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Supercritical processing of starch aerogels and aerogel-loaded poly (ε-caprolactone) scaffolds for sustained release of ketoprofen for bone regeneration☆

Leticia Goimil[a], Mara E.M. Braga[b], Ana M.A. Dias[b], José L. Gómez-Amoza[a], Angel Concheiro[a], Carmen Alvarez-Lorenzo[a], Hermínio C. de Sousa[b], Carlos A. García-González[a,*]

[a] Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, R+D Pharma group (GI-1645), and Health Research Institute of Santiago de Compostela (IDIS), Universidade de Santiago de Compostela, E-15782-Santiago de Compostela, Spain
[b] CIEPQPF, Chemical Engineering Department, FCTUC, University of Coimbra, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, Coimbra, 3030-790, Portugal

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ABSTRACT

Bone regeneration requires scaffolds with a suitable nanostructured 3D-network and decorated with bioactive functionalities to promote cell ingrowth and induce the intended biological responses. In this work, highly porous poly(ε-caprolactone) (PCL) scaffolds containing biodegradable mesoporous microparticles (starch aerogel microspheres) and a bioactive compound (ketoprofen, a NSAID) were designed and produced using supercritical technologies ($scCO_2$ impregnation/deposition and foaming) for bone regeneration purposes. One-micron-sized starch aerogel microspheres were processed for the first time. The effects of the incorporation of aerogel powders in the synthetic PCL-based scaffolds on the morphological, physico-chemical and mechanical properties of the construct were evaluated by N_2 adsorption-desorption analysis, scanning electron microscopy, mercury intrusion porosimetry, 3D-modeling, dynamic mechanical analysis and differential scanning calorimetry methods. Scaffolds containing starch aerogels presented an increased porosity and pore interconnectivity to promote the bone tissue growth processes at the expense of a minor decrease in the mechanical properties. The scaffolds showed sustained release of ketoprofen (37 °C, pH 7.4) in the timeframe of days with faster release rate in the case of scaffolds containing starch aerogels. Therefore, scaffolds containing starch aerogel microspheres and obtained by supercritical foaming are an attractive solution to obtain drug-loaded scaffolds with accurate pore structure (porosity, pore size distribution, interconnectivity) for cell in-growth and with sustained release profiles of bioactive compounds.

1. Introduction

Synthetic scaffolds are a promising alternative to biological grafts to promote bone repair in those situations where the natural self-regeneration is compromised. These synthetic constructs should give response to the typical problems associated with safety issues and to the scarcity of biological grafts with respect to the current increasing demand. Moreover, advanced synthetic scaffolds should have a superior performance to provide an accurate porous 3D-structure that guides and promotes tissue growth, enables the diffusion of nutrients and oxygen supplies and cell waste disposal, and acts as a provisional mechanical support [1]. Finally, bone repair materials should also have a performance aligned with the current social changes associated with the increase in life expectancy of population and with the world commitment of keeping older people autonomy enhancing their quality of life in the so-called “active ageing” [2].

Abbreviations: K, ketoprofen; LSD, least significant difference; MSCs, mesenchymal stem cells; NSAID, nonsteroidal anti-inflammatory drug; PBS, phosphate buffer solution; PCL, poly(ε-caprolactone); PGPR, polyglycerol polyricinoleate; PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); $scCO_2$, supercritical carbon dioxide; sc-drying, supercritical drying; St, starch; St_{raw}, starch in the native form; St_{aer}, starch aerogel; SEM, scanning electron microscopy; T_m, melting temperature.

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* Corresponding author at.
E-mail address: carlos.garcia@usc.es (C.A. García-González).

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Bioactive compounds can be incorporated in scaffolds for their local administration to promote bone healing efficiency by tackling several types of post-surgery complications due to infections and other biological processes that can impair the proper tissue regeneration [3]. Namely, it is necessary to reduce the time taken to solve severe foreign-body inflammatory responses [4]. Otherwise, poor osteointegration of the scaffold due to fibrous encapsulation and granuloma formation can occur [5,6]. Ketoprofen is a nonsteroideal anti-inflammatory drug (NSAID) that is orally administered during the post-operative period to relief pain and to reduce inflammation. The mode of action of ketoprofen is the non-selective inhibition of cyclooxygenases (COX-1 and COX-2), enzymes involved in the production of prostaglandins [7,8]. Ketoprofen does not inhibit bone healing process according to *in vivo* animal studies with goats and rabbits where no effects on MSCs proliferation and osteogenesis were observed after subcutaneous administration of daily doses of ketoprofen (*ca.* 2 mg/kg) at least for several weeks [9,10].

Several processing strategies are reported in the literature to incorporate bioactive compounds in bone scaffolds and to tune their release kinetics patterns to provide a local administration [11]. However, common scaffold processing techniques like solvent casting/particle leaching, phase separation or rapid prototyping have limited versatility to prepare drug-loaded scaffolds. These techniques usually need the use of either organic solvents, or high operating temperatures, or multi-step processing with leaching or purification steps, which may originate problems of cytotoxicity, premature drug degradation, low drug loading yields, reproducibility concerns or long processing times among others. Supercritical foaming of polymeric scaffolds emerges as the only one-step straightforward alternative able to overcome most of the above-mentioned drawbacks. This technique exploits the plasticizing and melting effects of supercritical carbon dioxide ($scCO_2$) on some biodegradable thermoplastic polymers such as poly-α-hydroxyesters (e.g. PLA, PLGA, PCL) to produce highly porous synthetic bone scaffolds in a simple, economical and reproducible procedure [12]. Nevertheless, the supercritical CO_2 foaming process has some limited control on the production of materials presenting well-defined pore size distributions and pore interconnectivities and, consequently, on the release of drug-loaded scaffolds.

Mesoporous silica particles incorporated in foamed polyester-based scaffolds have been shown to favour heterogeneous pore nucleation, to improve the mechanical properties and to refine the pore size distribution [13]. Also, inorganic aerogel particles tested for similar purposes were able to preserve the porous structure of the wet gel in the dry form [14]. Silica aerogel particles were observed to promote the survival and growth of fibroblastic and osteoblastic cells if incorporated in PCL-scaffolds obtained by solvent casting [15]. Nevertheless, the incorporation of silica in scaffold formulations is still a concern since rigorous downstream processes are required to remove remnants of toxic silica precursors and the full biodegradability, bioerosion and excretion profiles of silica aerogels are still not fully confirmed. The use of polysaccharide aerogels as a safer, biodegradable and even cheaper alternative mesoporous material to be incorporated in synthetic grafts seems promising [16] and it is prospected in this work.

Purely organic aerogel scaffolds can present high mesoporosity and good cytocompatibility [17–19], but weak mechanical properties for hard-tissue repair and limited control of macroporosity. Mechanical reinforcement of aerogels implies post-processing steps [18]. Techniques explored to confer macroporosity to aerogel scaffolds give restricted results concerning the overall macroporosity values and the control of the macropores sizes [17,19,20]. Differently, the combination of micron-sized polymeric aerogel particles with biodegradable polyesters using the supercritical CO_2 foaming technique to prepare scaffolds would assemble and combine the high porosity and mesopore volume of aerogels, the good mechanical properties of polyesters and the capacity of tuning macropores sizes and densities of the $scCO_2$ foaming method.

Starch aerogel powders emerge as an interesting admixture of polymeric-based scaffolds due to the intrinsic biocompatibility and biodegradability of starch and to the excellent textural properties of aerogels [21]. Moreover, starch is an abundant and relatively cheap polysaccharide of particular interest for regenerative medicine purposes [22,23]. Starch is able to interact with polyesters changing its crystallinity depending on the starch gelification method used [12]. Starch aerogels have been prepared from different sources (corn, pea, tapioca, potato), formats (monoliths, beads, particles) and using different gelation mechanisms (thermal, inclusion complexing, microwaves) [16,24–29]. Namely, aerogel particles reported in the literature have particle sizes in the order of hundreds of microns or larger for starch and of tens of microns or higher for other polysaccharides [30,31]. Nevertheless, starch aerogels in the size range of few microns would be ambitioned for several pharmaceutical and biomedical applications and, namely, for their incorporation in composite scaffolds presenting uniform physicochemical and mechanical properties.

The aim of this work was to prepare starch aerogel microspheres and to evaluate their potential roles once incorporated in ketoprofen-loaded PCL-based scaffolds on the control of the pore structure of the material and of the ketoprofen release over time. One-micron sized starch aerogel microspheres were developed by emulsion-gelation under ultrasound sonication followed by supercritical drying, for the first time. Ketoprofen was loaded in the starch aerogel particles by a supercritical CO_2 impregnation method or directly incorporated in the scaffold by a supercritical CO_2 foaming process for the sake of comparison. The resulting scaffolds were evaluated regarding their morphological, mechanical and physicochemical properties, as well as to their ketoprofen-release profiles.

2. Materials and methods

2.1. Reagents

PCL (PCL_{raw}, 50 kDa, T_m = 61.4 °C, 66.7% crystallinity) was purchased from Polysciences (Warrington, PA, USA) in the powdered form. Corn starch in the native form (St_{raw}, amylo N-460; 52.6% amylose content, 12.9% loss on drying, conform to USP and EP) was obtained from Roquette (Lestrem, France), and carbon dioxide (99.9% purity) was supplied by Praxair, Inc. (Madrid, Spain). Ketoprofen (K, T_m = 95.8 °C, 99.7% purity) was provided by Acofarma (Terrassa, Spain). Polyglycerol polyricinoleate (PGPR) was obtained from Palsgaard (Juelsminde, Denmark). Paraffin oil was provided by Panreac (Castellar del Vallès, Spain). Ethanol (99.8% purity) was obtained from Omnilab (Bremen, Germany) and diethyl ether (98% purity) from Labkem (Mataró, Spain).

2.2. Starch aerogel preparation

Starch gels in the form of microspheres were obtained by the emulsion-gelation method [25]. Briefly, a water-in-oil emulsion (w/o) was prepared from a mixture of paraffin oil and a starch aqueous solution 15% (w/w) in a 3:1 oil-to-water weight ratio. 3% (w/w) of PGPR with respect to the aqueous phase was added to the oil phase as an emulsifier. The mixture obtained (240 g) was autoclaved (Raypa Steam Sterilizer, Terrassa, Spain) at 121 °C and 1.1 bar for 20 min. After sterilization and partial cooling (95 °C), the mixture was homogenized using an ultrasound probe (450D, Branson Digital Sonifier, Nuevo Laredo, Mexico), using an