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Preparation and stability of dexamethasone-loaded polymeric scaffolds for bone regeneration processed by compressed CO_2 foaming☆

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ABSTRACT

Compressed CO_2 foaming allows for obtaining porous solid drug-loaded scaffolds for regenerative medicine under mild conditions and in the absence of organic solvents. However, a precise process design and optimization as well as certain modifications of the technology are still needed to tackle certain limitations like the control of the porosity of scaffolds from certain low molecular weight biopolymers. Besides, stability under storage is a critical quality attribute to define the usefulness of scaffolds. In the particular case of bone scaffolds, the information on this regard is still very limited and strongly dependent on each particular composition and architecture. In order to gain an insight into this issue, scaffolds composed of poly(lactic-*co*-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL) as polymeric matrix, with and without pre-gelified starch (St) as release-controlling agent and dexamethasone (DXMT) as bioactive compound were designed and prepared by a modified compressed CO_2 foaming technique (26 °C, 60 bar) and assessed regarding stability under storage. The scaffolds were stored at 25 °C and 65% relative humidity (zone II ICH-climatic conditions for Europe, USA and Japan) for up to three months to determine the effect of storage on the structural, physicochemical and mechanical properties, and DXMT release. Changes in the scaffolds point out the importance of stability assays and storage conditions. Namely, a decrease in the viscoelastic moduli of the scaffolds and a faster degradation rate were observed after prolonged storage periods. DXMT release from the scaffolds was erosion-controlled and thus modified for scaffolds stored for longer time periods.

1. Introduction

Bone tissue usually heals completely after damage without scarring [1]. Nevertheless, the overall rate of nonunion or delayed union represents 5–10% of the total fractures and depends on the type of bone injury and the patient condition. In Europe, around one million patients per year undergo a surgical bone reconstruction procedure, with expectations of increasing numbers owing to population ageing, a parameter that compromises bone regeneration [2].

Regenerative medicine is a rising discipline aiming to repair, replace or regenerate damaged tissues and organs. A suitable structure should be accordingly provided to cells for their attachment and proliferation, allowing the formation of a functional tissue similar to the one to be reconstructed [3]. In case of bone tissue regeneration, autografts (from the patient), allografts (from donors or corpses) and xenografts (from animals) are natural grafts that can act as mechanical support for tissue growth as well as a source of growth factors that promotes tissue regeneration [4].

Synthetic porous grafts (scaffolds) are being developed to overcome the problems of availability and post-implantation risks of natural grafts. Compressed CO_2 foaming is regarded as a key green technology for the processing of scaffolds [5]. This technology is based on the role of CO_2 as a porogen by putting in contact this compressed fluid with the matrix of the scaffold for CO_2 sorption and the subsequent expansion of

Abbreviations: DSC, differential scanning calorimetry; DXMT, dexamethasone; ICH, International Council for Harmonisation; MIP, mercury intrusion porosimetry; MSC, mesenchymal stem cells; PBS, phosphate buffer solution; PCL, poly(ε-caprolactone); PLLA, poly(L-lactic acid); PLGA, poly(lactic-*co*-glycolic) acid; St, pre-gelified starch; SEM, scanning electron microscopy; T_g, glass transition temperature; $Tm/Tm_1/Tm_2$, melting temperature values

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the scaffold (i.e. foaming) upon CO_2 depressurization through a pore nucleation and growth mechanism [6,7]. This technology is unique for preparing drug-loaded scaffolds in a solvent-free approach whilst avoiding leaching downstream steps [5]. Problems of cytotoxicity of the scaffolds, thermal degradation of their components and low incorporation yields are thus precluded by using this technique. Moreover, compressed CO_2 can also act as sterilizing agent for the scaffolds [8]. Nevertheless, compressed CO_2 foaming was not effective so far in the processing of certain biopolymers (e.g., PLGA) of low inherent viscosities ($< 0.5\ dL\ g^{-1}$) having attractive degradation profiles for scaffolding purposes [9,10]. Dramatic foam expansions have been obtained so far with this kind of matrices and a proper process design and optimization is urged to circumvent this limitation of the compressed CO_2 foaming technology.

The *in vivo* performance of the processed scaffolds depends primarily on their structure and composition [11]. Porosity must be at least 65%, ideally around 90% [12], while pore interconnectivity should be high to enable diffusion of oxygen and nutrients to the cells and removal of metabolites [11]. The presence of micro and mesopores in the scaffolds allows the circulation of biological fluids and cell adhesion, while small (5–10 μm) and large macropores (50–200 μm) are required to facilitate vessel formation and tissue growth, respectively [5]. At the same time, the mechanical properties of the scaffold should ideally be suitable to act as a temporary support while the growing tissue is still not able to withstand by itself the intrinsic mechanical demands of bone.

The composition of the scaffold must be precisely selected with a matrix able to provide a degradation rate that suits the growth rate of the bone tissue, and with growth and differentiation agents to promote the proper bone tissue growth. Various biodegradable synthetic (e.g., poly(lactic-*co*-glycolic) acid –PLGA– and poly(ε-caprolactone) –PCL– [12–14]) and natural (e.g., starch, collagen, gelatin, silk, alginate or chitosan [12,15–18]) polymers are common materials employed as scaffold matrices. Among the growth and differentiation agents, dexamethasone (DXMT), a drug belonging to the group of glucocorticoids, can induce the differentiation of mesenchymal stem cells (MSC) to osteoblasts [19]. DXMT-loaded scaffolds providing local delivery of the bioactive agent may have the advantage of inducing bone formation whilst avoiding or at least mitigating the undesired collateral effects derived from a systemic administration of glucocorticoids.

Stability under storage is a critical quality attribute to define the performance and the availability of scaffolds. A short shelf-life would hamper the supply of these grafts and raise the cost of the treatment [20]. Nitrogen atmospheres or vacuum conditions are the most common packaging options of the commercial scaffolds. In these cases, the direct exposition to climatic conditions is initially avoided, although it might occur if the packaging is accidentally damaged (e.g., with a microhole) resulting in loss of the inert atmosphere or breaking of vacuum. Systems like knitted polylactide scaffolds and knitted silk fibroin (SERI©) surgical scaffolds have been tested for up to 3 years under room conditions, maintaining their mechanical properties for that period [21,22]. Nevertheless, there is still a paucity of information on the effect of storage on the durability of scaffolds with more complex porous structures or loaded with drugs, which might be more sensitive to environmental factors.

In this work, synthetic scaffolds made of mixtures of PCL and PLGA of low inherent viscosity (50:50 w/w) loaded with and without DXMT were prepared using a novel compressed CO_2-assisted foaming method. The processing method was herein designed and optimized for DXMT-loaded scaffolds from a previously implemented one [10] so that it could operate in one-pot, during short processing times, without use of organic solvents and under mild temperatures. The role of the incorporation of starch (St) in controlling the DXMT release profile from the scaffolds was evaluated. Then, the scaffolds (without packaging) were stored in chambers (25 °C, 65% relative humidity) mimicking the zone II ICH-climatic conditions (Europe, USA and Japan) [23] and the stability of the scaffolds in terms of structural, physicochemical and mechanical properties as well as DXMT release profiles were monitored for 3 months.

2. Materials and methods

2.1. Materials

PLGA (50:50 lactic:glycolic ratio; M_w 16 kDa; amorphous; $T_g = 41.4$ °C) was purchased from Purac (Gorinchem, The Netherlands). PCL (M_w 50 kDa; semicrystalline; $T_m = 61.6$ °C; $\Delta H_m = 95.9$ J/g) was supplied by Polysciences (Warrington, PA, USA). Corn starch (Amylo N-460, 52.6% amylose content) was bought from Roquette (Lestrem, France). Dexamethasone (DXMT, 97% purity) was obtained from Sigma-Aldrich (Saint Louis, MO, USA). Carbon dioxide (99.8% purity) from Praxair (Madrid, Spain) was used to process the scaffolds. Milli-Q water (resistivity > 18 MΩ cm; MilliQ, Millipore®, Madrid, Spain), sulfuric acid (95–97% purity, Merck, Darmstadt, Germany) and acetonitrile (99.9% purity, Merck, Darmstadt, Germany) were also used.

2.2. Preparation of oven-dried starch gels (St)

10 g of corn starch were dispersed in 210 g of water under magnetic stirring and then heated for 20 min at 121 °C (Autoclave Raypa, model AES-12, Terrassa, Spain). Then, the starch dispersion was transferred to a fridge at 4 °C and maintained for 60 h for retrogradation, and subsequently poured on Petri dishes and put in an oven at 80 °C for water evaporation. The resulting dry solid films were ground in a ball mill (Mix MM 400, Retsch Inc., Newton, PA, USA) to obtain powdered starch particles of *ca.* 20 μm size.

2.3. CO_2 sorption in the scaffold polymers

A gravimetric method was used to monitor carbon dioxide sorption in PLGA and in PCL:PLGA (50:50 w/w) mixtures at elevated pressure. A high-pressure magnetic suspension balance (Rubotherm GmbH, Bochum, Germany) was used to measure the CO_2 uptake by PLGA as a function of time and simultaneously detecting the volume change by a high pressure view cell (Eurotechnica GmbH, Hamburg, Germany), at the operating conditions of 66 bar and 26 °C (compressed liquid) and 51 bar and 26 °C (compressed gas) for PLGA and of 60 bar and 26 °C (compressed gas) for the PCL:PLGA mixture. The polymer was previously filled in the powdered form into a glass vessel, molten at 70 °C, cooled down for solidification and then placed in the magnetic suspension balance (Fig. 1). The force resulting from the mass and the buoyancy of the polymer is transmitted to a microbalance at the outside of the autoclave via a magnetic coupling. From the evolution of the weight as a function of time, the diffusion process could be followed and the CO_2 solubility determined (< 5% deviation) once saturation was achieved [24].

2.4. Compressed CO_2 foaming of synthetic scaffolds

Polyester-based scaffolds were prepared using a multi-step process sketched in Fig. 2. The components of the scaffolds (Table 1) were homogenized in a mortar and mixed for 5 min (Mixer Wab, model T2C, Switzerland) and then compressed in an eccentric tableting machine (FE236FC, Korsch, Berlin, Germany) to obtain compacts of 400 mg with prismatic dimensions (14 × 10 × 2.6 mm).

The compacted materials were processed in a high pressure equipment (Thar Technologies, Pittsburgh, PA, USA) adapted for compressed CO_2 foaming. Briefly, the compacts were placed in a rotating basket (700 rpm) inside the foaming vessel and put in contact with CO_2 at 26 °C and 60 bar for 30 min. After this soaking period, pressure was lowered from 60 to 30 bar at a flow rate of 10 bar/min, and then