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Nanohydroxyapatite/poly(ester urethane) scaffold for bone tissue engineering

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Abstract

Biodegradable viscoelastic poly(ester urethane)-based scaffolds show great promise for tissue engineering. In this study, the preparation of hydroxyapatite nanoparticles (nHA)/poly(ester urethane) composite scaffolds using a salt-leaching-phase inverse process is reported. The dispersion of nHA microaggregates in the polymer matrix were imaged by microcomputed X-ray tomography, allowing a study of the effect of the nHA mass fraction and process parameters on the inorganic phase dispersion, and ultimately the optimization of the preparation method. How the composite scaffold's geometry and mechanical properties change with the nHA mass fraction and the process parameters were assessed. Increasing the amount of nHA particles in the composite scaffold decreased the porosity, increased the wall thickness and consequently decreased the pore size. The Young's modulus of the poly(ester urethane) scaffold was improved by 50% by addition of 10 wt.% nHA (from 0.95 ± 0.5 to 1.26 ± 0.4 MPa), while conserving poly(ester urethane) viscoelastic properties and without significant changes in the scaffold macrostructure. Moreover, the process permitted the inclusion of nHA particles not only in the poly(ester urethane) matrix, but also at the surface of the scaffold pores, as shown by scanning electron microscopy. nHA/poly(ester urethane) composite scaffolds have great potential as osteoconductive constructs for bone tissue engineering.

Keywords: Polyurethane; Composite; Hydroxyapatite; Microtomography; Scaffold

1. Introduction

Bioresorbable poly(urethane)-based scaffolds have recently attracted considerable attention, and their great potential in bone tissue engineering (TE) has recently been advocated [1–3]. In contrast to the majority of the synthetic scaffolds made of poly(lactic acid) and poly(glycolic acid), poly(urethane)s can have greater elasticity. This is given by the variety of chemistry and molecular weights of the various components, and by the molar ratios in which they react to form poly(urethane)s [2,3]. In fact, viscoelastic poly(urethane)s are beneficial for a large number of implantable devices, such as artificial skin, cardiovascular implant, bone graft and cartilage substitute [1]. More specifically, at the interface between bone and implant, elastic scaffolds can sustain shear forces and can establish an intimate contact with the native tissue. This may potentially facilitate the proliferation of osteogenic cells and the bone regeneration [2,4]. For example, two in vivo studies reported promising results using biodegradable viscoelastic poly(ester urethane)s scaffolds made of 1,4,3,6-dianhydro-D-sorbitol (ISO) or poly(ethylene glycol), 1,6-hexamethylene diisocyanate and poly(ε-caprolactone) diol (PCL), for the regeneration of iliac crest defects in sheep even in the absence of seeded cells [5,6]. Depending on the structure and geometry of the scaffolds obtained, moduli values ranging from 0.08 to 3.4 MPa have been measured [7]. These mechanical properties are far away from those of the most common bone substitutes, such as calcium phosphate ceramics. However, for a bone defect of critical size a fixation is normally used, the role of which is to carry most of the load applied to the bone and to stabilize the defect.

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In such a case, a biomaterial inserted into the bone defect does not experience compressive loads close to the ones in the real bone environment. However, the mechanical properties of scaffolds intended for bone TE should permit the application of a minimal mechano-stimulation of the seeded cells. A relatively stable structure for bone tissue ingrowth is thus provided and preserved until the newly grown tissue has completely inhabited the TE construct and is capable of taking the applied load at the defect site [8].

The bio- and cytocompatibility of poly(ester urethane) containing PCL segments have already been extensively reported, and micro- and macroporous poly(ester urethane) scaffolds have been used for studying biomechanical signal transmission to seeded chondrocytes and stem cells [9–11]. Osteogenic cells were shown to survive and proliferate in these scaffolds [12]. However, poly(ester urethane) with PCL segments did not demonstrate any outstanding osteoconductive property in vitro, and stem cells cultured in osteogenic medium have alkaline phosphatase and osteocalcin expression comparable to those cultured on a poly(D,L-lactic acid) surface [13]. Osteoconductivity is essential for the recruitment of cells capable of forming bone matrices and ultimately for successful bone ingrowth into scaffold [14]. Bone invasion in scaffolds can potentially be improved and accelerated by chemical surface modifications or by the addition of an osteoconductive component in the polymer [14]. Hydroxyapatite mineral is widely used to render non-osteoconductive materials more biologically responsive by formation of a coating [15]. Such calcium phosphate coatings have already been shown to promote bone formation and apposition on biomedical devices [16]. Moreover, hydroxyapatite nanoparticles (nHA) have shown better protein adsorption and bone cell (osteoblast) adhesion than micron-sized particles [17]. When using this approach, the nHA particles are more effective by being in direct contact with the cells. In a three-dimensional (3-D) poly(urethane) structure, particles must be present on all the pore surfaces. Distribution within the polymer matrix could also affect, and significantly improve, the mechanical properties of the poly(ester urethane) scaffold and possibly the cell response.

This study reports the synthesis and processing of nHA/poly(ester urethane) composite scaffolds using a salt-leaching-phase inverse process. Dispersion of nHA aggregates in the polymer matrix was imaged and optimized using micro-computed X-ray tomography. The influence of nHA content and process parameters on the microstructure and mechanical properties of the scaffold was investigated.

2. Materials and methods

2.1. Materials and scaffolds processing

Degradable poly(ester urethane) (PUR) scaffolds for bone graft substitutes were prepared from a biocompatible PUR [2]. The viscoelastic PUR was synthesized in a one-step solution polycondensation, as already described in detail elsewhere [7]. The reactants, used without further purification, were 1,6-hexamethylene diisocyanate (HMDI) (Sigma, Milwaukee, WI) and a PCL (Aldrich, Milwaukee, WI) with a number average molecular weight of 530 g mol^{-1} and a functionality of 2. 1,4,3,6-Dianhydro-D-sorbitol (ISO) (Aldrich, Milwaukee, WI) was used as a chain extender after purification and recrystallization in ethyl acetate. Dibutyltin dilaurate (DBDL) (Fluka, Buchs, CH) was used as a catalyst and the purest grade *N*,*N*-dimethylformamide (DMF) (Fluka, Buchs, CH) was used as the solvent. PCL and ISO were melted together at 60 ± 5 °C and any trace of water was removed under low vacuum (0.8 Torr) for at least 3 h before the addition of DBDL and HMDI. The HMDI:PCL:ISO molar ratio used was kept at 1:0.32:0.64 for all the polymerizations performed [2]. To prevent cross-link formation, the synthesis was performed at a temperature between 70 and 80 °C. The lack of cross-links in the network means that the synthesized PUR can be classified as a thermoplastic polymer. For the whole study and the preparation of the scaffolds, eight syntheses were performed, each yielding between 60 and 80 g of polymer. The PUR batches were mixed in a single master batch to avoid variations in scaffold properties due to discrepancies in molecular weight values between the PUR syntheses. The different syntheses and the final batch were characterized by viscosimetry, gel permeation chromatography and nuclear magnetic resonance. The experimental HMDI:PCL:ISO molar ratio was calculated, by integration of specific ^{1}H NMR peaks of the different PUR segments, to be 1:0.32:0.66 for the final batch; the PUR intrinsic viscosity, weight average molecular weight and polydispersity were, respectively, 1.29 dl g^{-1}, 503,000 g mol^{-1} and 2.03.

Hydroxyapatite nanoparticles, of 10–15 nm crystallite size (Biomaterials US, Ltd., USA), delivered in suspension in a solution of unknown composition, were used as ceramic fillers. The particles were collected, washed extensively with ethanol and dried at 100 °C under vacuum. The obtained dry powder was characterized by infrared spectroscopy and X-ray diffraction analysis to ensure the conservation of the hydroxyapatite crystalline structure during the nHA powder preparation, and transmission electron microscopy was performed to image aggregated and elongated nHA particles between 50 and 200 nm in size.

The PUR and nHA/PUR scaffolds were prepared by adapting a salt-leaching-phase inverse technique described in a previous study [7]. The general method consisted of mixing equal weights of the PUR solution and the porogen. The mixture was vigorously mixed by hand until a homogeneous paste was obtained, which was then poured into a mould and left in air for the solvents to evaporate slowly. The porogen was a sodium phosphate heptahydrate dibasic salt (Fluka, Buchs, CH) ground and sieved to a final particle size range of 90–300 μm. In order to minimize any interference from the experimental parameters, together with