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Biomineralized porous composite scaffolds prepared by chemical synthesis for bone tissue regeneration

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ABSTRACT

Scaffold design is a key factor in the clinical success of bone tissue engineering grafts. To date, no existing single biomaterial used in bone repair and regeneration fulfils all the requirements for an ideal bone graft. In this study hydroxyapatite/polycaprolactone (HA/PCL) composite scaffolds were prepared by a wet chemical method at room temperature. The physico-chemical properties of the composite materials were characterized by X-ray diffraction, Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy, while scaffold morphology was investigated by scanning electron microscopy (SEM) with energy-dispersive spectroscopy to validate the process used for synthesis. Finally, the response of bone marrow-derived human mesenchymal stem cells (hMSCs) in terms of cell proliferation and differentiation to the osteoblastic phenotype was evaluated using the Alamar blue assay, SEM and alkaline phosphatase activity. Microstructural analysis indicated that the HA particles were distributed homogeneously within the PCL matrix. The biological results revealed that the HA/PCL composite scaffolds are suitable for the proliferation and differentiation of MSCs in vitro, supporting osteogenesis after 15 days. All the results indicate that these scaffolds meet the requirements of materials for bone tissue engineering and could be used for many clinical applications in orthopaedic and maxillofacial surgery.

1. Introduction

Novel strategies for regenerating diseased or damaged bone tissue are necessary because of the limitations of established therapies for the treatment of trauma, congenital defects and other bone diseases. The development of new materials able to structurally, mechanically and biofunctionally interface with natural tissues is relevant to the success of regenerative strategies [1].

Bone is a highly organized tissue which assembles from the nano- to macro-scale levels, producing a complex structural network able to remodel itself in the presence of traumatic fractures [2]. However, the ability to naturally heal large defects is significantly reduced. In this context the use of synthetic scaffolds with tailored morphologies and appropriate functional responses promotes the correct growth of new bone tissue. However, optimal scaffold degradation kinetics, comparable with the rate of tissue formation, is a key aspect in preserving the mechanical response of the hybrid tissue at the bone defect site.

In this regard, several authors have investigated the use of biodegradable polymers in surgery and medicine [3]. For instance natural and synthetic polymers such as collagen, polylactic acid (PLA), polyglycolic acid (PGA), co-polymers of PLA and PGA (PLGA) and polycaprolactone (PCL) show suitable properties for their application in tissue engineering [4,5]. More specifically, PCL is a biodegradable and highly biocompatible polymer with chemical properties, namely hydrophobicity as well as a slow degradation rate, which make it an ideal bone substitute in bone regeneration [6]. However, it does not promote an osteogenic response, one of the main requirements in designing an ideal bone analogue to guide the cascade of biological events related to the formation of mineralized tissue.

Recently, composite materials comprising a bioactive phase within a biodegradable polymer matrix have been developed. In particular, the challenging idea of designing "tissue-inspired composite materials" has moved towards the synthesis of ceramic/polymer composites with advantages over either pure ceramic or pure polymer [7–10], resulting in superior materials for specific applications. Traditionally, calcium phosphate-based ceramics have proved to be attractive materials for biological applications. Among these bioceramics particular attention has been paid to hydroxyapatite (HA) – $Ca_{10}(PO_4)_6(OH)_2$ – with an atomic ratio of calcium to phosphorus (Ca/P) of 1.67.

There are numerous ways to synthesize HA. Widely used processes include aqueous colloidal precipitation and sol–gel, solid-state and mechano-chemical methods. HA may be synthesized at

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room temperature, providing the ability to directly control the particle and grain sizes [11,12]. In comparison with traditional strategies involving physical mixing of HA, they ensure a more controlled and finer distribution of crystallites in the polymer matrix. This provides an improved mechanical response in terms of strength, stiffness, toughness and fatigue resistance, to achieve complete mechanical compatibility [13].

In recent years studies of scaffold composite materials have been performed as to successfully reproduce the microenvironment required to support and improve the molecular interactions which occur within tissues, between cells and within the mineralized extracellular matrix (ECM) [14]. It is well known that the scaffold architecture plays a crucial role in initial cell attachment and subsequent migration into and through the matrix and in the mass transfer of nutrients and metabolites, providing sufficient space for development and later remodelling of the organized tissue [15,16].

Defined morphological features (i.e. pore size) are required to ensure cell adhesion, molecular transport, vascularization and osteogenesis. For instance, small pores (with a diameter of a few microns) favour hypoxic conditions and induce osteochondrum formation before osteogenesis occurs [17,18]. In contrast, scaffold architectures with larger pores (several hundred microns in size) rapidly induce a well-vascularized network and lead to direct osteogenesis [19].

In this work HA/PCL porous scaffolds for bone tissue engineering have been produced by chemical synthesis/phase inversion and salt leaching. First, a composite system was synthesized consisting of HA and PCL wherein the submicron HA particles were uniformly dispersed within the polymer matrix. The use of low temperature approaches to synthesize HA in the presence of a solvent in which the polymer is soluble provide a novel pathway to generate homogeneous composite materials preserving the microstructural features of the HA crystals. Then the pore architecture was developed by removing the solvents and the porogen system (i.e. NaCl crystals), of predefined particle size.

A careful investigation of the physico-chemical properties of the composite was carried out by X-ray diffraction, Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy. The in vitro cytotoxicity and biocompatibility of the porous composite scaffolds in direct contact with bone marrow-derived human mesenchymal stem cells (hMSCs) were also studied, to validate the potential utility of these scaffolds in bone tissue engineering applications.

2. Materials and methods

2.1. Synthesis of composite materials and scaffold preparation

HA/PCL (40/60 wt.%) composite scaffolds were prepared at room temperature from $Ca(NO_3)_2{\cdot}4H_2O$ (99%) (A.C.S. Reagent, Aldrich) and H_3PO_4 (85 wt.% solution in water) (A.C.S. Reagent, Aldrich) as Ca and P precursors, respectively. Tetrahydrofuran (THF) was used as the solvent, while NH_4OH was used to achieve a pH value of 11. PCL (molecular weight 65 kDa, Sigma–Aldrich) was chosen as the biodegradable polymer. The synthesis reaction in THF (Fluka) can be represented as follows:

$$10Ca(NO_3)_2 \cdot 4H_2O + 6H_3PO_4 + 20NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20NH_4(NO_3) + 58H_2O \quad (1)$$

The procedure consists of two steps (Fig. 1). The first involves the addition of H_3PO_4 to THF, followed by the slow addition of NH_4OH over 30 min, until the solution reaches a pH value higher than 10. $Ca(NO_3)_2{\cdot}4H_2O$ is added to the H_3PO_4 and NH_4OH solution and stirred for 60 min – the amount of $Ca(NO_3)_2{\cdot}4H_2O$ was chosen to produce a Ca/P ratio of 1.67. The reaction was allowed to

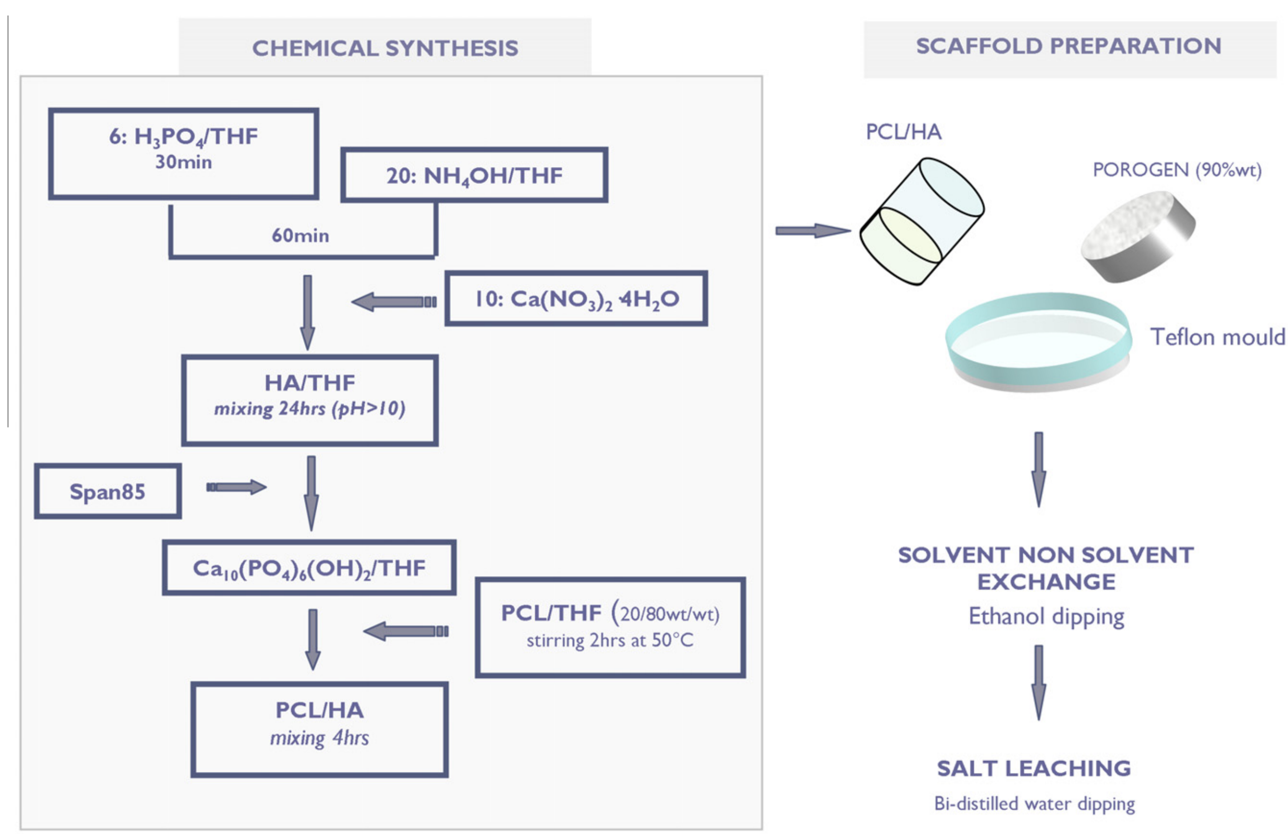

Fig. 1. Scheme of scaffold preparation by chemical synthesis and phase inversion/salt leaching.