



In-situ solvothermal processing of polycaprolactone/hydroxyapatite nanocomposites with enhanced mechanical and biological performance for bone tissue engineering



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ABSTRACT

The interest in biodegradable polymer-matrix nanocomposites with bone regeneration potential has been increasing in recent years. In the present work, a solvothermal process is introduced to prepare hydroxyapatite (HA) nanorod-reinforced polycaprolactone *in-situ*. A non-aqueous polymer solution containing calcium and phosphorous precursors is prepared and processed in a closed autoclave at different temperatures in the range of 60–150 °C. Hydroxyapatite nanorods with varying aspect ratios are formed depending on the processing temperature. X-ray diffraction analysis and field-emission scanning electron microscopy indicate that the HA nanorods are semi-crystalline. Energy-dispersive X-ray spectroscopy and Fourier transform infrared spectrometry determine that the ratio of calcium to phosphorous increases as the processing temperature increases. To evaluate the effect of *in-situ* processing on the mechanical properties of the nanocomposites, highly porous scaffolds (>90%) containing HA nanorods are prepared by employing freeze drying and salt leaching techniques. It is shown that the elastic modulus and strength of the nanocomposites prepared by the *in-situ* method is superior (~15%) to those of the *ex-situ* samples (blended HA nanorods with the polymer solution). The enhanced bone regeneration potential of the nanocomposites is shown via an *in vitro* bioactivity assay in a saturated simulated body fluid. An improved cell viability and proliferation is also shown by employing (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) (MTT) assay in human osteosarcoma cell lines. The prepared scaffolds with *in vitro* regeneration capacity could be potentially useful for orthopaedic applications and maxillofacial surgery.

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1. Introduction

Tissue engineering offers a new approach to regenerate diseased or damaged tissues such as bone [1]. The rapidly growing research in the bone tissue engineering area provides a new and promising approach for bone repair and regeneration [2]. Bone is a natural organic–inorganic composite consisting of collagen fibrils containing embedded, well-arrayed, nanocrystalline and plate-like inorganic materials with length of 25–50 nm [3,4]. Hydroxyapatite (HA), which is chemically similar to the inorganic component of

bone matrix, has been proved to be an osteoconductive material [5] with affinity toward many adhesive proteins and direct involvement in the bone cell differentiation and mineralization processes [6]. The close chemical similarity of HA to natural bone has led to extensive research efforts to use synthetic HA as a bone substitute and/or replacement in biomedical applications [7]. However, HA shows poor biomechanical properties such as high brittleness as well as low fatigue strength and flexibility that limit its load-bearing applications [8]. As a result, synthesis of HA particles with various sizes and morphologies and distribution of them inside biodegradable polymer matrixes have attracted significant interest in recent years [9]. The idea of combining bioactive ceramics and degradable polymers to produce three-dimensional (3D) scaffolds with high porosity is a promising strategy for the design and development of composite systems for bone tissue

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engineering [10]. Many composite scaffolds have been prepared either by distributing HA nanoparticles within a polymer matrix or by the mineralization of HA nanoparticles on the surface of polymeric substrates [10–13]. Much work has also been performed on the processing and applications of HA nanorods for bone tissue engineering as the bioactivity and mechanical properties are significantly enhanced compared to irregular-shaped particles [14–18].

In the present work, polycaprolactone (PCL) nanocomposites containing HA nanorods were prepared by *in-situ* solvothermal techniques. Polycaprolactone is a biodegradable polymer with remarkable toughness and good biocompatibility [19]. It is a semi-crystalline aliphatic polymer that has a slower degradation rate and higher fracture energy than most biocompatible polymers [20–23]. The intrinsic hydrophobic chemical nature of PCL as well as its poor surface wetting and interactions with biological fluids avoid cell adhesion and proliferation [11]. It has recently been shown that the biological and mechanical properties of PCL scaffolds can be tailored by osteogenic and osteoinductive inorganic phases, promoting bone regeneration [24–27]. Chen et al. [28] observed that relative cell survival of PCL-HA composites exceeded that of neat PCL and control samples in MTT assay. Nevertheless, poor interfacial adhesion is often observed when HA particles are *ex-situ* dispersed in a polymer matrix (due to different chemical nature of the components and their surface energy). Consequently, the *ex-situ* prepared composites may exhibit degraded mechanical properties. Li et al. [29] reported that surface treatment of HA nanoparticles by a kind of silane (γ -glycidoxypropyltrimethoxysilane), aiming to improve mechanical properties of PCL-HA composite, led to relative aggravation in biocompatibility and bioactivity (although the resulted values still exceeded that of control sample). A promising strategy to improve the distribution of the bioceramic in the polymer matrix with higher degree of phase interaction between the polymer and the inorganic filler is to synthesize the HA nanoparticles *in-situ* during processing of the scaffolds [11,30]. This procedure would avoid the extensive particle agglomeration typically seen in composites obtained by mechanical incorporation of HA particles into the polymer melt or solution [11]. Recently, coprecipitation and sol-gel methods have been utilized to synthesize HA nanoparticles-reinforced PCL nanocomposites [30–32]. Owing to the superior properties of HA nanorods, a few attempts have also made on processing of poly (D, L-lactide) (PDLLA) and polyvinyl alcohol (PVA) composites by employing solvothermal processes [33,34]. While the former polymer is expensive, the latter has weak mechanical characteristics. Therefore, we have adopted a new strategy for *in-situ* preparation of HA nanorods in the PCL matrix with fine particle distribution. Polycaprolactone is a FDA approved polymer which is relatively cheap with reliable mechanical strength [10,11,21]. Highly porous scaffolds (>90%) were prepared by salt leaching/freeze-drying techniques and their physicochemical, biological and mechanical properties were examined. The potential and benefits of the *in-situ* solvothermal processing compared to the *ex-situ* method is shown.

2. Experimental

2.1. Materials

Calcium hydrate ($\text{Ca}(\text{OH})_2$, Acros, USA) and phosphoric acid (85% H_3PO_4 , Merck, Germany) were used as Ca and P precursors for the synthesis of PCL/HA nanocomposites. Polycaprolactone with an average molecular weight of 80,000 was supplied by Sigma-Aldrich (USA). Analytical grades of acetone (Merck), dimethyl carbonate (DMC, Merck) and tetrahydrofuran (THF, Merck, Germany) were utilized as solvent. Ammonia (25%, Merck) was used to adjust the

pH. Hydroxyapatite powder with an average size of 60 nm was supplied by Merck (Germany). Sodium chloride crystals with sizes of 200–400 μm were provided by Merck (Germany) and utilized as porogen.

2.2. *In-situ* solvothermal process

Four grams of PCL was dissolved into 40 ml acetone under vigorous stirring for 6 h using a magnetic stirrer. The calcium precursor was added into the solution and mixed for 6 h. In a separate beaker, H_3PO_4 was added to acetone and mixed vigorously for 6 h. This solution was added to the PCL solution dropwise to achieve a Ca:P molar ratio of 1.67. The concentration of HA particles in the polymer matrix was set to 20 wt%. The HA concentration was selected based on the results of Rezaei et al. [30] that showed the highest tensile strength could be attained in PCL-20%HA nanocomposites. Ammonia was used to control the pH of the final sol to ~ 10 . This sol was transferred to a sealed autoclave and stirred vigorously for 24 h at different temperatures ranging from 60 to 150 °C. It is pertinent to point out that, some studies have reported that PCL can react with ammonia at temperatures roughly above 200 °C [35,36]; hence, the chemical nature of PCL is not affected in the examined range. The prepared PCL/HA nanocomposite was poured into a Petri dish and dried for 24 h at room temperature.

2.3. Fabrication of porous scaffolds

To prepare PCL/HA scaffolds, the nanocomposite film was dissolved in DMC under vigorous stirring for 8 h using a magnetic stirrer. To create open and large pores, sodium chloride crystals were added to the polymeric solution (30 vol%) and stirred for 2 h. The mixture was then poured into cylindrical aluminium moulds (1 cm diameter and 1.5 cm height) and freeze-dried for 24 h at -70 °C and 0.01 mbar. Afterwards, the scaffolds were immersed in double-distilled water for 7 days to remove the salt and extra ions formed during the solvothermal synthesis. To prepare *ex-situ* PCL/HA nanocomposite, the commercial HA powder and NaCl crystals were added to the polymeric solution and homogenized by stirring for 2 h. Freeze-drying and salt leaching techniques were utilized to prepare highly porous scaffolds (>90% porosity).

2.4. Materials characterization

To analyse the *in-situ* processed HA particles in the polymer matrix, the nanocomposite was dissolved in THF and centrifuged at 3000 rpm for 30 min. The extracted powder was dried and weighted. Morphological analysis was performed on HA and PCL/HA nanocomposites using a MIRA || TESCAN (Czech Republic) and a HITACHI S4160 field-emission scanning electron microscope (FE-SEM, Japan). Phase analysis was conducted using a STOE STADI P X-ray diffractometer (XRD, Germany). Fourier transform infrared (FTIR) spectroscopy (SHIMADZU, Japan) was used to identify the functional groups of HA and to determine the bonds between the ceramic and polymer phases in the composite material. FTIR spectrum was recorded in the range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1} . To determine the hydrophilicity of the prepared films, water contact angle was measured by an OCA15plus video based optical contact angle meter (Dataphysics Instruments GmbH, Filderstadt, Germany). An equal volume of deionized water (4 μl) was placed on the film by means of an electronic syringe unit forming a drop. Photos were taken to record the shape of the drops and the images were analyzed using the instrument software.

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