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ScienceDirect
Acta Biomaterialia 5 (2009) 2054–2062
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Dexamethasone-loaded scaffolds prepared by supercritical-assisted phase inversion

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Received 30 July 2008; received in revised form 28 November 2008; accepted 13 January 2009
Available online 7 February 2009

Abstract

The aim of this study was to evaluate the possibility of preparing dexamethasone-loaded starch-based porous matrices in a one-step process. Supercritical phase inversion technique was used to prepare composite scaffolds of dexamethasone and a polymeric blend of starch and poly(L-lactic acid) (SPLA) for tissue engineering purposes. Dexamethasone is used in osteogenic media to direct the differentiation of stem cells towards the osteogenic lineage. Samples with different drug concentrations (5–15 wt.% polymer) were prepared at 200 bar and 55 °C. The presence of dexamethasone did not affect the porosity or interconnectivity of the polymeric matrices. Water uptake and degradation studies were also performed on SPLA scaffolds. We conclude that SPLA matrices prepared by supercritical phase inversion have a swelling degree of nearly 90% and the material presents a weight loss of ~25% after 21 days in solution. Furthermore, in vitro drug release studies were carried out and the results show that a sustained release of dexamethasone was achieved over 21 days. The fitting of the power law to the experimental data demonstrated that drug release is governed by an anomalous transport, i.e., both the drug diffusion and the swelling of the matrix influence the release of dexamethasone out of the scaffold. The kinetic constant was also determined. This study reports the feasibility of using supercritical fluid technology to process in one step a porous matrix loaded with a pharmaceutical agent for tissue engineering purposes.

Keywords: Supercritical fluids; PLLA; Drug delivery; Tissue engineering; Phase inversion

1. Introduction

Tissue engineering is a promising therapeutic approach that combines cells, biomaterials, and bioactive compounds [1,2]. The emerging next generation of engineered tissues relies on the development of loaded scaffolds containing bioactive molecules in order to control the cellular function (e.g. growth or differentiation factors) or to act on the surrounding tissues (e.g. drugs such as anti-inflammatory agents or antibiotics) [3,4]. Hence, the strategy is to mimic matrix and provide the necessary information or signaling for cell attachment, proliferation and differentiation to meet the requirement of dynamic reciprocity for tissue engineering. This justifies the importance of drug delivery in tissue engineering applications [5,6].

Small molecular weight drugs that control proliferation and differentiation of cells can be incorporated into biodegradable scaffolds to induce cellular differentiation and tissue remodeling. The scaffold therefore plays an important role not only as a physical support but also in the cell proliferation and differentiation [7]. Dexamethasone is a relevant bioactive compound to be used in bone tissue engineering applications. This drug is used in osteogenic media to direct the differentiation of stem cells towards

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doi:10.1016/j.actbio.2009.01.047

the osteogenic lineage [8–10]. A wide variety of materials has been used for the preparation of scaffolds, from metals to ceramics and polymers. Synthetic biodegradable polymers have been widely used for tissue engineering. However, natural polymers have unique, intrinsic properties that make them appealing to be used as scaffolds [11]. Natural polymers are in general non-toxic, even in large concentrations, mucoadhesive, biocompatible, and biodegradable [11–13].

Starch-based polymers have been studied in our group for a wide range of bone-related therapy applications, ranging from tissue engineering scaffolds [14–17], to bone cements [18,19] and drug delivery systems [20,21]. Their natural origin, together with their mechanical properties and biocompatibility, support the potential of starch-based materials in the biomedical field. Our group has reported the success of starch-based microparticles, from starch-poly(L-lactic acid) (SPLA), to act as controlled delivery devices [22]. SPLA microparticles loaded with corticosteroids were able to release the drug up to 30 days.

One of the most important stages of tissue engineering is the design and processing of a porous 3D structure, with high porosity, high interconnectivity and uniform distribution. Conventionally, three-dimensional structures can be obtained by processes such as solvent casting-particle leaching [23], freeze-drying-particle leaching [24], thermally induced phase separation [25], compression moulding [26], injection moulding [27], extrusion [28], foaming [29], wet spinning [30], electrospinning [31], among others [32]. The main disadvantages of these methods are the use of large quantities of organic solvents or high temperatures. Supercritical fluids have been proposed as excellent alternatives to the conventional processes for polymer processing [33–35].

The supercritical-assisted phase inversion method is one of the proposed alternatives to reduce the use of large quantities of organic solvents. Different techniques have been proposed for the preparation of scaffolds for tissue engineering, namely gas foaming or phase inversion [36]. Gas foaming takes advantage of the plasticizing properties of carbon dioxide. In this technique, the polymer is exposed to carbon dioxide, which plasticizes it by reducing the glass transition temperature or melting point. On venting the CO_2 by depressurization, thermodynamic instability causes supersaturation of the carbon dioxide dissolved in the polymer matrix and hence nucleation of cells occurs [37]. This technique is limited by the high glass transitions of the crystalline polymers and is therefore more common to amorphous polymers.

The phase inversion method involves casting of a polymer solution onto an inert support followed by immersion of the support with the cast film into a bath filled with a non-solvent for the polymer. The contact between the solvent and the non-solvent causes the solution to be phase-separated. Several advantages exist if the non-solvent used is a supercritical fluid. One of the most important advantages of the use of carbon dioxide is the fact that simply by tuning the processing conditions, i.e. pressure and temperature, one can tailor the final structure of the product. Additionally, when carbon dioxide is used as a non-solvent a subsequent drying step is avoided and the porous structure obtained is a dry product free of any residual solvent.

Carbon dioxide is the most commonly used supercritical fluid as it has mild critical parameters, it is environmentally benign, non-toxic, non-flammable, non-corrosive, ready available and inexpensive. Its elimination and the recovery of final products are easier (no residue is left and a dry solid product is easily obtained, just by controlling the pressure), leading to processes with less energy consumption [38].

The use of carbon dioxide as a non-solvent for phase separation has been successfully reported in the literature, for example for PLLA [39,40], PMMA [41], Nylon 6 [42], PS [43], cellulose acetate [44,45], polysulfone [46,47] and polycarbonate/PEG [48]. Recently we have proposed the use of a supercritical-assisted process for the preparation of scaffolds from natural sources [49]. A starch-based blend (SPLA) was successfully processed by supercritical-assisted phase inversion method.

To our knowledge the use of a supercritical phase inversion technique for the preparation of composite matrices loaded with a bioactive agent is for the first time reported in this work. In this study, the possibility of preparing in a one-step process a porous matrix loaded with an active compound is evaluated. This methodology could open other possibilities of developing substrates for tissue engineering or bioengineering applications, where processing of biomaterials and incorporation of bioactive agents are combined using supercritical fluid technologies.

2. Experimental procedure

2.1. Materials

A commercial blend of starch and poly-(L-lactic acid) (SPLA 50:50) was supplied by Novamont. Dexamethasone (CAS 50-02-2, 98% purity) was purchased from Sigma and chloroform, (CAS 67-68-5, 99.9% purity) was purchased from Vaz Pereira. Carbon dioxide (99.998 mol.%) was supplied by Air Liquide. All chemicals were used with no further purification.

2.2. Supercritical-assisted phase inversion process

The phase inversion experiments were carried out in an apparatus specially designed and built for this work. The set up is schematically presented in Fig. 1 [49].

SPLA 50:50 is mixed with dexamethasone and dissolved in chloroform. This procedure was performed separately for each of the ratios of bioactive agent and polymer (5, 10 or 15% wt./wt. (dexamethasone:polymer). In each experiment a small amount (ca. 2 ml) of the polymer solution is loaded in a stainless steel cap 2 cm in diameter, which is placed inside the high pressure vessel. The vessel is heated (to 55 °C) by means of an electric thin band hea-

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