporation was performed overnight under gentle magnetic stirring at room temperature. The nanoparticles obtained were cleaned through three cycles of centrifugation and suspension in distilled water. The final product was dried under vacuum at 30°C for 1 day and then stored in a desiccator chamber.

### Preparation of gelatin nanoparticles loaded with methotrexate

Gelatin nanoparticles were prepared by a solvent evaporation procedure based on a single W/O emulsion previously described. Briefly, MTX was dissolved in a sodium hydroxide solution. A gelatin solution was prepared in phosphate buffered saline (PBS). This solution was mixed with a MTX solution to obtain the ‘water phase’. A PMMA solution was prepared by dissolving the polymer in a chloroform/toluene mixture to obtain the ‘oil phase’. The ‘water phase’ was added to the ‘oil phase’ under mixing using a high-speed homogenizer. Mixing was continued for 8 min at 23500rpm and then for 30 min at 400 rpm. Then cross-linking was performed by dropwise addition of a GTA–toluene solution. The system was maintained at 4°C overnight with magnetic stirring in order to allow the completion of the gelatin cross-linking reaction. The obtained nanoparticles were cleaned through three cycles of centrifugation and suspension in toluene and then two cycles in acetone. The final product was dried at room temperature to obtain a fine yellow powder.

### Scanning electron microscopy

To examine the morphological characteristics of the nanoparticles, samples were viewed using a scanning electron microscope (SEM) (Jeol T300). The samples were prepared on aluminum stubs and coated with gold prior to examination.

### Determination of drug loading

The total quantity of DXM contained in the PLGA nanoparticles was determined by the following method. Ten milligrams of nanoparticles were added with 20 ml of dichloromethane and, after complete dissolution, the drug was determined spectrophotometrically at $λ$=265.5 nm. Similarly, the total quantity of MTX contained in the gelatin nanoparticles was determined by dissolving 10 mg of nanoparticles in 30 ml of 6 N HCl under stirring at 60°C for 20 min. After filtration the drug was determined spectrophotometrically at $λ$=243 nm.

### In vitro release test

The release of DXM from PLGA nanoparticles and the release of MTX from gelatin nanoparticles were evaluated using a side-by-side diffusion chamber (Crown Glass, Sommerville, NJ, USA). This chamber consisted of two identical glass cells separated by a Millipore membrane LPVP 0.1 μm thick. The volume of each cell was 1.5 cm$^3$ and the surface area for material exchange was 0.64 cm$^2$. A nanoparticles suspension in phosphate buffer solution (PBS) was placed in one of the two cells (donor side) while pure PBS was placed in the other cell (acceptor side). The apparatus was held in a thermostat bath at 37°C and both donor and acceptor sides were maintained under stirring at 70 rpm. An aliquot of the solution was removed from the acceptor side of the chamber at regular time intervals and analyzed spectrophotometrically at $λ$=238.5 for DXM and at $λ$=303 nm for MTX.

### Table 1. Synthesized copolymers.

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>PDLA/PCL molar ratio</th>
<th>$M_w$ (g mol$^{-1}$)</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>40/60</td>
<td>305000</td>
</tr>
<tr>
<td>II</td>
<td>50/50</td>
<td>165000</td>
</tr>
<tr>
<td>III</td>
<td>50/50</td>
<td>866000</td>
</tr>
<tr>
<td>IV</td>
<td>60/40</td>
<td>659000</td>
</tr>
<tr>
<td>V</td>
<td>70/30</td>
<td>666000</td>
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Hollow microfibres were produced both by dry and wet spinning.

After having tested several solvents (chloroform, THF/dioxane mixtures, and acetone), solutions of
PLA–PCL in acetone were chosen for production of the fibres. In both cases deionized water was used as non-solvent. Acetone was chosen on the basis of the quality of the fibres produced and, taking into consideration its low cost, its good water miscibility and low toxicity.

The starting solutions were prepared by varying the polymer/solvent ratio from 1/1.5 to 1/10 (w/v) depending on the copolymer composition and extrusion conditions. The dried polymer was dissolved in acetone at room temperature with gentle stirring for 1–2h, and the solution then transferred into a syringe.

**Wet spinning**
The spinning apparatus is represented in Fig 2. Two syringes (one for the polymer solution and one containing water) were controlled with a Harvard Apparatus–Syringe Infusion Pump 22. Polymer solution and water were collected through 0.9-mm diameter needles and forwarded to a double annulus extrusion head immersed in a water-bath. As soon as the polymer solution exited from the spinneret, the polymer coagulated and a fibre with an internal cavity was formed. The fibre formed was collected on a glass or metal rotating bobbin.

**Dry spinning**
In this case, the spinning apparatus is similar to that
used for wet spinning, the main differences being the absence of the water bath and the vertical position of the extrusion head. The following operating parameters were varied in order to modulate morphology and the geometry of the fibres in both wet and dry spinning procedures:

- concentration of the starting polymer solution
- composition and molecular weight of the copolymer
- extrusion rate
- solvent/non solvent ratio
- bobbin rotational speed (stretching rate)
- coagulation bath temperature.

**Particle entrapment**

The entrapment of nanoparticles inside the fibre was performed as follows. The syringe, which pumps water into the internal part of the extrusion head, was filled with a suspension prepared adding water with weighed particles. The suspension was maintained under stirring using a very small magnetic stirrer (Fig 3) in order to prevent particle deposition inside the syringe. As stated above, because the coagulation of the fibre takes place immediately after extrusion, the particles remain entrapped inside the fibre.

**RESULTS AND DISCUSSION**

The results of morphological analysis (Fig 4) showed that PLGA nanoparticles were obtained with an average diameter of 200–300 nm. The total amount of DXM contained in these particles was about 38% relative to the initial amount used for sample loading.

A representative curve for the release of dexamethasone from PLGA nanoparticles prepared using an initial DXM/PLGA ratio of 1/3 is shown in Fig 5. The initial burst phase, observed during the first 70–80 h, is related to the drug located near the external surface of the particles. After this initial phase, a decrease in release rate can be observed, which is then followed by a new increase after about 150 h. This trend was observed in all the experiments performed. It can be hypothesized that the external aqueous phase diffuses into the polymeric matrix, inducing it to swell and thus favouring the subsequent release of the drug entrapped inside the matrix. Approximately 95% of the loaded DXM was released within 4 weeks.

The results of morphological analysis of the gelatin nanoparticles (Fig 6) showed that smooth and solid nanoparticles with an average diameter of 100–200 nm were obtained. The efficiency of MTX entrapment into the nanoparticles was calculated: it was found that the total quantity of MTX entrapped into the nanoparticles was about 10% relative to the initial amount used for sample loading.

Figure 7 shows a representative curve for the release
of MTX from nanoparticles prepared using an initial MTX/gelatin ratio of 1/8 (w/w). A burst phase can be observed during the first 10h, releasing about 40% of the loaded drug. This can be related to the drug located near the external surface of the particles that rapidly comes out. After this initial phase, a decrease in release rate can be observed followed by a new increase after about 30h. Approximately 97% of the MTX loaded was released in 10 days.

With regard to fibre preparation, it was observed that all the tested materials showed good properties with the exception of copolymer I (PLA/PCL=40/60) which showed inadequate mechanical properties leading to fibres with low stability. Irrespective of the elastomeric character, due to the PCL content, all the copolymers were able to support a very high shear rate, thus leading to fibres with extremely small dimensions.

Fibres produced employing copolymers II and III were not able to maintain their shape after spinning. The materials that resulted were soft and sticky so that the fibres collapsed from a circular to a flat cross-section and became attached to each other. Fibres produced employing copolymers IV and V were able to maintain their shape especially when extruded with low shear rates. Figures 8 and 9 show a fibre with its wall collapsed and a fibre which has maintained its shape, respectively. It must be emphasized that a flat shape is not considered a disadvantage of itself because purpose of the fibre is essentially to entrap the drug-loaded nanoparticles. However, the tendency to become flat indicates that the material can be too soft and very difficult to handle. Therefore, depending on the copolymer used, the spinning conditions, such as solution viscosity and extrusion rate, were varied in order to obtain a product with the desired properties.

The presence of particles inside the fibres was not easily detectable because of their very small dimensions. Therefore it was not surprising that SEM images of fibres, which had fractured under liquid nitrogen, did not clearly show the particles entrapped inside.

This could be explained in two different ways:

1) the particles depart through the fibre wall;
2) the particles remain entrapped in the fibre wall.

This second hypothesis was confirmed by the experimental evidence. First, SEM images (Fig 10) of a fibre surface defect show the presence of nanoparticles located inside the fibre wall. Second, images obtained by optical microscope of fibres produced using water added with Methylene Blue as non-solvent showed clearly that the blue-coloured water remained in the
fibre cavity (Fig 11). Therefore, if water molecules do not pass through the fibre wall it is reasonable to believe that nanoparticles suspended in water behave similarly.

Figures 12 and 13 show the release of dexamethasone and metotrexate respectively from PLGA and gelatin particles entrapped in the fibres. It can be observed that the trend of release of both the drugs is very similar to that observed in the case of free nanoparticles and the percentage of release resulted quite slow in comparison with that observed in the case of free nanoparticles.
CONCLUSIONS
A 'multiple' delivery system based on hollow fibres filled with drug-loaded nanoparticles has been developed. The release system is prepared in such a way that it could permit the drug release directly and exclusively to the desired body site, because the fibre keeps the nanoparticles in a fixed position. The biodegradation of both fibre and particles guarantees the complete release of the loaded drug and avoiding a procedure for removal of the system. Moreover, the system allows the contemporaneous release of various drugs simply using particles loaded with different drugs.

The biocompatibility and the mechanical properties of the fibres could allow the use of these systems in several applications. As an example, metallic stents are often used in transluminal coronary angioplasty to prevent restenosis. The inflammatory response of the host due to the contact with the stent could be locally treated by coating the stent with fibres containing particles loaded with anti-inflammatory agents. The elastic properties of the fibres could also allow this coating to follow the size changes that the stent undergoes to operate properly. In this case a further advantage is that the multiple delivery system would not require an additional surgical procedure to be implanted as it would be used as a coating of the metallic stent that is inserted by angioplasty.

Further potential applications that could be envisaged in other biomedical fields, such as odontoiatry and wound healing, in which local microbial infections must be treated. In these cases the system also obviates any surgical procedure for implanting and, in addition, allows the release of several drugs to the same target site.

REFERENCES