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Preparation and characterization of starch-poly-ε-caprolactone microparticles incorporating bioactive agents for drug delivery and tissue engineering applications

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Abstract

One limitation associated with the delivery of bioactive agents concerns the short half-life of these molecules when administered intravenously, which results in their loss from the desired site. Incorporation of bioactive agents into depot vehicles provides a means to increase their persistence at the disease site. Major issues are involved in the development of a proper carrier system able to deliver the correct drug, at the desired dose, place and time. In this work, starch-poly-ε-caprolactone (SPCL) microparticles were developed for use in drug delivery and tissue engineering (TE) applications. SPCL microparticles were prepared by using an emulsion solvent extraction/evaporation technique, which was demonstrated to be a successful procedure to obtain particles with a spherical shape (particle size between 5 and 900 μm) and exhibiting different surface morphologies. Their chemical structure was confirmed by Fourier transform infrared spectroscopy. To evaluate the potential of the developed microparticles as a drug delivery system, dexamethasone (DEX) was used as model drug. DEX, a well-known component of osteogenic differentiation media, was entrapped into SPCL microparticles at different percentages up to 93%. The encapsulation efficiency was found to be dependent on the polymer concentration and drug-to-polymer ratio. The initial DEX release seems to be governed mainly by diffusion, and it is expected that the remaining DEX will be released when the polymeric matrix starts to degrade. In this work it was demonstrated that SPCL microparticles containing DEX can be successfully prepared and that these microparticular systems seem to be quite promising for controlled release applications, namely as carriers of important differentiation agents in TE.

Keywords: Poly-ε-caprolactone; Starch-based microparticles; Emulsion-solvent evaporation; Drug delivery; Dexamethasone

1. Introduction

Materials of natural origin have been studied and proposed for a wide range of biomedical applications [1–4]. Materials such as collagen, alginate, hyaluronic acid, silk fibroin, chitosan and starch are among the most studied polymers with numerous advantages depending on the specific applications [5–13]. One of the most relevant benefits of using materials of natural origin is their biodegradability inside the human body. Biodegradable systems have the ability to function satisfactorily for a certain time and subsequently to degrade into products easily cleared from the body, with no need for surgery for their removal. This is a particularly desirable property for the design of carriers for the controlled delivery of therapeutic drugs, since it will permit the entrapped drug to be released slowly, allowing

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repeating dosages and ensuring the successful effect of the treatments [14] as the polymer carrier degrades.

Starch-based polymers have been studied and proposed in the last decade by Reis and coworkers [13,15–21] for several biomedical applications, such as drug delivery carrier systems, hydrogels and partially degradable bone cements, materials for bone replacement/fixation or fillers for bone defects, and porous structures to be used as scaffolds in tissue engineering of bone and cartilage. These materials were found to be biocompatible [16,22–23], noncytotoxic, biodegradable [24–27] and have shown a great processing versatility [13]. These blended materials have potential application as carriers for the controlled release of different bioactive agents in the form of microparticular systems. Indeed, biodegradable starch-based microparticles have been widely investigated and proposed as drug delivery systems [28–30]. For instance, starch microparticles using soluble potato starch have been developed and proposed for the release of a nonsteroidal anti-inflammatory drug [21]. Moreover, a blend of starch and polylactic acid have been used for the encapsulation of steroids, growth factors and bioactive glass in a microparticle system [31–33]. These studies showed that the starch–polylactic acid microparticles are suitable carriers for the controlled release of bioactive agents for bone tissue engineering applications. In addition, derivatives of starch, such as starch acetate or poly(acryl starch), have been described for the incorporation and release of peptides and proteins [34–36]. However, to our knowledge there has so far been no report in the literature on the development of microparticle systems based on starch–polycaprolactone blended materials. The combination of a hydrophilic natural material (starch) with a hydrophobic synthetic polymer (polycaprolactone), both biodegradable and biocompatible, in a single blended material constitutes the major advantage of these microparticles.

Numerous controlled release systems have been developed, ranging from implants [37,38] to novel osmotically driven pills [38]. The use of noninvasive delivery methods, such as injectable systems in the form of nano and microparticles, will bring substantial benefits when compared with some surgical techniques. It has already been reported that injectable systems made of nano and microparticles could be applied as carriers of different drugs and bioactive agents within the field of tissue engineering (e.g. differentiation agents and growth factors [39,40]). Dexamethasone (DEX) has been widely used in clinical applications to treat immuno-disorders [41,42], but a more specific and common use has been the control of the inflammatory response and tissue repair during organ transplantation [43]. In the last years, the use of this corticosteroid as an osteogenic agent has increased considerably in in vitro cell culture to induce the differentiation of stem cells into an osteoblastic lineage [41,44–46].

This study aims to establish experimental conditions for the production of a biodegradable and biocompatible microparticular system with different characteristics (e.g. size, size distribution, surface morphology) that can be used as a potential carrier for the delivery of important bioactive agents. For that, we have used a polymeric blend of starch with polycaprolactone. The microparticular system was characterized in terms of particle size, size distribution, surface morphology and chemical structure. The carrier potential was evaluated by encapsulating DEX into the microparticles and its release behavior studied in vitro.

2. Materials and methods

2.1. Materials

A polymeric blend of corn starch with poly-ε-caprolactone (SPCL, 30–70 wt.%) was used in this study. More details about the thermal properties of this polymeric blend can be found elsewhere [47]. Methylene chloride and polyvinyl alcohol (PVA) were obtained from Sigma, and used as received. Unless otherwise indicated, the molecular weight (MW) of the PVA used was in the range 30,000–70,000 g mol^{-1} DEX (97%, cell culture tested, Sigma) was used as a bioactive molecule for the encapsulation studies. Solvents for high-performance liquid chromatography (HPLC) (acetonitrile and water) were HPLC grade (LABSCAN). Triamcinolone was used as internal standard for DEX quantification. Potassium bromide (KBr) for IR spectroscopy ($\geqslant$99.5%) was obtained from Sigma. Other chemicals were of reagent grade, all from Sigma, and used as received.

2.2. Preparation of SPCL microparticles

SPCL microparticles were prepared by using an emulsion solvent extraction/evaporation technique [48]. Briefly, SPCL was dissolved in 5 ml of methylene chloride under vigorous stirring. This solution was dropped into a 200 ml PVA solution, and emulsified for 4 h at different stirring rates. Different experimental conditions were evaluated, and the details of each condition are summarized in Table 1. The microparticles where then collected by filtration, washed with distilled water and vacuum dried in a desiccator. For the selected condition to be loaded with DEX, SPCL was mixed with the steroid at different percentages (5, 10 and 15% (w/w), relatively to polymer weight) and dissolved in methylene chloride. The same procedure was performed as described for unloaded microparticles. The reaction medium was stored at 4 °C for later quantification of unloaded DEX. All experiments were carried out in triplicate.

2.3. Physicochemical characterization of SPCL microparticles

2.3.1. Morphological analysis: scanning electron microscopy (SEM) and micro-computed tomography (μ-CT)

To analyze the morphology and surface of the microparticles obtained under the different experimental conditions, the samples were mounted onto aluminium stubs with a

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